The effects of liraglutide and dapagliflozin on cardiac function and structure in a multi-hit mouse model of Heart Failure with Preserved Ejection Fraction

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Short title: Liraglutide and dapagliflozin in a HFpEF mouse model

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ABSTRACT

Aims
Heart failure with preserved ejection fraction (HFpEF) is a multifactorial disease that constitutes several distinct phenotypes, including a common cardiometabolic phenotype with obesity and type 2 diabetes mellitus. Treatment options for HFpEF are limited, and development of novel therapeutics is hindered by the paucity of suitable preclinical HFpEF models that recapitulate the complexity of human HFpEF. Metabolic drugs, like Glucagon Like Peptide Receptor Agonist (GLP-1RA) and Sodium Glucose Transporter 2 inhibitors (SGLT2i), have emerged as promising drugs to restore metabolic perturbations and may have value in the treatment of the cardiometabolic HFpEF phenotype. We aimed to develop a multifactorial HFpEF mouse model that closely resembles the cardiometabolic HFpEF phenotype, and evaluated the GLP-1 RA liraglutide and a SGLT2i dapagliflozin.

Methods & Results
Aged (18-22 months old) female C57BL/6J mice were fed a standardized chow (CTRL) or high fat diet (HFD) for 12 weeks. After 8 weeks HFD, Angiotensin-II (ANGII), was administered for 4 weeks via osmotic mini-pumps. HFD+ANGII resulted in a cardiometabolic HFpEF phenotype, including obesity, impaired glucose handling and metabolic dysregulation with inflammation. The multiple-hit resulted in typical clinical HFpEF features, including cardiac hypertrophy and fibrosis with preserved fractional shortening but with impaired myocardial deformation, atrial enlargement lung congestion, and elevated blood pressures. Treatment with liraglutide attenuated the cardiometabolic dysregulation and improved cardiac function, with reduced cardiac hypertrophy, less myocardial fibrosis, and attenuation of atrial weight, natriuretic peptide levels, and lung congestion. Dapagliflozin treatment improved glucose handling, but had mild effects on the HFpEF phenotype.

Conclusions
We developed a mouse model that recapitulates the human HFpEF disease, providing a novel opportunity to study disease pathogenesis and development of enhanced therapeutic approaches. We furthermore show that attenuation of cardiometabolic dysregulation may represent a novel therapeutic target for treatment of HFpEF.

Translational Perspective
The failure of many treatment modalities for HFpEF may –at least in part– be explained by the lack of an adequate animal model. The diverse etiology of HFpEF is still largely neglected in pre-clinical research. In this study we developed a murine model that includes advanced age, female sex, in concert with co-morbidities: elevated blood pressure, obesity and T2DM. We demonstrate that this model recapitulates the human cardiometabolic HFpEF phenotype. We showed that contemporary glucose lowering drugs, liraglutide and dapagliflozin, which are both under study for HFpEF, have positive results. Our model may be useful to evaluate novel cardiometabolic, anti-fibrotic, and anti-inflammatory treatments for HFpEF.

Keywords: HFpEF, liraglutide, dapagliflozin, mouse model, cardio-metabolic
A translational cardiometabolic HFpEF model

HFpEF Phenotype

| Comorbidities      | Structural and molecular changes |
|--------------------|----------------------------------|
| Obesity            | Preserved EF                     |
| T2DM               | Concentric LVH                   |
| Elevated blood pressure | Left atrial hypertrophy       |
| Lung Congestion    | Diastolic dysfunction            |
|                    | Elevated natriuretic peptides    |
|                    | Inflammatory metabolic profile   |

Liraglutide attenuates the unfavorable cardiometabolic changes and preserves cardiac function in murine HFpEF

- Obesity
- T2DM
- Lung Congestion
- Fibrosis

- Concentric LVH
- Left atrial hypertrophy
- Diastolic dysfunction
- Natriuretic peptides
### Non-standard Abbreviations and Acronyms

| Abbreviation | Description |
|--------------|-------------|
| Aorta Pmax   | aorta maximal pressure |
| Aorta Pmin   | aortic minimal pressure |
| Aorta Pmean  | aortic mean pressure |
| ANGII        | Angiotensin-II |
| ANP          | atrial natriuretic peptide |
| CCD          | central committee of animal experiments |
| CO           | cardiac output |
| Col1a1       | collagen-1a1 |
| Col3a1       | collagen-3a1 |
| CSA          | cross sectional area |
| CTRL         | Low fat equivalent chow |
| Dapa         | dapagliflozin |
| EF           | ejection fraction |
| FS           | fractional shortening |
| Gal-3        | galectin-3 |
| GDF-15       | growth differentiation factor-15 |
| GLP-1        | glucagon like peptide 1 |
| GLP-1-RA     | glucagon like peptide 1 receptor antagonist |
| GLS          | global longitudinal strain |
| HFpEF        | heart failure with preserved ejection fraction |
| HFrEF        | heart failure with reduced ejection fraction |
| HFD          | high fat diet |
| HFD+ANGII    | high fat diet + Angiotensin-II |
| HR           | heart rate |
| IL-6         | interleukin-6 |
| Lira         | liraglutide |
| LVAWd        | left ventricular anterior wall thickness in diastole |
| LVAWs        | left ventricular anterior wall thickness in systole |
| LVEF         | left ventricular ejection fraction |
| LVIDd        | left ventricular internal diameter in diastole |
| LVIDs        | left ventricular internal diameter in systole |
| LVPWd        | left ventricular posterior wall thickness in diastole |
| LVPWs        | left ventricular posterior wall thickness in systole |
| MCCA         | mouse clinic for cancer and aging |
| MI           | myocardial infarction |
| OGTT         | oral glucose tolerance test |
| RPLSR        | reverse peak longitudinal strain |
| SGLT2i       | sodium glucose co-transporter 2 inhibitor |
| T2DM         | type-2 diabetes mellitus |
| TIMP-1       | tissue inhibitor of metalloprotease-1 |
1. INTRODUCTION

Patients with heart failure (HF) are commonly subdivided into those with a reduced (HFrEF) and preserved (HFpEF) ejection fraction \(^1\). The prevalence of HFpEF is expected to steeply increase during the following years, and will likely become the dominant HF subtype in the near future \(^2\). Prognosis of HFpEF is poor, with a mortality of 10%-30% over 5 years, and burden of the disease is substantial \(^3\). Risk factors and pathophysiological processes leading to HFrEF and HFpEF differ largely: HFrEF is mostly seen in men with a history of myocardial infarction (MI) or cardiomyopathy, whereas HFpEF is a multifactorial disease expressed as several distinct phenotypes, including a common cardiometabolic phenotype characterized by obesity, type-2 diabetes mellitus (T2DM), hypertension, advanced age and a female predominance \(^4\text{–}^6\). In contrast to HFrEF, no currently available evidence-based treatments can improve symptoms and prognosis in HFpEF patients. In addition, the development of novel treatments is hampered by the lack of animal models that can sufficiently represent the complex human HFpEF phenotype\(^7,^8\).

In this study we describe the development of a mouse model that features typical aspects of the cardiometabolic HFpEF phenotype. This model is of particular interest for the exploration of novel therapeutic interventions that target the cardiometabolic system in HFpEF, such as glucagon like peptide receptor agonists (GLP-1 RA) and sodium glucose co-transporter 2 inhibitors (SGLT2i). GLP-1 is a hormone primarily produced in the intestine with an important role in glycemic control, body weight and appetite \(^9\). SGLT2i inhibits reabsorption of glucose in the kidney which results in glycosuria, natriuretic and diuretic effects\(^10\). Both GLP-1 RA and
SGLT2i have emerged as novel and promising cardioprotective drug, as they reduced the risk of cardiovascular events in patients with type 2 diabetes and may reflect a novel promising therapy to improve cardiovascular outcomes and delay the onset and progression of HFrEF. In addition, several studies have also reported improved diastolic function in animals and patients with HFpEF treated with SGLT2i and GLP-1 RA. However, the effects of these treatments on cardiac structure and function in experimental HFpEF models are lacking. We therefore studied the effects of the GLP-1 RA liraglutide and the SGLT2i dapagliflozin on cardiac structure and function in a novel cardiometabolic HFpEF mouse model.
2. MATERIALS AND METHODS

2.1 Animals

All animal studies were approved by the Central Committee of Animal experiments (CCD) license number AVD105002016487 and the Animal Care and User Committee of the Groningen University (permit number 16487-04-01 and 16487-04-02) and conducted in accordance with the ARRIVE guidelines, the general principles governing the use of animals in experiments of the European Communities (Directive 2010/63/EU) and Dutch legislation (The revised Experiments on Animals Act, 2014). Female, 18-22 months old C57BL6/J mice were purchased from Jackson laboratory and provided via the Mouse Clinic for Cancer and Aging (MCCA), Groningen. Mice were housed on a 12h light/12h dark cycle with ad libitum access to chow and water. We conducted echocardiography, mini pump placement, and sacrifice under anesthesia with oxygen and 2-3% isoflurane, as published before. Isoflurane is used extensively in rodent research and drug-drug interactions between liraglutide or dapagliflozin have not been described. Animals were euthanized by dissecting the diaphragm under isoflurane anesthesia, after which organs were harvested.

2.2 Experimental design

Mice were fed a high fat diet (HFD; 60% kcal fat, 20% kcal protein, 20% kcal carbohydrates; Research Diets D17041409), or a low-fat equivalent chow (CTRL; 20% kcal fat, 20% kcal protein, 60% kcal carbohydrates; Research diets D17041407) for 12 consecutive weeks, as described. After 8 weeks of diet, mice were anesthetized with oxygen and isoflurane (2-3%), and a mouth
mask was put over the nose and mouth of the mice to deliver this mixture throughout the pump placement, which lasted 10 minutes. The mice were then allowed to wake up and put back in their cage. All mice underwent surgery and an ALZET® osmotic mini pump (Model 2004) was implanted in a subcutaneous pocket on the back. For Sham-treated mice, the subcutaneous pocket was closed without placement of a pump. Mice were infused with Angiotensin-II (ANGII) (1.25 mg/kg/day) (Bachem) for 4 weeks, as previously described. Mice were randomized to respective treatment groups based upon bodyweight, and a total of 4 experimental groups were studied: 1) CTRL – control diet for 12 weeks, Sham surgery; 2) CTRL + ANGII – control diet for 12 weeks with 4 weeks of ANGII- infusion via osmotic mini-pump; 3) HFD – high fat diet for 12 weeks, Sham surgery; 4) HFD + ANGII - high fat diet for 12 weeks with 4 weeks of ANGII- infusion via the osmotic mini-pump. A schematic overview of the experiment is shown in Figure 1A.

