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1 **Diabetic Cardiomyopathy:**

2 **The need for adjusting experimental models to meet clinical reality**

3
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29

1 **Abstract:**

2 Diabetic cardiomyopathy (CM), occurring in the absence of hypertension, coronary
3 artery disease and valvular or congenital heart disease, is now recognized as a
4 distinct, multifactorial disease leading to ventricular hypertrophy and abnormal
5 myocardial contractility that correlates with an array of complex molecular and
6 cellular changes.

7 While animal models provide the unique opportunity to investigate mechanistic
8 aspects of diabetic CM, important caveats exist when extrapolating findings obtained
9 from preclinical models of diabetes to humans. Indeed, animal models do not
10 recapitulate the complexity of environmental factors, most notably the duration of the
11 exposure to insulin resistance that may play a crucial role in the development of
12 diabetic CM. Also, most preclinical studies are performed in animals with uncontrolled
13 or poorly controlled diabetes, whereas most patients undergo therapeutic
14 intervention. Finally, while T2DM is a disease of the elderly in humans, most rodent
15 models employ juvenile or young adult animals.

16 The aim of this review is to identify the current limitations of rodent models, and to
17 discuss how future mechanistic and preclinical studies should integrate key
18 confounding factors to better mimic the diabetic CM phenotype.

19

20 **Key Words:**

21 Diabetic cardiomyopathy; Type 2 diabetes mellitus; Insulin resistance; organ-to-organ
22 interaction; Heart Failure

23

1 **Introduction:**

2 The prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide,
3 afflicting all ages, sexes, and socioeconomic classes, ultimately leading to frailty and
4 compromising healthy ageing.¹ Cardiovascular (CV) complications are the leading
5 causes of morbidity and mortality in T2DM patients, accounting for about two-thirds of
6 overall deaths as evidenced by the Framingham Heart Study.² One of the specific CV
7 complications in T2DM is the onset of diabetic cardiomyopathy (CM), originally
8 described as an early diastolic dysfunction progressing to systolic dysfunction and
9 heart failure (HF) in the absence of hypertension, coronary artery disease and
10 valvular or congenital heart disease.³ The CARDIA study provided strong evidence
11 for the diagnosis of diabetic CM,³ identifying subtle diastolic and/or systolic
12 myocardial abnormalities preceding the onset of overt CM and HF.⁴⁻⁶

13 Diabetic CM is now recognized as a distinct, multifactorial disease leading to
14 ventricular hypertrophy and abnormal myocardial contractility that correlates with an
15 array of complex molecular and cellular changes (Figure 1).⁷ Both insulin resistance
16 and chronic hyperglycaemia contribute to impaired cardiac contractility and structure
17 via reduced Ca²⁺ influx through L-type Ca²⁺ channels, abnormal PI3K/Akt pathway
18 signalling, enhanced production of reactive oxygen species (ROS), advanced
19 glycation end products (AGEs), and toxic fatty acid (FA) metabolites, as well as
20 potentially involving less well studied mechanisms such as altered autophagy, and
21 epigenetic control.⁸ Asserting the “uniqueness” of diabetic CM will require
22 determination of the role of the «metabolic exposome», including diet, lifestyle,
23 glycemic disorders, obesity and sedentary behavior, alongside other confounders
24 such as systemic hypertension, sex and ageing, which are intertwined in the
25 pathogenesis of diabetic CM.⁹

1 Moreover, the complex impact of these environmental factors are magnified by other
2 processes linking the heart to key metabolic organs, i.e., the adipose tissue, liver,
3 kidney and the gut (microbiota) that may separately exert noxious cardiac effects
4 through a crosstalk mediated by pro-inflammatory cytokines, pro-fibrotic factors,
5 microvesicles, miRNAs and immune cells. This endocrine organ-crosstalk evolves
6 into a paracrine cellular-crosstalk between cardiomyocytes, fibroblasts, endothelial
7 cells and immune cells in the myocardium (Figure 2).

8 While animal models provide the unique opportunity to investigate mechanistic
9 aspects of diabetic complications, including diabetic CM, important caveats exist
10 when extrapolating findings obtained from preclinical models of diabetes to humans.
11 Indeed, animal models do not recapitulate the complexity of environmental factors,
12 most notably the duration of the exposure to insulin resistance that may play a crucial
13 role in the development of diabetic CM. Also, cardiac physiology such as heart rate,
14 calcium fluxes, sarcomere composition and vessel function is different in rodents
15 versus humans, and rodent models are less likely to develop micro- and
16 macrovascular diseases, which are quite prevalent in T2DM patients. Important
17 differences also exist in the hormonal milieu and the concentrations of various lipid
18 species. Furthermore, most preclinical studies are performed in animals with
19 uncontrolled or poorly controlled diabetes, whereas most patients undergo
20 therapeutic intervention. Finally, while T2DM is a disease of the elderly in humans,
21 most rodent models employ juvenile or young adult animals.

