SGLT2 Inhibition and Exercise for the Diabetic Right Ventricle

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Abstract

Diabetes mellitus (DM) is a leading cause of death in the world, and significant risk factor for cardiovascular disease (CVD). Though DM is characterized by elevated blood glucose, significant diabetes-related morbidity and mortality is due to cardiovascular diseases. To date, the majority of diabetic cardiomyopathy research has been conducted in the left ventricle. However, in most disease contexts, right ventricular (RV) function predicts survival, yet little is known about the diabetic RV nor are there specific RV-targeted therapies. Sodium glucose co-transporter-2 inhibitors (SGLT2i) and exercise have clear cardioprotective effects, yet whether these interventions protect the RV in the setting of diabetes is unknown. To determine whether SGLT2i and exercise protect the diabetic RV, diabetic Sprague Dawley rats induced by streptozotocin (30 mg/kg) and a high-fat diet underwent a 13-week intervention of the SGLT2i canagliflozin (3mg/kg/day in 0.5% methylcellulose) with and without access to exercise. Indicators of diabetic cardiomyopathy were assessed by myocyte cell size, fibrosis by trichrome staining and expression of fibrotic mediators, and expression of molecular signature genes in failing heart (fetal gene program). Expression of pro-fibrotic mediators (collagen and α-SMA) were significantly attenuated with SGLT2i and exercise compared to sedentary controls. RV histology demonstrated significant evidence of diabetes-induced hypertrophy, and fibrosis that was attenuated in SGLT2i and exercise groups compared to sedentary controls and exercise and SGLT2i monotherapies. Together, we demonstrate that SGLT2i and exercise have protective effects on the RV in diabetic rats. The clinical implication of this data suggests a potential for therapeutic interventions that target the RV in diabetic cardiomyopathy.

Introduction:

Diabetes mellitus (DM) is an epidemic that affects more than 422 million patients worldwide (Liu et al., 2018). DM can be defined as a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action, or both (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Goldenberg, & Punthakee, 2013). Among the most significant outcomes of prolonged elevated blood glucose is damage to the heart and vasculature. Resultantly, 70% of patients with diabetes die from cardiovascular disease (CVD) complications.

The bulk of scientific and clinical knowledge of diabetic complications to date have been studied in the left ventricle. However, diabetes impacts the often-overlooked right ventricle (RV) as well. It is important to study the right ventricle because improved left ventricle function is insignificant if treatments cannot also improve the right ventricle function (Kaplan et al., 2018). In most disease contexts, right ventricle function predicts survival, yet there are no right ventricle

specific therapies (Walker & Buttrick, 2013). Several medications exist for diabetic patients, yet the increased risk of CVD poses a complicated problem for pharmaceutical companies creating cardioprotective interventions (Brown & Everett, 2019). It is crucial to understand how diabetes, medications, and exercise affect the heart to create effective treatment plans.

Over the last decade, antidiabetic medications have been studied in regard to long term cardiovascular (CV) benefits. Of these medications, sodium glucose co-transporter-2 inhibitors (SGLT2i) demonstrated CV protective effects, albeit limited to the left ventricle (Lytvyn, Bjornstad, Udell, Lovshin, & Cherney, 2017). SGLT2i manages hyperglycemia by preventing reabsorption of glucose in the kidneys (Kalra, 2014). Canagliflozin, a sub-class of SGLT2is is used to manage blood glucose levels in Type II diabetic patients. Notably, canagliflozin has been recognized by the US FDA because of its beneficial CV outcomes in studies with high CV risk patients (Sarraju et al., 2020), however these benefits have not been explored in the right ventricle. It is necessary to study its effects on the RV to assess its potential in decreasing CVD associated mortality in diabetic patients. In effort to bridge the gap between CVD and diabetic pharmaceutical interventions, exercise has been introduced as a cardioprotective target for diabetic cardiomyopathy. Previous studies indicate that exercise has regulated cardiac mitochondrial metabolism, improved myocardial fibrosis, vascular disorders and more in the left ventricle (Seo et al., 2019), yet these studies exclude potential right ventricle benefits. Given this understanding, exercise has been used in conjunction with other therapeutic targets in the left ventricle. This, however, is insufficient when considering potential therapeutic targets for diabetic cardiomyopathy in the whole heart, which includes the right ventricle. Further research is necessary to assess how exercise interacts with cardioprotective drugs, i.e., canagliflozin, in the right heart to create more targeted interventions

Purpose: The purpose of this research is to study the impact of canagliflozin and exercise on the right ventricle functions in rats with diabetes induced with Streptozotocin (STZ).