2.3 Liraglutide and dapagliflozin treatment

A separate group of mice were subjected to the combinatorial HFD + ANGII intervention. At week 8, after mini-pumps were placed, mice were randomly assigned to receive treatment for 28 days. Administration of subcutaneous liraglutide (Lira) (1mg/kg/day, Novonordisk) or saline was initiated daily and injection volume was adjusted to bodyweight daily, this dose has been shown to be effective. Dapagliflozin (Dapa) (1.5mg/kg/day, Sigma), a dose previously described was mixed with the HFD chow.

2.4 Fasting glucose levels and glucose tolerance
After 12h of fasting, fasting blood glucose levels were measured using an Accu-Chek Aviva glucose analyser (Roche Diagnostics) in week 12. A subset of mice was subjected to an oral glucose tolerance test (OGTT) in week 8 and 12 (n= 7-8 per group). After 12 hours of fasting mice were administered an oral bolus of glucose (2g/kg) and repetitive tail vein blood samples were obtained at multiple time points (time from glucose bolus: 0 min; 15 min; 30 min; 60 min; 90 min; 120 min). To determine glucose tolerance we calculated the area under the curve per treatment group, as published 25.

2.5 Measurements of body mass composition

Body composition (fat mass, fluid mass and lean mass) was determined using minispec LF90II body composition analyser (Bruker Optics) 2 days prior to sacrifice. The procedure was performed according to the guidelines provided by the supplier.

2.6 Echocardiography

Three days prior to sacrifice, 2D transthoracic echocardiography (Vevo 3100 system with 40-MHz MXX550D linear array transducer (FUJIFILM VisualSonics) was performed to assess cardiac dimensional and functional parameters, as described 20. Briefly, mice were anesthetized with oxygen and isoflurane (2-3%), and a mouth mask was put over the nose and mouth of the mice to deliver this mixture throughout the echocardiography, which lasted 15 minutes. The mice were then allowed to wake up and put back in their cage. Parasternal short axis views were obtained at Left Ventricular (LV) mid-papillary level. Vevo LAB software version 3.1.1 (FUJIFILM VisualSonics) was used to measure fractional shortening (FS), left ventricular ejection fraction.
(EF), LV wall thickness (LVAW, LVPW) and LV dimensions (LVIDd, LVIDs). LV parasternal long axis B-mode images were obtained with 300 frames per second for offline speckle tracking analysis (VEVO strain).

Myocardial performance, and specifically myocardial deformation was determined by global longitudinal systolic strain (GLS %) analysis. Reverse peak longitudinal strain rate (RPLSR) was used to measure the speed of myocardial deformation during (early) relaxation. Three consecutive cardiac cycles were analyzed, and semi-automated tracing of the endocardium was performed. LV GLS and reverse strain rates were calculated using speckle tracking algorithms and frame by frame endocardial border tracking according to the guidelines provided by the supplier (VEVO systems). A blinded observer performed all measurements.

2.7 Aortic catheterization and pressure measurements

Mice were anesthetized with oxygen and isoflurane (2-3%), and a mouth mask was put over the nose and mouth of the mice to deliver this mixture throughout the procedure. Before sacrifice, in vivo pressure measurements were performed by aortic catheterization to measure blood pressures. A catheter (Mikro-Tip pressure catheter 1.4F, Millar Instruments, TX, USA) was inserted via the left carotid artery and aortic pressures were measured. Aortic maximal pressure (Aorta Pmax), aortic minimal pressure (Aorta Pmin) and aortic mean pressure (Aorta Pmean) were registered.
2.8 Tissue procurement and organ morphometry

Following aortic catheterization, mice were anesthetized with isoflurane and blood was collected by apical puncture, collected in EDTA tubes, spun down and plasma was stored. The thoracic cavity was opened by cleavage of the sternum, the diaphragm was cut and the heart was excised and rinsed in icecold 1M potassium Chloride (KCl) (Merck Millipore). Right Ventricle (RV) and atria were dissected from LV, and all compartments were weighed and stored separately. LV mid-ventricular transverse sections were fixed in 4% paraformaldehyde before paraffin embedding, as described previously. The remainder of the LV was snap frozen for molecular analysis. Lungs were excised and their wet weight was measured. Additionally, liver, spleen and kidneys were excised, weighed and partly snap-frozen for molecular analyses and partly fixed in 4% neutral buffered formalin and then processed and embedded in paraffin.

2.9 Quantitative real time polymerase chain reaction (qRT-PCR)

Total RNA from LV tissue was extracted using TRI Reagent® (Sigma-aldrich), as described. From total RNA, 0.5 μg was reverse transcribed to produce cDNA using the RNeasy Mini kit (Qiagen Inc, Valencia, USA). cDNA was subjected to quantitative real-time PCR using the C1000 Thermal Cycler CFX384 Real-Time PCR Detection System (Bio-Rad) using iQ SYBR green super mix (Biorad). Quantification of gene expression levels were performed with Bio-Rad CFX Manager 2.0. All gene expression levels were normalized to the reference gene 36B4 and were presented as fold change to the control group. Primer sequences that were used for quantitative PCR analyses are displayed in supplemental table 3.
2.10 Immunohistochemistry on paraffin-embedded LV tissues

Mid-Transverse LV sections, kidney sections, and lung sections were processed and prepared according to previously described protocols 19,27,31. Paraffin-embedded LV tissues were cut into 4µm sections for histological analyses. Stained sections were automatically scanned using a Nanozoomer 2.0-HT digital slide scanner (Hamamatsu, Japan).

To quantify fibrosis, Masson trichrome staining was performed. The amount of fibrosis per section was determined using Aperio’s Imagescope software and was quantified as percentage of the entire section at 40x magnification as described 28.

Capillary density was determined with antibodies against CD31 with subsequent incubation of IgG secondary antibodies coupled to peroxidase followed by diaminobenzidine staining. Total amount of capillaries was quantified per mm2. Capillary density was calculated by manually counting five squares of 100 µm² in stained sections using Aperio’s ImageScope software.

Pulmonary vasculature remodeling was determined using Verhoeff-Van Gieson staining kit (Sigma Aldrich), as per manufacturer’s instructions 82

2.11 Immunofluorescence on paraffin-embedded LV tissues

Cardiomyocyte cross sectional area (CSA) (µm²) was visualized with immunofluorescent with germ agglutinin fluorescein isothiocynatae (WGA-FITC) (Sigma-Aldrich) staining. Fluorescent images were made at 20x magnification using a Leica AF6000 microscope. CSA was measured of ± 30-50 cardiomyocytes per animal, using Fiji software. The observer where blinded to the conditions.
2.12 Plasma Biomarkers

The O-Link mouse exploratory panel (Olink Proteomics, Uppsala, Sweden) was used to measure plasma levels of 92 proteins in a broad range of biological function and pathways. Measurement of these 92 biomarkers was performed using a validated proximity extension assay. The protein concentrations were reported as Normalized Protein eXpression (NPX), which are relative units on a log₂-scale. The abbreviations, full names and uniProt ID-codes of the 92 biomarkers are presented in Supplemental table 4. The difference in biomarker levels between groups was compared using Mann-Whitney tests and expressed as log₂-fold change values. For biological relevance a cut-off of 0.5-fold change was chosen. The p-values were corrected for multiple comparisons using the Benjamini–Hochberg and Bonferroni method with a false discovery cut-off of 5%. These analyses were carried out using R v.3.6.0. Plasma levels of growth differentiation factor 15 (GDF-15), fibroblast growth factor 21 (FGF-21), vascular endothelial growth factor (VEGF), TIMP-1 and P-selectin were measured using multiplex assays (Luminex R&D systems) according to manufacturer instructions.

2.13 RNA-Sequencing

RNA was extracted from pulverized LV tissue using Lysing matrix D beads (MP Biomedicals) and standard TRIzol extraction (ThermoFisher scientific). RNA quality was determined using RNA Pico Chips on Bioanalyzer 2100 (Agilent) and Truseq stranded mRNA libraries (Illumina) were generated from high quality total RNA (RNA integrity number > 8.0). Samples were subjected to single end sequencing on Next Seq 500 platform (illumina). Reads were aligned to mouse
reference genome (mm10) using STAR 2.4.2a \(^{32}\) and readcount analysis was performed using \texttt{htseq-count} 0.6.1 \(^{33}\). Differential expression (DE) analysis was performed using \texttt{DeSeq2} 1.24.0 \(^{34}\). Gene set enrichment analysis (GSEA) was performed using \texttt{Fgsea} 1.10.0 \(^{35}\) and \texttt{MsigDB} v6.2 \(^{36}\). GSEA Gene Ontology (GO) terms with focus on biological process and Kyoto Encyclopedia of Genes (KEGG) library annotation were used. The analyzed data can be found in NCBI GEO, GSE153923 \(^{83}\).

### 2.14 Statistical analysis

All values are presented as means ± standard errors of the mean (SEM). Normality of data was tested with Shapiro-Wilk test. Grubbs test was performed to identify statistically significant outliers, and outliers were removed if appropriate. One-way analysis (ANOVA), followed by Tukey test was performed for multi-group comparison. Kruskal-Wallis test was done for multi-group analysis for variables that were not-normally distributed, followed by Mann-Whitney-U to correct for intra-group comparisons. All statistical analyses were performed using GraphPad Prism version 7.02 or SPSS version 23. A p-value of <0.05 was considered statistically significant.
3. RESULTS

3.1 A combination of high fat diet (HFD) and ANGII infusion results in cardiometabolic dysregulation with impaired glucose handling and obesity

HFD resulted in a steep and significant increase in bodyweight within 8 weeks (+60%, $P<0.001$) (Figure 1B-C). This increase was mostly due to a significant increase in fat mass (CTRL: $7.76\pm0.7$g and HFD: $24.70\pm1.2$g $P<0.001$) (Figure 1B). HFD also impaired glucose handling, resulting in higher glucose levels during oral glucose tolerance test (area under the curve (AUC) 1100 and 1569, respectively) and significantly elevated fasting glucose levels (Figure 1D-E). Concomitant infusion of ANGII (during an HFD) did not affect body weight or glucose levels (Figure 1B and E).