22
23 The aim of this review is to identify the current limitations of rodent models, and to
24 discuss how future mechanistic and preclinical studies should integrate key
25 confounding factors to better mimic the diabetic CM phenotype.

1 Authors of this review are all partners of the CARDIATEAM consortium, a H2020
2 Innovative Medicine Initiative (IMI) funded project, aiming to assess the uniqueness
3 of diabetic CM and develop an innovative approach involving deep clinical
4 phenotyping for back translation to tailored preclinical models recapitulating human
5 diabetic CM.

6 7 **Investigating diabetic cardiomyopathy in preclinical models: the importance of** 8 **clinical confounding factors:**

9 Most of the molecular mechanisms involved in the pathogenesis of diabetic CM have
10 been investigated in rodent models of type 1 or type 2 diabetes mellitus (Table 1).
11 The models consist of animals with defective insulin actions or signalling, altered
12 cardiac glucose and/or FA utilization, enhanced oxidative stress, and/or cardiac
13 fibrosis.^{10, 11} The most popular animal models include the chemical ablation of the β -
14 cells of the pancreas by Streptozotocin, genetic interference with leptin signalling
15 (*ob/ob* and *db/db* mice, ZDF rats), the induction of insulin resistance by exposure to
16 high fat diet (HFD) and transgenic animals with a cardiac-specific lipotoxicity.¹⁰
17 However, important confounding factors contributing to cardiac remodelling and
18 dysfunction are rarely considered when dissecting the signalling pathways leading to
19 diabetic CM in rodent models. Experimental approaches that more closely mimic the
20 clinical scenario in T2DM patients are detailed below:

21 **▪ Ageing**

22 Many of the cardiac abnormalities (i.e., increased wall thickness and interstitial
23 myocardial fibrosis, cardiomyocyte hypertrophy) found in diabetic CM are analogous
24 to those induced by ageing.^{12, 13} Recent studies suggest that T2DM accelerates the
25 ageing of the heart and may therefore represent a form of premature senescence

1 leading to premature onset of HF.¹⁴ Indeed, T2DM has a dramatic impact on cellular
2 senescence of different types of stem cells, including cardiac stem cells and
3 potentiates the accumulation of senescent cells in the heart.¹⁵
4 Senescent cells display a complex phenotype including DNA damage and genomic
5 instability, endoplasmic reticulum stress, mitochondria dysfunction, impaired
6 contractile function, hypertrophic growth and change in gene expression involving a
7 rise of a unique secretory phenotype (senescence-associated secretory phenotype:
8 SASP) and induction of senescence-associated beta-galactosidase positivity.
9 Senescent cardiomyocytes secrete growth factors, creating a profibrotic
10 microenvironment and promoting activation of cardiac fibroblasts, which is harmful to
11 the myocardium and triggers processes associated with maladaptive cardiac
12 remodelling.^{13, 16} Furthermore, T2DM induces epigenetic alterations, such as
13 hypermethylation of CpG islands, increased trimethylation of Histone 3 (H3) at lysine
14 (K)4, H3K9, H3K27, and H4K20, as well as a decreased monomethylation and
15 acetylation of H3K9. These epigenetic modifications contribute to senescence
16 through changing the access of transcription factors to promoter/enhancer regions
17 and are complemented by noncoding RNA regulation by microRNA (i.e., miR 34a)
18 and long-noncoding RNA.¹⁷
19 To underscore the importance of senescence in the pathogenesis of diabetic CM,
20 senolytic drugs have been shown to alleviate myocardial hypertrophy, fibrosis, and
21 diastolic dysfunction in db/db obese mice.¹⁸ Such observations have suggested
22 diabetic CM as a model of premature cardiac aging and that senolytic therapy can
23 prevent this T2DM-related complication.¹⁹