Hypothesis: It is hypothesized that canagliflozin and exercise will improve hypertrophy and fibrotic markers in the right ventricle of rats with diabetes induced Streptozotocin (STZ).

Significance of the Study: Our research intends to help medical professionals prescribe diabetes medications that promote beneficial changes in the right ventricle and permit cardiovascular adaptations to exercise. If treatments such as canagliflozin and exercise can help improve cardiovascular function in the right ventricle of rats, we are optimistic that diabetic patients can incorporate lifestyle changes in addition to pharmaceutical treatments.

Independent: Canagliflozin, exercise

Dependent: markers of CVD; aSMA, fibrosis, hypertrophy of the right ventricle

Categorical: N/A

Control: animal model (rat), sex (male), age (adult), diet (high fat +streptozotocin)

Extraneous: rat hearts were shipped in foil rather than standard Eppendorf tubes

Operationally defining terms:

Cardiovascular diseases (CVD) are "heart and circulatory system disorders" (Francula-Zaninovic & Nola, 2018; Lytvyn et al., 2017).

Diabetes is defined as "a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion" (Kharroubi & Darwish, 2015; Lytvyn et al., 2017).

SGLT2i are sodium-glucose cotransporters type 2 inhibitors.

Streptozotocin (STZ) induces "pancreatic islet beta-cell destruction" (Wang-Fischer & Garyantes, 2018) to imitate diabetes.

Basic assumptions: Diabetes affects the left ventricle (heart), the right ventricle is affected like the right ventricle, rats are comparable models to the human heart

Limitations: My samples were collected in the course of other experiments, thus I am only able to perform post-mortem analyses. Analysis only included male rats.

Delimitations: Diabetes in the right ventricle of adult male rats

Methods:

Sample

The current study was a continuation of a previous study, in which male Sprague Dawley rats were treated with 30 mg/kg of STZ and a high-fat diet to induce a model of type 2 diabetes beginning at four-weeks (Damasceno et al., 2014). The animals were randomized into treatment groups to assess the impact of SGLT2i and exercise over a 12-week intervention (Linden et al., 2019). Treatment groups include vehicle (0.5% methyl cellulose) sedentary (VEH SED), vehicle exercise (VEH EX), canagliflozin (3 mg/kg/d) sedentary (SGLT2i SED), and canagliflozin exercise (SGLT2i EX) (Linden et al., 2019). Canagliflozin (3 mg/kg/d) and VEH (0.5% methylcellulose) were administered orally once daily in respective treatment groups. Exercise tests were conducted over the 12 weeks with five acclimatization sessions and seven VO2 peak tests on a treadmill until volitional fatigue (Linden et al., 2019). All exercise tests and pre-mortem analyses were conducted by (Linden et al., 2019) in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Pfizer Institutional Animal Care and Use Committee. After discontinuation of the study, animals were humanely euthanized at 16 weeks, and the hearts were sent to the University of Wyoming to continue post-mortem analyses.

Procedure

To begin to understand how diabetes and its treatments impact the right ventricle, the right ventricle was dissected from the left. A variety of molecular and biochemical assays were conducted thereafter. These include assessing myocyte cell size by wheat-germ agglutinin staining of sectioned right ventricles, assessing fibrosis by trichrome staining frozen sections, and assessing expression of fibrotic mediators, markers of hypertrophy, and molecular signaling of the failing heart (the fetal gene program) by western blotting and real-time PCR. ThermoFisher Scientific technology were used for all fetal gene program biochemical assays. Staining procedures were also conducted with the use of the Thermo Shandon Cryotome E Cryostat and Masson's Staining procedures (Schipke et al., 2017).

Staining procedures were conducted using wheat germ agglutinin (WGA) to bind glycoproteins on the cardiomyocyte membrane. This is a routine staining procedure to assess cross sectional area and myocyte density, both of which are used to quantify hypertrophy, a common CVD marker (Emde, Heinen, Godecke, & Bottermann, 2014). Similarly, Masson's Trichrome Staining measures collagen deposition in the heart with a blue stain (fibrous collagen) and red counterstain (myocyte) (Schipke et al., 2017). Myocyte size and fibrotic tissue data are then quantified via ImageJ software.