3.2 Combination of HFD + ANGII results in concentric hypertrophy with preserved fractional shortening and diastolic dysfunction

None of the perturbations of (HFD or ANGII or HFD + ANGII) impaired fractional shortening (Figure 2A). A single perturbation of either HFD or ANGII induced concentric hypertrophy, and this remained in the HFD + ANGII group (Figure 2 B and C). However, in the HFD + ANGII group concentric hypertrophy was even more pronounced and deformation of the myocardium (global longitudinal strain (GLS %)) was most impaired in this group (CTRL vs CTRL+ANGII $p=0.007$, CTRL vs HFD+ANGII $p<0.001$) (Figure 2D). The combination of HFD + ANGII also reduced myocardial deformation in diastole (reverse peak longitudinal strain rate (RPLSR)) and especially the combination of both perturbations resulted in marked diastolic dysfunction ($p=0.009$) (Figure 2E).
3.3 Mice subjected to HFD and ANGII infusion have significantly elevated blood pressure, as well as higher left ventricular weights, atria weights and, lung weights

LV weight increased due to HFD (Figure 3A), while atrial weights were not influenced by single perturbations with HFD or ANGII infusion (Figure 3B). Lung weight increased after ANGII infusion, but not in HFD alone (Figure 3C). The combinatorial model of HFD + ANGII consistently increased lung weights and atria weights and maximal aortic pressure (figure 3F and supplemental table 1B).

3.4 Combination of HFD + ANGII reduces angiogenesis, induces cardiac hypertrophy and is associated with significantly more cardiac fibrosis

The cross-sectional area (CSA) of cardiomyocytes was significantly increased (Figure 3 D and E), while the capillary density was significantly decreased in the HFD + ANGII group (Table 1A). In addition, cardiac fibrosis and the expression of pro-fibrotic genes was significantly increased in the HFD + ANGII group compared to control mice (Figure 3E, Table 1B). Furthermore, atrial natriuretic peptide (ANP) gene expression levels in the left ventricle were significantly increased in the HFD+ANGII group (Figure 3F)

3.5 The effect of HFD + ANGII on extra-cardiac tissue

To determine the effect of HFD + ANGII on extra-cardiac tissue we analyzed kidney and pulmonary tissue. Although HFD + ANGII increased gene expression levels of markers that are related to kidney injury and inflammation (KIM-1 and Clusterin), there was no increase of renal fibrosis on a histological level (supplemental figure 2). In line with these findings, we also
observed no changes in altered remodeling in pulmonary vessels, as displayed by similar
degrees of vascular muscularization, collagen deposition and (neo)intimal development in all
intervention groups (supplemental figure 3).

3.6 HFD + ANGII results in inflammation and metabolic dysregulation
To better understand the effects of HFD and ANGII on the entire organism, we assessed a large
panel of biomarkers. We observed that 21 plasma biomarkers were significantly changed in the
HFD+ANGII group (Table 2). Among these biomarkers were pro-inflammatory and vascular
calcification markers, such as TNF Receptor Superfamily Member 12A (TNFRSF12A) and vascular
Osteoprotegerin (TNFRSF12B), as well as apoptosis-related proteins (FAS, CASP3, SNAP23). Also,
fibrosis and related secreted cytokines such as transforming growth system (TGF-B) factors,
Activins (ACVRL1) and Follistatins (FSTL-3) were elevated in the HFD+ANGII group. In an
additional assay (the Luminex assay) we detected several markers of inflammation and fibrosis,
among which growth differentiation factor 15 (GDF-15), fibroblast growth factor 21 (FGF-21),
TIMP-1 and P-selectin, that were all significantly higher in the HFD+ANGII group (supplemental
figure 1).

3.7 HFD + ANGII results in gene enrichment of extracellular matrix components, immune
system signaling, TGF-B signaling and energy house holding pathways
Given the differences in biomarkers levels, thereafter we performed RNA-sequencing on
HFD+ANGII and CTRL heart tissues followed by gene set enrichment analyses (GSEA) to identify
functional biological processes and pathways that were affected. In general, the extent of
upregulated genes for the comparison of HFD+ANGII versus CTRL group belonged to the collagen gene family. Upregulated genes in HFD+ANGII group were significantly enriched for “extracellular structure organization” and “focal adhesion” pathways, mostly driven by increased collagen gene family expression. Furthermore, increased expression of TGF, TNF, and tyrosine phosphatase gene families revealed enrichment of inflammatory response pathways such as “TGF-beta signaling”. Downregulated pathways included “oxidative phosphorylation” and “mitochondrial respiratory chain complex”, marked by decreased expression of all components of the mitochondrial complex I-IV (e.g. SDHA, COX, NDUF and UQCRC1). A full list with top 25 of enriched pathways and the lead edge genes can be found in supplemental table 5.

Given the unfavorable metabolic and inflammatory profile we observed in our model we decided for treatment with metabolic modulators, i.e. the GLP-RA liraglutide and the SGLT2i dapagliflozin.

3.8 Effects of liraglutide (Lira) and dapagliflozin (Dapa) on food intake, body weight and fat mass and plasma glucose levels

Treatment with Lira reduced body weight with 30%, mainly by a decrease in fat mass, whereas treatment with Dapa slightly reduced body weight (Figure 4A-B). All mice have an average food intake of 3 gram per day of the CTRL or HFD diet, respectively. When treated with liraglutide we observed that the mice eat less, but that food intake normalize after 7-10 days. In contrast, food intake remained unchanged in Dapa treated mice. Treatment with Lira and Dapa improved
insulin sensitivity and glucose tolerance and significantly lower fasting glucose levels as compared to non-treated mice (figure 4D, 4E).

3.9 Effect of treatment with liraglutide and dapagliflozin on cardiac function and structure

Figure 5A-C displays the effect of Lira or Dapa on echocardiography parameters of cardiac structure and function. We validated that fractional shortening remains normal in untreated HFD+ANGII mice, and this remained unchanged after treatment with Lira or Dapa (Figure 5A). LV wall thickness was reduced in Lira treated mice compared to untreated animals (Figure 5B). Wall thickness did not significantly change in Dapa treated animals. Global longitudinal strain (GLS) (Figure 5C) was reduced in untreated mice and was improved both in Lira treated mice and Dapa treated mice.

3.10 Effect of treatment with liraglutide on cardiac hypertrophy and lung congestion

Treatment with Lira significantly reduced LV, atrial weight and lung weights (Figure 5D-F). Treatment with Dapa did not attenuate LV, nor atrial, nor lung weights. Lira reduced mRNA gene expression levels of ANP compared to controls, while Dapa did not (Table 1C). Lira reduced fibrosis and pro-inflammatory genes in our HFP EF mouse model (Table 1 C).

3.11 Treatment with liraglutide attenuates cardiac hypertrophy and reduces the amount of fibrosis

We observed a reduced amount of cardiac fibrosis in mice treated with liraglutide or dapagliflozin (Figure 6A, 6D). Treatment with liraglutide also increased capillary density (figure
6B, 6D) with a concomitant decrease in cardiomyocyte size (figure 6C, 6D). Treatment with dapagliflozin had no effect on capillary density or cardiomyocyte size (figure 6B-6D).
4. DISCUSSION

We describe the development of a multiple hit mouse model of HFpEF. We combined a number of perturbations, including advanced age, female sex, and an HFD with ANGII infusion (1.25 mg/kg/day). The combination of these factors resulted in a cardiometabolic HF phenotype that resembles the human HFpEF phenotype to a large extent. Specifically, this multifactorial model resulted in concentric LV hypertrophy, elevated blood pressure, increased LV fibrosis and collagen deposition, with functionally preserved fractional shortening, but reduced diastolic function; as well as left atrial enlargement, pulmonary congestion and raised natriuretic peptides – all hallmarks of clinical HFpEF. Furthermore, this model induces a cardiometabolic unfavorable profile including obesity and impaired glucose handling and metabolic dysregulation. Further, in the cardio-metabolic HFpEF mouse model, we demonstrate that treatment with the GLP-1 RA liraglutide attenuates the cardiometabolic dysregulation. Liraglutide improved GLS, reduced cardiac hypertrophy, and reduced atrial weights, fibrosis and inflammation and attenuates pulmonary congestion. The SGLT2i dapagliflozin also improved GLS and tissue fibrosis, but had less clear effects on cardiac structure or function.

Although HFpEF is no longer synonymous with diastolic heart failure we performed echocardiographic characterization of diastolic function in our model. A recent paper of Schnelle et al. showed that the routine assessment of E/A and E/é ratio in the mouse heart is unreliable, given that the E and A peaks are often fused with high heart rate. E/é prime was also reported to be difficult to assess and highly variable. On the other hand, GLS and reverse peak longitudinal strain rate (RPLSR) were identified as the preferred indices of diastolic dysfunction.
in the mouse and human heart\textsuperscript{38–40}. The impaired GLS and RPLSR, in combination with atrial enlargement, concentric LV hypertrophy and fibrosis, and lung congestion, as detected in our mouse model, are in accordance with contemporary clinical HFpEF criteria as commonly observed\textsuperscript{41–43}.

HFpEF is a multifactorial disease which etiology is not fully understood. Although HFpEF patients can have various phenotypes, they do share several common cardiac features including impaired diastolic function with increased fibrosis. But many patients also have extracardiac manifestations such as T2DM and obesity\textsuperscript{6}. Therefore, the importance of a combinatorial definition is recognized by the two most recent HFpEF scores\textsuperscript{44,45}. When we compared our model with the American H\textsubscript{2}FPEF score (Table 3) our model recapitulates obesity, advanced age, elevated blood pressure, and diastolic dysfunction. Mice do not spontaneously develop atrial fibrillation or pulmonary hypertension (PH), so these criteria cannot be met in murine models. Also, according to HFA–PEFF score our model scores a definite diagnosis of HFpEF (5 points, Table 3), with abnormalities in all the three categories of this score: functional abnormalities (abnormal GLS), structural remodeling (left ventricular hypertrophy and atrial enlargement), and elevated natriuretic peptides\textsuperscript{45}.