24 ▪ **Sex**

1 Women with T2DM, independent of age, are at higher risk of developing CV diseases
2 (CVD) compared to age-matched men, and tend to manifest a more severe cardiac
3 remodelling in diabetic CM.^{20, 21} Interestingly, this sex-dependent aggravation of
4 diabetic CM has been successfully recapitulated in several female rodent models.
5 For instance, ZDF female rats exhibit cardiac hypertrophy with reduced capillary
6 density and increased myocardial structural damage, even though males develop
7 more pronounced fibrosis.²² Increased hypertrophy and endothelial **dysfunction**
8 have also been shown in female GK rats compared to males.²³ In the db/db mouse
9 model left ventricular pro-hypertrophic and pro-oxidant gene expression were
10 exaggerated in female leading to an increased cardiomyocyte size as compared with
11 males.²⁴ This difference is probably due to sex hormones and neurohormonal
12 diversity coupled with gender-specific activation of molecular pathways involved in
13 cardiac metabolism/remodelling.^{25, 26} For instance, sex differences have been
14 demonstrated in terms of a dichotomous fatty acid handling pattern: differential FA
15 activation together with acylcarnitine (AC) and triglyceride (TG) metabolism has been
16 reported in the GK rat model, with increased accumulation of AC species and
17 reduced TG in female compared to male rats, thus providing enhanced production of
18 ROS in cardiomyocytes.²⁷

19 Therefore, exploring both sexes in preclinical models of CVD is recommended.^{26, 28}

20 ▪ **Obesity / adiposity**

21 Adipose tissue represents an intersection of pathways involved in longevity, genesis
22 of age-related chronic diseases, metabolic dysfunction, and low-grade inflammation.
23 Obesity and adiposity are causally linked to the development of T2DM and strongly
24 contribute to diabetic CM.²⁹ The cardiac risk of obesity per se, without diabetes and
25 other co-morbid conditions, is underscored by its close association with structural,

1 functional, metabolic, and haemodynamic changes in the heart, leading to a condition
2 clinically termed as obesity CM.³⁰ Cardiac alterations related to obesity include
3 progressive increase in left ventricular (LV) mass, LV remodelling with interstitial
4 fibrosis and systolic dysfunction that may lead to HF in both patients and rodent
5 models^{31, 32} and less energetic efficiency with reduced ATP delivery in obese CM
6 hearts.³³ Several wild type high fat diet (HFD) or genetically modified rodent models
7 have shown to partially recapitulate features of human obesity CM (Table 1).
8 Although conflicting data exists as per the ability of HFD to induce myocardial
9 dysfunction these are likely explained by differences in mouse strains, the duration
10 and timing of dietary intervention, and composition of diet.^{33, 34} Overall, the
11 mechanisms by which adiposity contributes to cardiac alterations largely overlap with
12 those reported for diabetic CM and include oxidative stress, inflammation, apoptosis,
13 dysregulated autophagy, hypertrophy, interstitial fibrosis, lipotoxicity and metabolic
14 defects. It seems that not only the degree of adiposity counts but the location of body
15 fat accumulation influences the risk of cardiac dysfunction: ectopic adiposity (visceral,
16 pericardial and epicardial) carries a higher risk than subcutaneous fat^{35, 36} through the
17 release of pro-inflammatory and pro-fibrotic factors.^{37, 38} Interestingly, the effects of
18 HFD on cardiac remodelling are reversible, as a switch from HFD to standard diet for
19 8 weeks reduced lipid accumulation, myocardial hypertrophy, and fibrosis, and
20 improved myocardial function in 16-week HFD mice.³⁹ These preclinical data are in
21 line with clinical intervention studies, such as gastric bypass, caloric restriction or
22 exercise, intended to reduce myocardial structural and functional consequences of
23 diabetes or obesity.^{40, 41}

24 ▪ **Sedentary life/exercise**

1 Physical exercise is an important non-pharmacological treatment in T2DM, with high
2 efficacy in delaying or preventing diabetic CM.⁴² Preclinical studies have identified
3 some mechanisms underlying the exercise-related benefits. Exercise inhibits the
4 pathological processes of myocardial apoptosis, fibrosis, and microvascular
5 alterations through improving myocardial metabolism (improved glucose oxidation
6 and reduced FA oxidation), restoring the physiological regulation of Ca²⁺ (normalizing
7 depressed expression and function of SERCA2a in HFD + streptozotocin rats) and
8 protecting mitochondrial function.⁴³ Beneficial cardiac effects of exercise are also
9 mediated by a decrease in adipose tissue senescence and its related pro-fibrotic
10 secretome, independently of improvement in metabolic status in HFD mice.⁴⁴