The fetal gene program utilizes gel electrophoresis (Western blotting) and PCR to identify proteins and gene expression (Cox & Marsh, 2014). CVD markers such as collagen and alpha smooth muscle actin (aSMA), a protein that contributes to vascular motility and contraction (Yuan, 2015) were identified using Western blots and verified by PCR thereafter. PCR verifies expression of proteins identified via Western blots by amplifying genes that encode collagen and aSMA (Cox & Marsh, 2014). These procedures provide a quantification of CVD marker expression.

Power and statistical analyses

A previous study reported that the mean collagen level in the ventricles was 2.85 mg/g (± 0.18 mg/g) in diabetic rats compared to 2.29 mg/g (± 0.04 mg/g) of collagen in control rats (Spiro & Crowley, 1993), resultant in a 24.45% increase between conditions. From these values, an effect size of 1.11 was estimated for a paired t-test, and a sample size of 3 per group was calculated to achieve a power of 0.8 with an alpha error probability of 0.05.

Two-way analyses of variance (ANOVA) were performed to compare differences in collagen area and percent of fibrotic area in trichrome stained VEH, VEH EX, SGLT2i, and SGLT2i EX groups. If the data indicated significant difference between the variables, independent t-tests were used to compare the difference between treatment groups. A type I-error rate of ≤0.05 was used as a marker of significance difference. Effect size was evaluated using Cohen's D equation; $= \frac{(M_1 - M_2)}{SD_{pooled}}$, with small, moderate, and large correlations set at ES < 0.5, 0.5 < ES < 0.8, and ES > 0.8 respectively (Lakens, 2013). All statistical analyses were performed using SPSS.

Results

Table 1. Masson's Trichrome Stain for Fibrosis

	VEH SED	**VEH WR**	**SGLT2i SED**	**SGLT2i WR**
Total Fibrotic Area (pixels) at 20x/10µm	3819647.86± 1458577.02	2687079.45± 798932.152	2065896.27± 664130.31	1358479.43± 572893.624
Percent Fibrotic Area (%)	38.79±14.84	27.67±8.35	20.96±6.76	14.33±5.32