So far, preclinical HFpEF models have mainly focused on the use of single perturbations to mimic the human HFpEF phenotype, and these models have struggled with reproducibility of the myriad of symptoms HFpEF has in humans. For example, a pressure overload model such as transverse aortic constriction (TAC) results in hypertension and diastolic dysfunction, but this
initial diastolic impairment with concentric hypertrophy progresses towards a phenotype of LV dilatation and systolic dysfunction within 4-8 weeks, and such a progression is not typical for the natural course of HFpEF in humans. Diabetic models - such as leptin-resistant db/db mice – are characterized by obesity and type 2 diabetes, but their cardiac phenotype is rather mild when compared to humans. Additionally, it has been speculated that the acute onset of load and T2DM in these mice – in humans the onset of hypertension or T2DM progresses over decades making HFpEF development a slow process – could explain why single perturbations were not successful to induce HFpEF in experimental settings.

Exploring aging, the model with senescence-accelerated mice (SAM) did not deliver what was hoped for: albeit a model of slow disease progress due to ageing, SAM developed diastolic dysfunction without hypertension or other comorbidities, thus not reflecting a human HFpEF state. Several models of diabetic cardiomyopathy have been developed to investigate the effect of diabetes on cardiac remodelling, but these models are typically useful to ascertain the contribution of a single factor to the HFpEF phenotype. Only recently, an interesting study from Schiattarella and colleagues described a two-hit preclinical mouse model to recapitulate the multi-organ syndrome HFpEF in humans. In this study, mice were subjected to a combination of metabolic stress (obesity and metabolic syndrome) and hypertension induced by suppression of NO synthase with L-NAME. We cannot provide a direct head-to-head comparison between our models. We chose ANGII, as one of the hallmarks of heart failure is an activated renin-angiotensin-aldosterone-system (RAAS). Further, we added advanced age and female sex.
as additional risk factors for HFpEF. We thus advocate that a multifactorial model is required to induce a HFpEF phenotype that resembles the human situation.

Age is the hallmark of HFpEF and it has been demonstrated that older, obese women are at higher risk for development of HFpEF\(^5\)\(^5\)\(^6\)\(^7\). It is thought that during aging, the heart becomes more prone to remodeling, and several underlying structural and molecular changes contribute to the development of systolic and diastolic dysfunction\(^5\)\(^8\). Clearly, aging affects both sexes, and HFpEF also presents in male patients. Therefore, we also subjected male mice to our multi-hit HFpEF model. We observed that aged male mice developed a phenotype that has characteristics of HFrEF, with features of eccentric remodeling, such as LV dilatation and reduced systolic function (supplemental figure 3). Therefore, the use of aged female mice appears to result in a phenotype that better mimics the human clinical reality\(^5\)\(^9\). We did not study sex-specific effects in detail, and future studies should identify factors that explain the propensity of female sex towards the HFpEF phenotype, and the propensity of male sex towards the HFrEF.

We showed that on a molecular level, our HFpEF phenotype involves genes associated with pathways of fibrosis, inflammation, lipid metabolism and, mitochondrial energy house holding. This is in accordance with observations in humans and these pathways thus be considered crucial for development of a cardiometabolic HFpEF phenotype\(^6\)\(^0\)\(^,\)\(^6\)\(^1\). Treatment of HFpEF, however, remains challenging as there are no drugs have been shown to effectively reduce morbidity or mortality, and no treatment is recommended in treatment guidelines. Our data reveal that inflammation, fibrotic pathways and metabolic pathways are involved in the cardiometabolic HFpEF phenotype. Therefore, we hypothesized that targeting these pathways
could be a novel and successful intervention strategy. Interventions that target glycemic control and food intake may attenuate the unfavorable cardiometabolic profile described by us and could potentially exert cardiac remodeling protective effect which can improve the HFpEF phenotype.

GLP-1 is an incretin hormone that is primarily produced in intestine cells. It has an important role in postprandial metabolism, by regulating glucose metabolism through increased insulin-release, inhibiting glucagon secretion, but also regulates appetite and food intake. Although it is not entirely clear how GLP-1-RA confer their cardioprotective properties \(^62\), it is thought that they are the result of indirect benefits that results from weight loss, but also due to direct CV effects that are initiated by vasodilation and increased natriuresis and prevention of cardiac fibrosis \(^63\)–\(^66\). In line with our results, Bizino and colleagues showed that in humans with T2DM, treatment with liraglutide unloaded the left ventricle, improved diastolic function, and exerted anti-atherosclerotic effects through an anti-inflammatory mechanism \(^63\). At baseline, these subjects were without HF, albeit at high risk for new onset HF. The authors from this study speculated that treatment with liraglutide may postpone onset of HFpEF as it may delay the onset of diabetic cardiomyopathy \(^67\). These results therefore warrant larger studies that unravel the potential CV benefits of GLP-1-RA in patients with HFpEF with and without DM2. The ongoing SELECT study will address if semaglutide (an analogue of liraglutide with a longer half-life) is effective in reducing CV end points, including HF outcomes (NCT03574597).
Dapagliflozin is a SGLT2i and acts by inhibiting glucose reabsorption in the proximal tubule of the kidney\textsuperscript{68}. This results in glycosuria, and a subsequent increase in diuresis. The recent DAPA-HF trial showed that treatment with dapagliflozin, in diabetic and non-diabetic patients with HFrEF, improved HF outcomes\textsuperscript{69}. Tanaka et al. previously showed that improvement of diastolic parameters (GLS and E/e') in patients with T2DM and chronic HF may be achieved with dapagliflozin, and our data validate this effect in a mouse model\textsuperscript{70}. In our study, dapagliflozin lowered glucose levels but did not substantially lower body weight. Likely, in our model, modulation of the primary drivers is necessary to reverse the phenotype. Since female sex and advanced age are clearly not amenable, targeting either the angiotensin-II derived signaling or the obesity are needed at the least to reverse the phenotype. Treatment with dapagliflozin reduced histological fibrosis, as reported by others\textsuperscript{71,72}, however was not paralleled by decreases in pro-fibrotic genes. Since myocardial fibrosis is an extremely dynamic and complex phenomenon\textsuperscript{73}, the effects of dapagliflozin on fibrosis requires further study. The efficacy of SGLT2 inhibitors is, at least, partly explained by their natriuretic actions. The beneficial effect of dapagliflozin may rather be the result of improved volume homeostasis than due to structural improvements in the heart\textsuperscript{74}. In our model we have used ANGII to induce high blood pressure, but associated increased sodium retention may have attenuated the efficacy of the SGLT2-inhibitor. In human trials, however, it has been suggested that the beneficial effects of SGLT2 inhibitors are achieved independent from RAAS activation\textsuperscript{75}. We therefore, believe that the use of this dose of ANGII in our multifactorial model, if any, will only have had a very limited effect on the efficacy of dapagliflozin. Alternatively, the brief period of treatment may have been too
limited to demonstrate the long-term beneficial effects of dapaglipizin. The ongoing DELIVER trial (NCT03619213) will show if dapaglipizin exerts beneficial effects in patients with HFpEF.

4.1 Limitations

In this study we used a multiple hit model to induce HFpEF that resembles human HFpEF. Although we were able to design and develop a model that includes ageing, obesity, impaired glucose handling and female sex, the model is still not representative for the entire spectrum of HFpEF patients. The number of co-morbidities in humans with HFpEF often is even larger. For example, HFpEF patients oftentimes suffer from chronic obstructive pulmonary disease and atrial fibrillation, factors we could not include. As always, experimental models are limited to the factors that they include. However, we do believe that this model has translational value since it is one of the first that uses multiple perturbations to induce HFpEF, and — as far as we know — is the first to include advanced aging and female sex.

We did not acquire invasive left ventricle hemodynamics, and as a result we cannot relate the abnormalities in our model to absolute intracardiac pressure levels $^{19,26-28}$. Although capillary density was reduced in our model, we did not measure myocardial blood flow.

Sham surgery was performed without placement of a saline-filled mini pump. Previous studies, comparable to the sham surgeries performed in this experiment, have revealed that implantation of saline-filled mini pumps does not cause differences in function or molecular signature of the heart $^{20,22,28}$. 
Another limitation of this study is that we only used aged female mice that underwent several perturbations to induce HFpEF. In patients, the HFpEF phenotype is more often found in ageing women and less common in ageing men \(^\text{76}\), but clearly our results cannot be directly translated to male sex.

Mice that were treated with liraglutide had a reduced food intake and weight loss, although after 1 week, food intake normalized and food intake became comparable with other groups. We cannot rule out the observed effects of liraglutide may be explained, at least in part, by caloric restriction, however, previous studies have repeatedly shown that the beneficial effects of liraglutide are independent of weight loss, as liraglutide also exerts cardioprotective effects in a lower dose that not affects food intake \(^\text{77,78}\).

### 4.2 Future perspectives

To date, large randomized trials that aimed to improve morbidity and mortality in HFpEF have been disappointing. Nevertheless, since prevalence of HFpEF is still rising and this HF-subtype is expected to become the major HF-subtype, novel therapeutic strategies are urgently needed. In this study we show that treatment with the GLP-1 RA liraglutide (to a large extent) and the SGLT2i dapagliflozin (to a lesser extent) exert cardioprotective effects in a cardiometabolic driven HFpEF mouse model, through modulation of its unfavorable cardiometabolic profile. This provides background for development of future studies that focus on cardiometabolic HFpEF. Furthermore, in-depth studies are needed to unravel the mechanisms how cardiometabolic dysregulation is associated with the development of HFpEF.
Our cardiometabolic HFpEF model is a pre-clinical mouse model with enhanced fibrotic and inflammatory state. Systemic chronic inflammation is thought to play an important role in HFpEF, and is hypothesized that anti-inflammatory and anti-fibrotic therapies could also improve HFpEF outcomes. The development of this model may help to design future studies that can be used to evaluate the effect of anti-inflammatory and anti-fibrotic drugs on HFpEF development.