11 **▪ Left ventricular pressure overload**

12 Left ventricular pressure overload occurs in a variety of conditions such as advanced
13 age, hypertension, valvular heart disease, often in association with obesity, and
14 diabetes. Its deleterious consequences, i.e., myocardial fibrosis and hypertrophy, are
15 mediated by neuro-hormonal factors involving the sympathetic nervous system and
16 the renin–angiotensin–aldosterone system (RAAS). The sympathetic nervous system
17 provides the most powerful, but also deleterious, stimulation of cardiac function, via
18 catecholamines and their post-synaptic β -adrenergic receptors (β -AR) including β 1-
19 AR, β 2-AR, and β 3-AR subtypes.⁴⁵ Interestingly, diabetic CM, similar to other forms
20 of HF, displays alterations of autonomic control with reduction of parasympathetic
21 activity and an increase of sympathetic nervous system activity, which promotes
22 decreased β -AR responsiveness.⁴⁶ The latter increases heart rate, stroke volume
23 and peripheral vascular resistance, and stimulates the RAAS, exacerbating left
24 ventricular dysfunction. At the molecular level, elevated sympathetic drive enhances
25 β 1-AR signalling, which promotes hypertrophy, interstitial fibrosis, cardiomyocyte

1 apoptosis and impairs energy metabolism and myocardial function.⁴⁵ Additional
2 mechanistic studies suggested that a canonical downstream effector of β -AR, the
3 cyclic AMP-dependent protein kinase A (PKA) may be involved in the deficient
4 ventricular performance and metabolism in the mouse diabetic heart,⁴⁷ potentially
5 giving way to other cAMP effectors, such as the Epac proteins.⁴⁸ Interestingly, the
6 relationship between insulin resistance and β -AR signalling is emerging as an
7 important focal node in the pathogenesis of diabetic CM since hyperinsulinemia may
8 play a role in desensitization of β -AR signalling in T2DM. This is well illustrated in a
9 study showing that in a diabetic CM murine model induced by HFD, myocardial injury
10 and dysfunction could be reversed by pharmacologically inhibition of β 2-AR or G
11 protein-coupled receptor kinase 2 (GRK2) activity.⁴⁹ In contrast to cardiac β 1- and
12 β 2-AR, the role of the β 3-AR in the diabetic heart has been minimally investigated. It
13 was reported that a β 3-adrenoceptor-mediated negative inotropic effect contributes to
14 the altered positive inotropic response induced by β -adrenoceptor activation in
15 diabetic rat heart.⁵⁰

16 ▪ **Intermittent hypoxia**

17 Prevalence and severity of obstructive sleep apnoea (OSA) is higher among diabetic
18 individuals compared to non-diabetic subjects.⁵¹ OSA is associated with metabolic
19 and CV co-morbidities including hypertension, arrhythmia, stroke, coronary heart
20 disease, which supports OSA as a major health burden. Mechanistic studies in
21 rodents subjected to chronic intermittent hypoxia (the pathophysiologic basis of OSA)
22 found that OSA-induced CV dysfunction (vascular remodelling, endothelial
23 dysfunction, early atherosclerosis and increased arterial blood pressure) depends on
24 oxidative stress- and HIF1 α -driven sympathetic overactivity. Specifically, increased
25 levels of ROS and HIF α activate chemoreflex and suppress baroreflex, thereby

1 stimulating the sympathetic nervous system, increasing LV afterload, and contributing
2 to insulin resistance and T2DM.⁵²

3

4

5 **Crosstalk between metabolic organs and the heart beyond cardiac glucose**
6 **toxicity:**

7 There is need to consider each major organ, heart, liver, adipose tissue, skeletal
8 muscle, lung and kidney operating as an integrated network within the human body in
9 response to dysregulated metabolism. In particular, T2DM is associated with
10 progressive microvascular disorders and systemic inflammatory processes, inducing
11 fibrosis in several organs, including CV system, liver, adipose tissue, and skeletal
12 muscle. During the progression of these fibro-inflammatory processes, there are
13 significant haemodynamic and metabolic interactions between these organs, which
14 need to be assessed to predict health trajectories in T2DM patients⁵³ and more
15 specifically the progression towards diabetic CM and HF with preserved ejection
16 fraction (HFpEF).^{54, 55} Indeed, dysregulation of both the immune system and
17 microcirculation through endothelial cell dysfunction and procoagulant changes
18 contributes to diabetic CM beyond hyperglycaemia, insulin resistance, and metabolic
19 derangements.^{8, 53} Importantly, the microcirculation impacts on insulin sensitivity by
20 affecting the delivery of insulin and glucose to skeletal muscle. Thus, endothelial
21 dysfunction and extracellular matrix remodelling promote the progression from
22 prediabetes to diabetes and the development of diabetic CM and other T2DM
23 complications, including HFpEF and chronic kidney disease (CKD).^{56, 57} Thus, whilst
24 HFpEF was initially considered as a disorder characterized by hypertension, cardiac

1 hypertrophy and diastolic dysfunction, the pandemics of obesity and T2DM have
2 modified the HFpEF syndrome. As a result, HFpEF is now recognized as a
3 multisystem disorder involving the heart, lungs, kidneys, skeletal muscle, adipose
4 tissue, vascular system, and immune and inflammatory signalling.^{54, 58}

5 How inter organ crosstalk during T2DM specifically contributes to diabetic CM
6 requires further exploration. Animal models of T2DM represent a unique approach to
7 test the mechanisms of such organ interactions and to assess how the pathological
8 state developing in one organ, can lead to deleterious functional and structural
9 consequences in the heart.