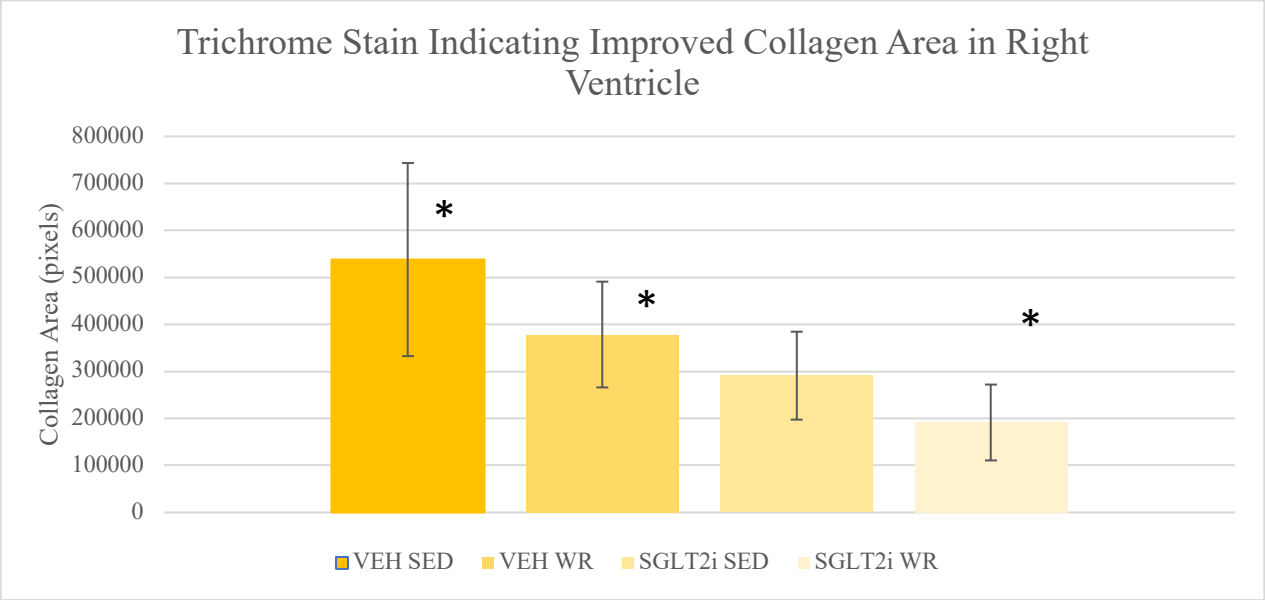

Figure 1. Collagen measured using Masson's Trichrome staining protocol in the right-ventricle of male Sprague Dawley rats with diabetes induced with STZ (n = 6).

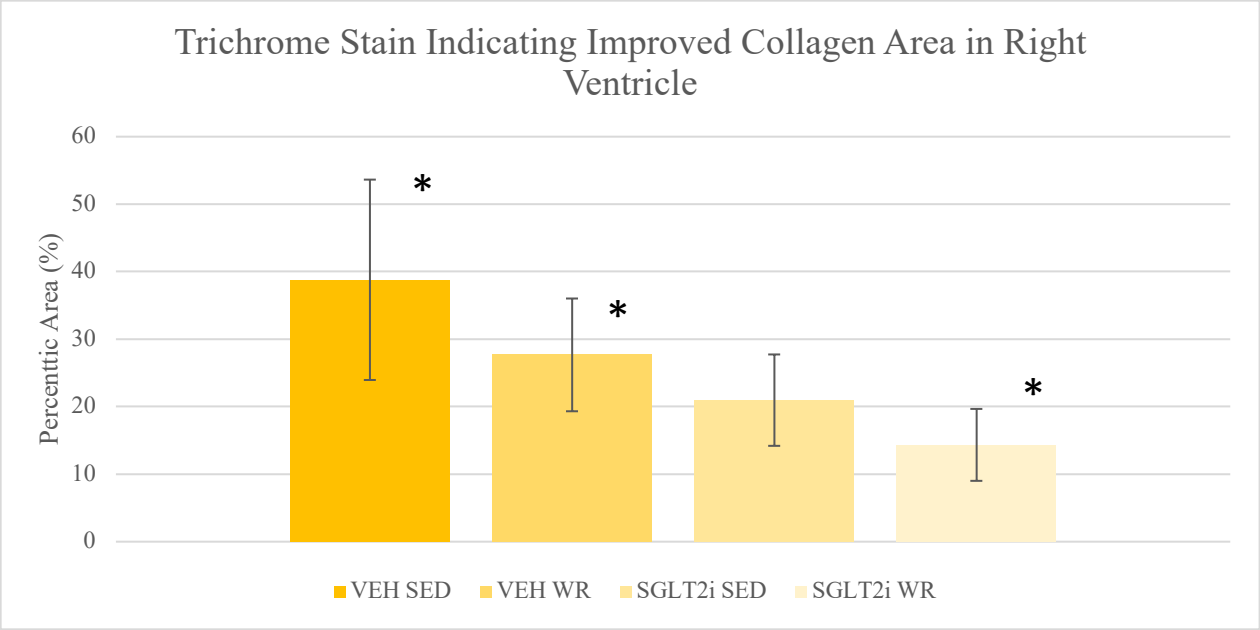

Figure 2. Percent area of collagen measured using Masson's Trichrome staining protocol in the right-ventricle of male Sprague Dawley rats with diabetes induced with STZ (n = 6)