5. Conclusions

In conclusion, we describe a multifactorial cardiometabolic mouse HFpEF model that reflects human HFpEF. This model is unique in its kind, and relies upon multiple perturbations including HFD, Angiotensin-II infusion on top of advanced aged and female sex. The combination of perturbations showed herein advocates the use of multiple hit models to achieve translational capacity. Moreover, in this cardio-metabolic HFpEF mouse model, treatment with the GLP-1 RA liraglutide improved cardiometabolic dysregulation, cardiac structure and function. The SGLT2i dapagliflozin also improved cardiac function and tissue fibrosis, but had no ancillary effects on cardiac structure.
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Conflict of interest

The UMCG, which employs Dr. De Boer has received research grants and/or fees from AstraZeneca, Abbott, Bristol-Myers Squibb, Novartis, Novo Nordisk, and Roche. Dr. de Boer received speaker fees from Abbott, AstraZeneca, Novartis, and Roche.
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Statement of contributions

Coenraad Withaar and Rudolf de Boer conceived the study and study design. Coenraad Withaar performed the experiments. Laura M.G. Meems and Herman W. Silljé contributed to the main conceptual ideas and proof outline and supervised the work. George Markousis-Mavrogenis analyzed the plasma biomarker profile data. Cees J. Boogerd performed the RNA sequencing and helped to analyze results. E. Marloes Schouten carried out animal experiments. Martin M. Dokter performed immunohistochemical staining and analysis. Adriaan A. Voors, B. Daan Westenbrink and Carolyn S.P. Lam interpreted results and provided important intellectual input.
to the manuscript. All authors discussed the results and provided important intellectual input to the manuscript. All authors read and approved the final version of the manuscript.

**Data availability statements**

The data underlying this article are available in the article and in its online supplementary material. The RNA sequencing data are available in NCBI GEO, and can be accessed with GSE153923. 

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References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, Van Der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J* 2016;37:2129-2200m.

2. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev* 2017;3:7–11.

3. Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol* 2017;14:591–602.

4. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiue SE, Cushman M, Delling FN, Deo R, De Ferranti SD, Ferguson JF, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Lutsey PL, MacKey JS, Matchar DB, Matsushita K, Mussolino ME, Nasir K, O’Flaherty M, Palaniappan LP, Pandey A, Pandey DK, Reeves MJ, Ritchey MD, Rodriguez CJ, Roth GA, Rosamond WD, Sampson UKA, Satou GM, Shah SH, Spartano NL, Tirschwell DL, Tsao CW, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P. Heart disease and stroke statistics - 2018 update: A report from the American Heart Association. *Circulation* 2018;137:E67–E492.

5. Ho JE, Enserro D, Brouwers FP, Kizer JR, Shah SJ, Psaty BM, Bartz TM, Santhanakrishnan R, Lee DS, Chan C, Liu K, Blaha MJ, Hilleg HE, van der Harst P, van Gilst WH, Kop WJ, Garin JM, Levy D, Gottdiener JS, de Boer RA, Larson MG. Predicting Heart Failure With Preserved and Reduced Ejection Fraction CLINICAL PERSPECTIVE. *Circ Hear Fail* 2016;9:e003116.

6. Shah SJ, Kitzman DW, Borlaug BA, van Heerebeeck L, Zile MR, Kass DA, Paulus WJ. Phenotype-Specific Treatment of Heart Failure With Preserved Ejection Fraction. *Circulation* 2016;134:73–90.

7. Conceição G, Heinonen I, Lourenço AP, Duncker DJ, Falcão-Pires I, Heinonen I, Lourenço AP, Duncker DJ, Falcão-Pires I. Animal models of heart failure with preserved ejection fraction. *Netherlands Hear J* 2016;24:275–286.

8. Valero-Muñoz M, Backman W, Sam F. Murine Models of Heart Failure With Preserved Ejection Fraction: A “Fishing Expedition.” *JACC Basic to Transl Sci* 2017;2:770–789.

9. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol (Lausanne)* 2019;10:155.

10. De Leeuw AE, De Boer RA. Sodium-glucose cotransporter 2 inhibition: Cardioprotection by treating diabetes-a translational viewpoint explaining its potential salutary effects. *Eur Hear J - Cardiovasc Pharmacother* 2016;2:244–255.

11. Marso SP, Daniels GH, Brown-Brandes K, Kristensen P, Mann JFE, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravns LS, Steinberg WM, Stockner M, Zimman B, Bergenstal RM, Buse JB. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016;375:311–322.

12. Nauck MA, Meier JJ, Cavender MA, El Aziz MA, Drucker DJ. Cardiovascular actions and clinical outcomes with glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Circulation* 2017;136:849–870.

13. McMurray JJV, Solomon SD, Inzucchi SE, Kober L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Lohlavek JB, Bohm M, Chiang CE, Chopra VK, De Boer RA, Desai AS, Diez M, Drozdz J, Dukat A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O’Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjostrand M, Langkilde AM. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med*
2019;381:1995–2008.

14. Nguyen TD, Shingu Y, Amorim PA, Schenkl C, Schwarzer M, Doenst T. GLP-1 Improves Diastolic Function and Survival in Heart Failure with Preserved Ejection Fraction. J Cardiovasc Transl Res 2018;11:1–9.

15. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Furtado RHM, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Sabatine MS. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet 2019;393:31–39.

16. Lambadiari V, Pavlidis G, Kousathana F, Varoudi M, Vlastos D, Maratou E, Georgiou D, Andreadou I, Parissis J, Triantafyllidou H, Lekakis J, Iliodromitis E, Dimitriadis G, Ikonomidis I. Effects of 6-month treatment with the glucagon like peptide-1 analogue liraglutide on arterial stiffness, left ventricular myocardial deformation and oxidative stress in subjects with newly diagnosed type 2 diabetes. Cardiovasc Diabetol 2018;17:8.

17. Hammoudi N, Jeong D, Singh R, Farhat A, Komajda M, Mayoux E, Hajjar R, Lebeche D. Empagliflozin Improves Left Ventricular Diastolic Dysfunction in a Genetic Model of Type 2 Diabetes. Cardiovasc Drugs Ther 2017;31:233–246.

18. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. Animals 2013;4:35–44.

19. van der Pol A, Gil A, Silljé HHW, Tromp J, Ovhchinnikova ES, Vreeswijk-Baudoin I, Hoes M, Domian U, van de Sluis B, van Deursen JM, Voors AA, van Veldhuisen DJ, van Gilst WH, Berezikov E, van der Harst P, de Boer RA, Bischoff R, van den Meer P. Accumulation of 5-oxoproline in myocardial dysfunction and the protective effects of OPLAH. Sci Transl Med 2017;9:eaam8574.

20. Meems LMG, Cannon M V, Mahmud H, Voors AA, van Gilst WH, Silljé HHW, Ruifrok WPT, de Boer RA. The vitamin D receptor activator paricalcitol prevents fibrosis and diastolic dysfunction in a murine model of pressure overload. J Steroid Biochem Mol Biol 2012;132:282–289.

21. Cannon M V, Silljé HHW, Sijbesma JWA, Khan MAF, Steffensen KR, van Gilst WH, de Boer RA. LXRα improves myocardial glucose tolerance and reduces cardiac hypertrophy in a mouse model of obesity-induced type 2 diabetes. Diabetologia 2016;59:634–643.

22. Yu L, Ruifrok WPT, Meissner M, Bos EM, Van Goor H, Sanjabi B, Van Der Harst P, Pitt B, Goldstein U, Koerts JA, Van Veldhuisen DJ, Bank RA, Van Gilst WH, Silljé HHW, De Boer RA. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. Circ Hear Fail 2013;6:107–117.

23. Knudsen LB. Liraglutide: The therapeutic promise from animal models. Int J Clin Pract 2010;64:4–11.

24. Obermeier MT, Yao M, Khanna A, Koplowitz B, Zhu M, Li W, Komoroski B, Kasichayanula S, Discenza L, Washburn W, Meng W, Ellsworth BA, Whaley JM, Humphreys WG. In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos 2010;38:405–414.

25. Yin M, van der Horst ICC, van Melle JP, Qian C, van Gilst WH, Silljé HHW, de Boer RA. Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure. Am J Physiol - Heart Circ Physiol 2011;301:H459–H68.

26. Du W, Piek A, Marloes Schouten E, van de Kolk CWA, Mueller C, Mebazaa A, Voors AA, de Boer RA, Silljé HHW. Plasma levels of heart failure biomarkers are primarily a reflection of extracardiac production. Theranostics 2018;8:4155–4169.
27. Booij HG, Yu H, De Boer RA, Van De Kolk CWA, Van De Sluis B, Van Deursen JM, Van Gilst WH, Silljé HHW, Westenbrink BD. Overexpression of A kinase interacting protein 1 attenuates myocardial ischaemia/reperfusion injury but does not influence heart failure development. Cardiovasc Res 2016;111:217–226.

28. Cannon M V, Silljé HH, Sijbesma JW, Vreeswijk-Baudoin I, Ciapaite J, van der Sluis B, van Deursen J, Silva GJ, de Windt LJ, Gustafsson J, van der Harst P, van Gilst WH, de Boer RA. Cardiac LXr α protects against pathological cardiac hypertrophy and dysfunction by enhancing glucose uptake and utilization. EMBO Mol Med 2015;7:1229–1243.

29. De Boer RA, Pokharel S, Flesch M, Van Kampen DA, Suurmeijer AJH, Boomsma F, Van Gilst WH, Van Veldhuisen DJ, Pinto YM. Extracellular signal regulated kinase and SMAD signaling both mediate the angiotensin II driven progression towards overt heart failure in homozygous TGR(mRen2)27. J Mol Med 2004;82:678–687.

30. Meijers WC, Maglione M, Bakker SJL, Oberhuber R, Kieneker LM, de Jong S, Haubner BJ, Nagengast WB, Lyon AR, van der Vegt B, van Veldhuisen DJ, Westenbrink BD, van der Harst P, van Gilst WH, de Boer RA. Heart Failure Stimulates Tumor Growth by Circulating Factors. Circulation 2018;138:678–691.

31. Van Der Feen DE, Dickinson MG, Bartelds B, Borgdorff MAJ, Sietsma H, Lévy M, Berger RMF. Egr-1 identifies neointimal remodeling and relates to progression in human pulmonary arterial hypertension. J Hear Lung Transplant 2016;35:481–490.

32. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 2013;29:15–21.

33. Anders S, Pyl PT, Huber W. HTSeq-A Python framework to work with high-throughput sequencing data. Bioinformatics 2015;31:166–169.

34. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.

35. Sergushichev AA. An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. BioRxiv 2016:060012.

36. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–15550.

37. Schnelle M, Catibog N, Zhang M, Nabeebaccus AA, Anderson G, Richards DA, Sawyer G, Zhang X, Toischer K, Hasenfuss G, Monaghan MJ, Shah AM. Echocardiographic evaluation of diastolic function in mouse models of heart disease. J Mol Cell Cardiol 2018;114:20–28.

38. Ternacle J, Wan F, Sawaki D, Surendra M, Pini M, Meredes R, Ernande L, Audureau E, Dubois-Rande JL, Adnot S, Hue S, Czibik G, Derumeaux G. Short-term high-fat diet compromises myocardial function: A radial strain rate imaging study. Eur Heart J Cardiovasc Imaging 2017;18:1283–1291.

39. Ferferieva V, Van den Bergh A, Claus P, Jasaityte R, La Gerche A, Rademakers F, Herijgers P, D’hooge J. Assessment of strain and strain rate by two-dimensional speckle tracking in mice: comparison with tissue Doppler echocardiography and conductance catheter measurements. Eur Heart J Cardiovasc Imaging 2013;14:765–773.
40. Gorscan J, Tanaka H. Echocardiographic assessment of myocardial strain. J Am Coll Cardiol 2011;58:1401–1413.

41. DeVore AD, McNulty S, Alenezi F, Erbsoll M, Vader JM, Oh JK, Lin G, Redfield MM, Lewis G, Semigran MJ, Anstrom KJ, Hernandez AF, Velazquez EJ. Impaired left ventricular global longitudinal strain in patients with heart failure with preserved ejection fraction: insights from the RELAX trial. Eur J Heart Fail 2017;19:893–900.

42. Henein M, Mörner S, Lindmark K, Lindqvist P. Impaired left ventricular systolic function reserve limits cardiac output and exercise capacity in HFrEF patients due to systemic hypertension. Int J Cardiol 2013;168:1088–1093.

43. Kosmala W, Rojek A, Przewlocka-Kosmala M, Mysiak A, Karolko B, Warwick TH. Contributions of nondiastolic factors to exercise intolerance in heart failure with preserved ejection fraction. J Am Coll Cardiol 2016;67:659–670.

44. Reddy YNV, Carter RE, Obokata M, Redfield MM, Borlaug BA. A simple, evidence-based approach to help guide diagnosis of heart failure with preserved ejection fraction. Circulation 2018;138:861–870.

45. Pieske B, Tschöpe C, De Boer RA, Fraser AG, Anker SD, Donal E, Edelmann F, Fu M, Guazzi M, Lam CSP, Lancellotti P, Melenovsky V, Morris DA, Nagel E, Pieske-Kraigher E, Ponikowski P, Solomon SD, Vasen RS, Rutten FH, Voors AA, Ruschitzka F, Paulus WJ, Seferovic P, Filippatos G. How to diagnose heart failure with preserved ejection fraction: The HFA-PEFF diagnostic algorithm: A consensus recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). Eur Heart J 2019;40:3297–3317.

46. Mohammed SF, Storlie JR, Oehler EA, Bowen LA, Korinek J, Lam CS, Simari RD, Burnett JC, Redfield MM. Variable Phenotype in Murine Transverse Aortic Constriction (TAC). Cardiovasc Pathol 2012;21:188–198.

47. Hampton C, Rosa R, Campbell B, Kennan R, Gichuru L, Ping X, Shen X, Small K, Madwed J, Lynch JJ. Early echocardiographic predictors of outcomes in the mouse transverse aortic constriction heart failure model. J Pharmacol Toxicol Methods 2017;84:93–101.

48. Gao C, Ren SV, Yu J, Baal U, Thai D, Lu J, Zeng C, Yan H, Wang Y. Glucagon Receptor Antagonism Ameliorates Progression of Heart Failure. JACC Basic to Transl Sci 2019;4:161–172.

49. Mori J, Patel VB, Alrob OA, Basu R, Altamimi T, DesAulniers J, Wagg CS, Kassiri Z, Lopaschuk GD, Oudit GY. Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. Circ Hear Fail 2014;7:327–339.

50. Alex L, Russo I, Holoborodko V, Frangogiannis NG. Characterization of a mouse model of obesity-related fibrotic cardiomyopathy that recapitulates features of human heart failure with preserved ejection fraction 2018;315:H934–H949.

51. Gevaert AB, Shakeri H, Leloup AJ, Van Hove CE, De Meyer GRY, Vrints CJ, Lemmens K, Van Craenenbroeck EM. Endothelial Senescence Contributes to Heart Failure with Preserved Ejection Fraction in an Aging Mouse Model. Circ Hear Fail 2017;10:e003806.

52. Van Bilsen M, Daniels A, Brouwers O, Janssen BJA, Derks WIA, Brouns AE, Munts C, Schalkwijk CG, Van Der Vusse GJ, Van Nieuwenhoven FA. Hypertension is a conditional factor for the development of cardiac hypertrophy in type 2 diabetic mice. PLoS One 2014;9:e85078.

53. Tate M, Prakoso D, Willis AM, Peng C, Deo M, Qin CX, Walsh JL, Nash DM, Cohen CD, Rofe AK, Sharma A, Kiriazis H, Donner DG, De Haan JB, Watson AMD, De Blasio MJ, Ritchie RH. Characterising an Alternative Murine Model of Diabetic Cardiomyopathy. Front
54. Schiattarella GG, Altamirano F, Tong D, French KM, Villalobos E, Kim SY, Luo X, Jiang N, May HI, Wang Z V., Hill TM, Mammen PPA, Huang J, Lee DI, Hahn VS, Sharma K, Kass DA, Lavandero S, Gillette TG, Hill JA. Nitrosative stress drives heart failure with preserved ejection fraction. Nature 2019;568:351–356.

55. Savji N, Meijers WC, Bartz TM, Bambhani V, Cushman M, Nayor M, Kizer JR, Sarma A, Blaha MJ, Gansevoort RT, Gardin JM, Hillege HL, Ji F, Kop WJ, Lau ES, Lee DS, Sadreyev R, van Gilst WH, Wang TJ, Zanni M V., Vasan RS, Allen NB, Psaty BM, van der Harst P, Levy D, Larson M, Shah SJ, de Boer RA, Gottdiener JS, Ho JE. The Association of Obesity and Cardiometabolic Traits With Incident HfPef and HFrEF. JACC Hear Fail 2018;6:701–709.

56. Ho JE, Enserro D, Brouwers FP, Kizer JR, Shah SJ, Psaty BM, Bartz TM, Santhanakrishnan R, Lee DS, Chan C, Liu K, Blaha MJ, Hillege HL, Van Der Harst P, Van Gilst WH, Kop WJ, Gansevoort RT, Vasan RS, Gardin JM, Levy D, Gottdiener JS, De Boer RA, Larson MG. Predicting Heart Failure with Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Inftypes. Circ Hear Fail 2018;3:215–224.

57. Lourenço AP, Leite-Moreira AF, Balligand JL, Bauersachs J, Dawson D, de Boer RA, de Windt LJ, Falcão-Pires I, Fontes-Carvalho R, Franz S, Giacca M, Hilfiker-Kleiner D, Hirsch E, Maack C, Mayr M, Pieske B, Thum T, Tocchetti CG, Butsaert DL, Heymans S. An integrative translational approach to study heart failure with preserved ejection fraction: a position paper from the Working Group on Myocardial Function of the European Society of Cardiology. Eur J Heart Fail 2018;20:216–227.

58. Martens P, Mathieu C, Verbrugge FH. Promise of SGLT2 Inhibitors in Heart Failure: Diabetes and Beyond. Curr Treat Options Cardiovasc Med 2017;19:23.

59. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. Circ Res 2014;114:1788–1803.

60. Skov J, Pedersen M, Holst JJ, Madsen B, Goetze JP, Rittig S, Jonassen T, Frøkiaer J, Dejgaard A, Christiansen JS. Short-term effects of liraglutide on kidney function and vasoactive hormones in type 2 diabetes: a randomized clinical trial. Diabetes Obes Metab
Koska J, Sands M, Burciu C, D'Souza KM, Ravarikar K, Liu J, Truran S, Franco DA, Schwartz EA, Schwenke DC, D'Alessio D, Migrino RQ, Reaven PD. Exenatide protects against glucose and lipid-induced endothelial dysfunction: Evidence for direct vasodilation effect of glp-1 receptor agonists in humans. *Diabetes* 2015;64:2624–2635.

Gaspari T, Welungoda I, Widdop RE, Simpson RW, Dear AE. The GLP-1 receptor agonist liraglutide inhibits progression of vascular disease via effects on atherogenesis, plaque stability and endothelial function in an ApoE-/- mouse model. *Diabetes Vasc Dis Res* 2013;10:353–360.

Bizino MB, Jazet IM, Westenberg JJM, Van Eyk HJ, Paiman EHM, Smit JWA, Lamb HJ. Effect of liraglutide on cardiac function in patients with type 2 diabetes mellitus: Randomized placebo-controlled trial. *Cardiovasc Diabetol* 2019;18:55.

Saeed MA, Narendran P. Dapagliflozin for the treatment of type 2 diabetes: A review of the literature. *Drug Des Devel Ther* 2014;8:2493–2505.