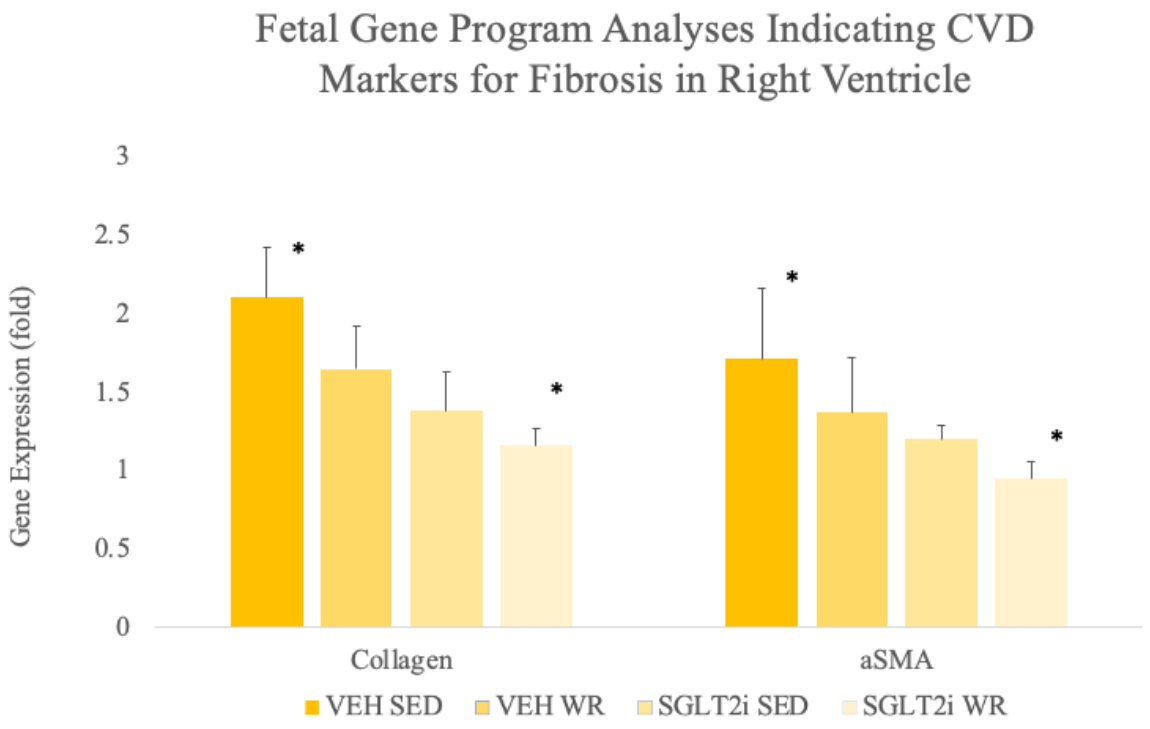

Figure 3. Collagen and α-smooth muscle actin gene expression in the right-ventricle of male Sprague Dawley rats with diabetes induced with STZ (n=6)

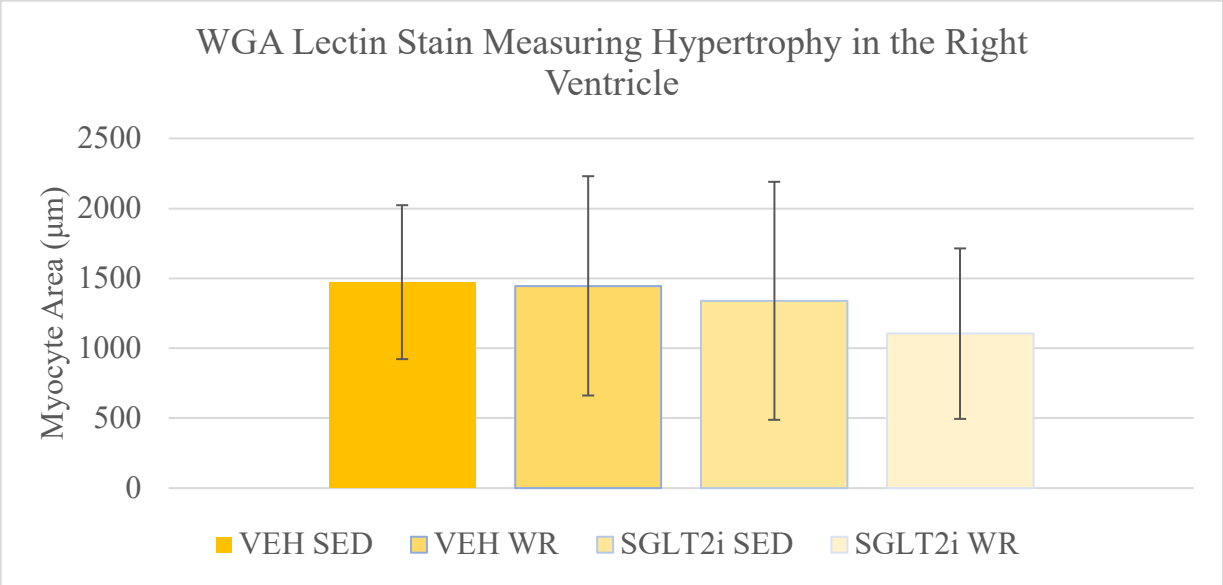

Figure 4. Myocyte cell size measured using Wheat Germ Agglutinin, Lectin staining in male Sprague Dawley rats with diabetes induced with STZ (n=6)