Mcmurray JJV, DeMets DL, Inzucchi SE, Køber L, Kosiborod MN, Langkilde AM, Martinez FA, Bengtsson O, Ponikowski P, Sabatine MS, Sjöström M, Solomon SD, Mcmurray JIV, DeMets DL, Inzucchi SE, Køber L, Kosiborod MN, Langkilde AM, Martinez FA, Ponikowski P, Sabatine MS, Sjöström M, Solomon SD, Díez M, Nicolau J, Katova T, O'Meara E, Howlett J, Verma S, Ge J, Belohlavek J, Schou M, Böhm M, Merkely B, Chopra V, Kitakaze M, de Boer RA, Drozdz J, Tereshchenko S, Dukat A, Ljungman C, Chiang CE, Petrie M, Desai A, Anand I, Pham VN, Pfeffer MA, Pocock S, Swedberg K, Rouleau JL, Chaturvedi N, Ivanovich P, Levey AS, Christ-Schmidt H, Held C, Varenhorst C, Christersson C, Mann J, Holmgren P, Hallberg T, Langkilde AM, Sjöström M, Denison H, Reicher B, Bengtsson O, Fox Y, Forsby M, Alenhag EL, Nilsson A, Kazanowska K, Olofsson EL, Karup C, Ekedahl-Berggren M, Klockargård AL, Kempe K, Selvén M. A trial to evaluate the effect of the sodium-glucose co-transporter 2 inhibitor dapagliflozin on morbidity and mortality in patients with heart failure and reduced left ventricular ejection fraction (DAPA-HF). *Eur J Heart Fail* 2019;21:665–675.

Tanaka H, Soga F, Tatsumi K, Mochizuki Y, Sano H, Toki H, Matsumoto K, Shite J, Takaoka H, Doi T, Hirata K. Positive effect of dapagliflozin on left ventricular longitudinal function for type 2 diabetic mellitus patients with chronic heart failure. *Cardiovasc Diabetol* 2020 191 2020;19:1–9.

Lee TM, Chang NC, Lin SZ. Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts. *Free Radic Biol Med* 2017;104:298–310.

Shi L, Zhu D, Wang S, Jiang A, Li F. Dapagliflozin Attenuates Cardiac Remodeling in Mice Model of Cardiac Pressure Overload. *Am J Hypertens* 2019;32:452–459.

de Boer RA, De Keulenaer G, Bauersachs J, Brutsaert D, Cleland JG, Diez J, Du XJ, Ford P, Heinzel FR, Lipson KE, McDonagh T, Lopez-Andres N, Lunde IG, Lyon AR, Pollesello P, Rasokat SK, Toddergård AL, Kempe K, Selvén M. Towards better definition, quantification and treatment of fibrosis in heart failure. A scientific roadmap by the Committee of Translational Research of the Heart Failure Association (HFA) of the European Society of Cardiology. *Eur J Heart Fail* 2019;21:272–285.

Arow M, Waldman M, Yadin D, Nudelman V, Shainberg A, Abraham NG, Freimark D, Kornowski R, Aviram D, Hochhauser E, Arad M.
Sodium-glucose cotransporter 2 inhibitor Dapagliflozin attenuates diabetic cardiomyopathy. *Cardiovasc Diabetol* 2020;19:7.

75. Burns KD, Cherney D. Renal Angiotensinogen and Sodium-Glucose Cotransporter-2 Inhibition: Insights from Experimental Diabetic Kidney Disease. *Am J Nephrol* 2019;49:328–330.

76. Brouwers FP, de Boer RA, van der Harst P, Voors AA, Gansevoort RT, Bakker SJ, Hillege HL, van Veldhuisen DJ, van Gilst WH. Incidence and epidemiology of new onset heart failure with preserved vs. reduced ejection fraction in a community-based cohort: 11-year follow-up of PREVEND. *Eur Heart J* 2013;34:1424–1431.

77. Gaspari T, Brdar M, Lee HW, Spizzo I, Hu Y, Widdop RE, Simpson RW, Dear AE. Molecular and cellular mechanisms of glucagon-like peptide-1 receptor agonist-mediated attenuation of cardiac fibrosis. *Diabetes Vasc Dis Res* 2016;13:56–68.

78. Noyan-Ashraf MH, Shikatani EA, Schuiki I, Mukovozov I, Wu J, Li RK, Volchuk A, Robinson LA, Billia F, Drucker DJ, Husain M. A glucagon-like peptide-1 analog reverses the molecular pathology and cardiac dysfunction of a mouse model of obesity. *Circulation* 2013;127:74–85.

79. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol* 2013;62:263–271.

80. Lam CSP, Voors AA, de Boer RA, Solomon SD, van Veldhuisen DJ. Heart failure with preserved ejection fraction: from mechanisms to therapies. *Eur Heart J* 2018;39:2780–2792.

81. [dataset] Withaar C, Meems LMG, Markousis-Mavrogenis G, Booger CL, Silljé HHW, Schouten EM, Dokter EM, Voors AA, Westenbrink BD, Lam CSP, de Boer RA de. The effects of liraglutide and dapagliflozin on cardiac function and structure in a multi-hit mouse model of Heart Failure with Preserved Ejection Fraction. 2020. NCBI GEO, GSE153923. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153923
Tables

|                  | CTRL | HFD | CTRL+ | HFD+ |
|------------------|------|-----|-------|------|
| **A) Histology** |      |     |       |      |
| LV fibrosis (fold change) | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.4 ± 0.1 | 2.1 ± 0.2*† |
| Capillary density (number/mm²) | 25.8 ± 1.3 | 25.4 ± 0.8 | 21.3 ± 1.2 | 18.8 ± 0.9* |
| **B) LV RNA expression levels** |      |     |       |      |
| ANP              | 1.0 ± 0.3 | 1.0 ± 0.2 | 1.4 ± 0.2 | 2.8 ± 0.5**† |
| Collagen 1a1     | 1.0 ± 0.1 | 1.8 ± 0.2 | 2.3 ± 0.3 | 2.7 ± 0.3**† |
| Collagen 3a1     | 1.0 ± 0.2 | 1.6 ± 0.2 | 1.6 ± 0.1 | 2.6 ± 0.3**† |
| TIMP-1           | 1.0 ± 0.2 | 1.9 ± 0.4 | 3.3 ± 0.4 | 5.1 ± 0.9**† |
| Galectin-3       | 1.0 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 | 2.3 ± 0.3**† |
| IL-6             | 1.0 ± 0.5 | 0.3 ± 0.0 | 0.7 ± 0.1 | 2.4 ± 0.7 |

|                  | HFD+ | HFD+ | HFD+ |
|------------------|------|------|------|
| **C) LV RNA expression levels** |      |      |      |
| ANP              | 2.4 ± 0.5 | 0.9 ± 0.2* | 3.3 ± 0.5 |
| Collagen 1a1     | 2.6 ± 0.4 | 1.2 ± 0.2* | 2.5 ± 0.4 |
| Collagen 3a1     | 1.7 ± 0.1 | 1.1 ± 0.1* | 2.1 ± 0.2 |
| TIMP-1           | 2.7 ± 0.5 | 1.3 ± 0.3* | 3.2 ± 0.4 |
| Galectin-3       | 2.0 ± 0.3 | 0.9 ± 0.2* | 1.4 ± 0.1 |
| IL-6             | 2.9 ± 0.6 | 1.8 ± 0.4* | 2.8 ± 0.4 |

Table 1. Histology and RNA expression

**A) Histology.** LV fibrosis = Histological analysis of fibrosis by Masson staining. Capillary density is number of positive CD31 cells per mm².

**B) and C) LV RNA expression.** Gene expression was normalized to 36B4 and presented as fold change to CTRL. ANP = Atrial Natriuretic Peptide. Collagen 1a1 = Collagen 1a1. Collagen 3a1 = Collagen 3a1. TIMP-1 = tissue inhibitor of metallopeptidase 1. Galectin-3 = Galectin-3. IL-6 = Interleukin-6.

CTRL = control chow. HFD = high fat diet. CTRL+ANGII = Angiotensin-II treated group on control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet. HFD+ANGII+Saline = high fat diet + Angiotensin-II with daily saline injections. HFD+ANGII+Lira = high fat diet + Angiotensin-II with daily liraglutide injection. HFD+ANGII+Dapa = high fat diet + Angiotensin-II with dapagliflozin treatment. Statistical testing was performed with Kruskal Wallis test followed by Mann-Whitney-U. * compared to CTRL. † compared to HFD. Data are presented as means ± standard error of the mean.
### HFD+ANGII vs CTRL

| Biomarkers | NPX | p-value |
|------------|-----|---------|
| EPO        | 1.770 | 0.0022  |
| TNFRSF12A  | 1.336 | 0.0000  |
| IL10       | 1.064 | 0.0010  |
| CASP3      | 0.946 | 0.0036  |
| SNAP29     | 0.885 | 0.0007  |
| FLJ1       | 0.853 | 0.0070  |
| EDA2R      | 0.845 | 0.0002  |
| GHRL       | 0.833 | 0.0036  |
| PLIN1      | 0.770 | 0.0045  |
| CA13       | 0.715 | 0.0086  |
| TNFRSF11B  | 0.665 | 0.0045  |
| AXIN1      | 0.663 | 0.0036  |
| GDNF       | 0.564 | 0.0000  |
| ACVR1      | 0.537 | 0.0005  |
| KITLG      | 0.525 | 0.0017  |
| FSTL3      | 0.483 | 0.0000  |
| FAS        | 0.475 | 0.0005  |
| APBB1IP    | 0.318 | 0.0000  |
| TGFB1      | 0.311 | 0.0086  |
| LGMN       | 0.290 | 0.0106  |
| IL23R      | -0.485 | 0.0005  |

#### Table 2. Biomarkers profile of the HFD+ANGII group

Significantly altered biomarkers obtained with O-link mouse panel expressed at log₂ fold change. NPX are normalized protein expression (NPX) wherein a high value correspondent to higher protein expression in the HFD+ANGII group (n=11-12 mice per group). The difference in biomarkers levels were determined on log₂ fold change-values by volcano plot.