Data from Masson's Trichrome stain (Table 1) indicate that the un-medicated, sedentary (VEH SED) diabetic rats had the highest amount of collagen, and a downward trend followed for the exercise (VEH WR) and medicated (SGLT2i SED) monotherapies and combination (SGLT2i WR) groups. Total fibrotic area (Figure 1) decreased significantly between VEH SED and VEH WR ($p=0.048$), VEH SED and SGLT2i SED ($p=0.0029$), VEH SED and SGLT2i WR ($p=0.0013$), VEH WR and SGLT2i WR ($p=0.0015$), and SGLT2i SED and SGLT2i WR groups ($p=0.034$). Percent fibrotic area (Figure 2) followed the same trend with significance between VEH SED and SGLT2i SED ($p=0.0029$), VEH SED and SGLT2i WR ($p=0.0015$), VEH WR and SGLT2i WR ($p=0.0018$), and SGLT2i SED and SGLT2i WR groups ($p=0.044$).

Biochemical tests for fibrosis (Figure 3) indicated a similar trend with less significance, as did WGA Lectin staining (Figure 4) for hypertrophy. The western blot analysis in particular showed significance between the VEH SED and SGLT2i WR ($p = 0.021$) as well as between VEH SED and SGLT2i SED ($p = 0.092$) when Type I error rate is set at p-value ≤ 0.10. Interestingly, the p-value between the VEH WR and SGLT2i SED groups is high ($p = 0.454$), indicating no significant difference between exercise treatment and medication monotherapies. The WGA lectin stain shows a similar trend with significance between VEH SED and SGLT2i EX ($p = 0.002$), and between VEH EX and SGLT2 EX ($p = 0.019$), but a high p-value between VEH EX and SGLT2i SED ($p = 0.522$).

Discussion

Before we could assess the impact of exercise and SGLT2i therapeutics on the right heart, it was necessary to establish that diabetes is affecting the right heart. The VEH SED group was crucial in establishing the presence of markers of diabetic cardiomyopathies. We consistently found high levels of collagen and markers for hypertrophy in this control group. This reassured us that the streptozotocin and high fat diet model were resulting in diabetic cardiomyopathies in the right ventricle. This acknowledgement alone provided a foundation that fibrosis and hypertrophy exist that exercise and canagliflozin could improve upon.

There was evidence that both therapies impact fibrosis in the heart, but the verdict is still out considering the impact of either monotherapy on hypertrophy in the right ventricle. We know that exercise causes a hypertrophic response on a young adult heart, therefore hypertrophy may not be solely a marker of cardiac remodeling from diabetes. This limitation cannot be ruled out and further analysis of cardiac function by echocardiography is necessary to establish the relationship between diabetes, hypertrophy, and exercise in the right ventricle. Nevertheless, the results from this research leave us with two conclusions; first that there was no significant difference between exercise alone or canagliflozin alone, and second that the combination of exercise and canagliflozin significantly attenuate the diabetic cardiomyopathies in the right ventricle. This is important because care providers often diagnose a patient with diabetes and rely on medication to alleviate the effects of diabetes on the heart yet exercise alone may be a comparable option when medication is not available. Nevertheless, neither monotherapy is the most beneficial. We found that the only consistently significant treatment on markers of diabetic cardiomyopathies in the right heart was a combination of both anti-diabetic canagliflozin and exercise. Care providers should take this finding into consideration when prescribing treatment because right-sided heart failure is the biggest indicator of mortality in cardiovascular disease. Similarly, while there is evidence of benefits from exercise and SGLT2i co-therapy in the right-heart, further research is necessary to explore the potential of diabetic therapies that target the right ventricle.

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