Statistical testing was performed with Kruskal Wallis test followed by Mann-Whitney-U. * = P-values controlled for false discovery rate using the Benjamini–Hochberg method (≤ 5%). NPX=Normalized Protein Expression. CTRL = control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet.
| Clinical diagnostic criteria | Features of the mouse model |
|------------------------------|-----------------------------|
| **H₂FPEF score**<sup>44</sup> |                             |
| Obesity                      | ✓ Increased body weight     |
| Atrial fibrillation          | x Not applicable            |
| Age >60                      | ✓ Female >18 months old C57BL6/J |
| Treatment with 2 antihypertensives | x Not applicable |
| Echocardiographic diastolic function | ✓ atrial weight↑ lung weight↑ and RPLSR ↓ as surrogate for E/é |
| Echocardiographic PASP       | x Not feasible in mice      |
| **HFA–PEFF score**<sup>45</sup> |                             |
| Functional abnormalities     | ✓ 1 point: GLS = 14.1 ( = < 16%), RPLSR ↓ |
| Structural abnormalities     | ✓ 2 points: concentric LVH ↑ and atrial enlargement↑ |
| Elevated natriuretic peptides | ✓ 2 points: ANP expression↑ |

Table 3. Comparison of clinical diagnostic criteria with the HFpEF model

GLS = global longitudinal strain. RPLSR = reverse peak longitudinal strain rate measured during early filling. PASP = pulmonary arterial systolic pressure. LVH = left ventricle hypertrophy. ANP = atrial natriuretic peptide.
Figure legends

Figure 1. High fat diet (HFD) induces obesity and impaired glucose; this phenotype is not affected by addition of Angiotensin-II (ANGII)

A) Overview of experimental design and an example of a CTRL and HFD+ANGII mouse. B) Body mass composition (fluid, fat, and lean mass) measured by minispec LF90 body composition analyser (n=7-11 mice). C) Weight development over time (weeks) (n=7-11 mice per group). D) Plasma glucose levels over time (minutes) after glucose loading (2 gram/kg body weight) in an oral glucose tolerance test (n=7-9 mice per group). To determine glucose tolerance, we calculated area under the curve (AUC) per treatment group. E) Fasting glucose levels per group (n=6-12 mice per group). LBM = Lean body mass.

CTRL = control chow. HFD = high fat diet. CTRL+ANGII = Angiotensin-II treated group on control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet. Data are presented as mean + SEM. * = Kruskal Wallis test followed by Mann-Witney-U P<0.05 is considered significant.

Figure 2. Combination of HFD + ANGII results in a phenotype of concentric LV hypertrophy with preserved fractional shortening and diastolic dysfunction

A) Fractional shortening (FS, systolic function) (n=7-11 mice per group). B) Left ventricle anterior wall thickness in diastole (LVAWd) (n=7-11 mice per group). C) representative M-mode echocardiographic images of LV. D) Global longitudinal strain (GLS) as marker of myocardial deformation per treatment group (n=6-9 mice per group). E) Quantification of reverse peak longitudinal strain rate (RPLSR) as marker of diastolic function (n=6-10 mice per group). F) Pmax aorta measured by intra cardiac pressure measurements (n=5-9 mice per group). CTRL = control chow. HFD = high fat diet. CTRL+ANGII = Angiotensin-II treated group on control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet. Data are presented as mean + SEM. * = Kruskal Wallis test followed by Mann-Witney-U P<0.05 is considered significant.

Figure 3. Combination of HFD + ANGII increases left ventricular, atrial and pulmonary weights and increased myocardial cross-sectional area, and cardiac stress marker ANP

A) LV/tibia = left ventricle weight corrected for tibia length (n=7-10 mice per group). B) Atria weight/tibia length (n=6-11 mice per group). C) Lung weight/tibia length (n=7-10 mice per group). D) Cross sectional area of cardiomyocytes quantified by WGA-FITC staining (30-50 cells per animal, n=6-9 mice per group). E) Representative images of histological staining depicting the cardiomyocyte size (WGA), capillary density (CD31+), and fibrosis (Masson). F) LV mRNA expression levels of Atrial Natriuretic Peptide (ANP) (n=7-9 mice per group).

CTRL = control chow. HFD = high fat diet. CTRL+ANGII = Angiotensin-II treated group on control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet. Data are presented as mean + SEM. * = Kruskal Wallis test followed by Mann-Witney-U P<0.05 is considered significant.
**Figure 4. Effects of liraglutide (Lira) and dapagliflozin (Dapa) on body weight, glucose tolerance test, and food intake**

A) Body weight from start of treatment over time (days) (n=13-14 mice per group). B) Body mass composition (fluid, fat, lean mass) measured by minispec LF90 II body composition analyser (n=13-14 mice per group). C) Total food intake per 3 days (n=13-14 mice per group). D) Fasting glucose levels per group measured 2 days before sacrifice (n=13-14 mice per group). Dotted line represents control reference value. E) Plasma glucose levels over time (minutes) after glucose loading (2 gram/kg body weight) in an oral glucose tolerance test (n=9-10 mice per group). To determine glucose tolerance, we calculated area under the curve (AUC) per treatment group. CTRL = control group. HFD+ANGII+Saline = high fat diet + Angiotensin-II with saline injections. HFD+ANGII+Lira = high fat diet + Angiotensin-II with daily liraglutide injection. HFD+ANGII+Dapa = high fat diet + Angiotensin-II with dapagliflozin treatment. Data are presented as mean + SEM. * = Kruskal Wallis test followed by Mann-Witney-U. P<0.05 is considered significant.

**Figure 5. Effects of treatment with liraglutide or dapagliflozin on cardiac function, cardiac structure and cardiac remodeling**

A) Fractional shortening (FS, systolic function) (n=8-13 mice per group). B) Left ventricle anterior wall thickness (LV/AWd) (n=12-14 mice per group). C) Global longitudinal strain (GLS) as marker of myocardial deformation (n=9-13 mice per group). D) LV/tibia = left ventricle weight corrected for tibia length (n=13 mice per group). E) Atria/tibia = atria weight corrected for tibia length (n=13-15 mice per group). F) Lung weight/tibia length (n=13-14 mice per group). Dotted lines represent control reference values. HFD+ANGII+Saline = high fat diet + Angiotensin-II with saline injections. HFD+ANGII+Lira = high fat diet + Angiotensin-II with daily liraglutide injection. HFD+ANGII+Dapa = high fat diet + Angiotensin-II with dapagliflozin treatment. Data are presented as mean + SEM. * = Kruskal Wallis test followed by Mann-Witney-U. P<0.05 is considered significant.

**Figure 6. Treatment effects of liraglutide or dapagliflozin on cardiac fibrosis and cardiac hypertrophy**

A) Histological analysis of fibrosis, expressed in fold-change compared to reference control values (n=10-11 mice per group). B) Capillaries density determined by number of capillaries per mm2 positive stained by CD31+ (n=10 mice per group). C) Cross sectional area of cardiomyocytes quantified by WGA-FITC staining (30-50 cells per animal, n=9-13 mice per group). D) Representative images of histological staining depicting the cardiomyocyte size (WGA), capillary density (CD31+), and fibrosis (Masson). CTRL = control diet. HFD = high fat diet. CTRL+ANGII = Angiotensin-II treated group on control chow. HFD+ANGII = Angiotensin-II treated group on high fat diet.
HFD+ANGII+Saline = high fat diet + Angiotensin-II with saline injections. HFD+ANGII+Lira = high fat diet + Angiotensin-II with daily liraglutide injection. HFD+ANGII+Dapa = high fat diet + Angiotensin-II with dapagliflozin treatment. Data are presented as mean ± SEM. * = Kruskal Wallis test followed by Mann-Witney-U P<0.05 is considered significant.
Figure 4

A

Body weight (g)

Time (day)

HFD+ANGII+Saline
HFD+ANGII+Dapa
HFD+ANGII+Lira

B

Weight (g)

Saline
Lira
Dapa

HFD+ANGII

Fluid
Fat
Lean

C

Food intake (g)

Period (days)

1-3
4-6
7-9
10-12
13-16
17-19

HFD+ANGII+Saline
HFD+ANGII+Dapa
HFD+ANGII+Lira

D

Plasma glucose (mmol/L)

Saline
Lira
Dapa

HFD+ANGII

E

Plasma glucose (mmol/L)

Time (min)

AUC: HFD+ANGII vs HFD+ANGII+Lira p<0.001
AUC: HFD+ANGII vs HFD+ANGII+Dapa p<0.05
Figure 6

A

Fibrosis (fold change)

Saline | Lira | Dapa

HFD+ANGII

B

Capillaries (number/mm²)

Saline | Lira | Dapa

HFD+ANGII

C

Cross sectional area (µm²)

Saline | Lira | Dapa

HFD+ANGII

D

HFD+ANGII + Saline | HFD+ANGII + Lira | HFD+ANGII + Dapa

WGA

CD31+

MASSON
A translational cardiometabolic HFpEF model

HFpEF Phenotype

| Comorbidities       | Structural and molecular changes |
|---------------------|----------------------------------|
| Obesity             | Preserved EF                     |
| T2DM                | Concentric LVH                   |
| Elevated blood pressure | Left atrial hypertrophy          |
| Lung congestion     | Diastolic dysfunction            |
|                     | Elevated natriuretic peptides    |
|                     | Inflammatory metabolic profile   |

Liraglutide attenuates the unfavorable cardiometabolic changes and preserves cardiac function in murine HFpEF

↓ Obesity
↓ T2DM
↓ Lung congestion
↓ Fibrosis

GLP-1 RA - liraglutide

↓ Concentric LVH
↓ Left atrial hypertrophy
↓ Diastolic dysfunction
↓ Natriuretic peptides

SGLT2i - dapagliflozin
Liraglutide attenuates the unfavorable cardiometabolic changes and preserves cardiac function in murine HFpEF

- **Comorbidities**
  - Obesity
  - T2DM
  - Elevated blood pressure
  - Lung congestion

- **Structural and molecular changes**
  - Preserved EF
  - Concentric LVH
  - Left atrial hypertrophy
  - Diastolic dysfunction
  - Elevated natriuretic peptides
  - Inflammatory metabolic profile

**Graphical Abstract**

- **A transnal cardiometabolic HFpEF model**
- **Female**
- **Aged**
- **Obesity**
- **Elevated blood pressure**
- **T2DM**
- **High Fat Diet**
- **Angiotensin-II**
- **Left atrial hypertrophy**
- **Diastolic dysfunction**
- **Elevated natriuretic peptides**
- **Obesity**
- **T2DM**
- **Lung congestion**
- **Fibrosis**

**HFpEF Phenotype**

- **GLP-1 RA - liraglutide**
- **SGLT2i - dapagliflozin**

- **↓ Concentric LVH**
- **↓ Left atrial hypertrophy**
- **↓ Diastolic dysfunction**
- **↓ Natriuretic peptides**