10 ▪ **Kidney**

11 T2DM is directly related to both CKD and CVD. Patients with diabetes and CVD are
12 twice as likely to develop CKD than those without CVD.⁵⁶⁻⁵⁹ Furthermore, the co-
13 incidence of HFpEF and CKD is very strong since approximately 50% of the patients
14 with HFpEF also suffer from CKD.^{55, 60} To further underscore a pathophysiological
15 crosstalk, activated inflammatory cascades and endothelial dysfunction in renal injury
16 promote features of HFpEF, such as cardiomyocyte stiffening and myocardial
17 fibrosis.⁵⁵ Beside clinical evidence, a causal relationship between CKD and HFpEF
18 came from an experimental rat model of CKD induced by nephrectomy, which
19 resulted in a cardiac HFpEF-like phenotype, with left ventricular hypertrophy and
20 diastolic dysfunction.⁶¹ The kidney-heart relationship is also achieved by complex
21 interactions involving neuro-hormonal pathways.⁶² This is well illustrated with the
22 RAAS system, which is overactivated during CKD and causes a cascade of events
23 leading to vasoconstriction, increased sodium retention, and reduced water excretion.
24 All of which increase blood volume expansion and restore perfusion pressure and
25 therefore may contribute to the development of HFpEF.⁶² Besides its renal effects,

1 aldosterone directly promotes cardiac fibrosis, left ventricular hypertrophy, and
2 coronary microvascular dysfunction.⁵⁸ Additional renal factors such as uremic toxins
3 and galectin 3 may also have a direct impact on the heart and/or coronary
4 microvasculature and therefore may play a role in the pathogenesis of HFpEF.^{63, 64}
5 These important interactions between T2DM, renal dysfunction and diabetic CM
6 induce a downward spiral of deleterious events, whose interruption represents a
7 novel therapeutic opportunity.⁶⁵

8 ▪ **Adipose tissue**

9 A growing body of evidence supports the existence of a two-way adipose-myocardial
10 axis in which products released from fat affect myocardial metabolism and function,
11 whilst peptides secreted from the heart affect FA disposal. Accumulation of ectopic
12 fat in various organs, e.g., in heart, liver, pancreas and kidney has been identified as
13 an important marker in the pathogenesis of T2DM in both human and animal
14 studies.⁶⁶⁻⁶⁸ Although the causal relationship between the pathophysiological status
15 of white adipose tissue and cardiac lipotoxicity remains elusive, elevated lipolytic rate
16 in adipose tissue has been demonstrated to contribute to the overall augmentation of
17 plasma lipid levels, as observed in the majority of patients suffering from HF.
18 Excessive release of FA from adipose tissue contributes to myocardial insulin
19 resistance with subsequent metabolic inflexibility characterised by a shift in cardiac
20 energy expenditure towards a near-exclusive and less oxygen-efficient FA oxidation.
21 The perpetuation of this metabolic deregulation leads to the development of cardiac
22 lipotoxicity.^{69, 70} Cardiac lipid overload promotes the formation of cytotoxic
23 intermediates (diacyl-glycerols and ceramides) and enhances ROS generation
24 through exacerbated peroxisomal and mitochondria FA oxidation. Both intermediate

1 lipotoxic species and ROS affect mitochondrial function and Ca²⁺ handling proteins
2 promoting cardiac dysfunction.^{71, 72}

3 Adipose tissue is an important source of inflammatory mediators (TNF- α , IL6, IL-8,
4 MCP-1) and adipokines (leptin, resistin, and omentin), which may act in an autocrine,
5 paracrine, and endocrine manner ultimately furthering cardiac injury.^{38, 73} In contrast,
6 the anti-inflammatory adipokine, adiponectin, has been found to be inversely
7 correlated with myocardial adiposity. Whilst visceral adipose tissue contributes to a
8 low-level and sustained systemic inflammation, pericardial and epicardial fat can
9 directly affect the underlying myocardium by local diffusion of secreted inflammatory
10 mediators.⁷⁴⁻⁷⁶ Another ectopic fat source known to influence the heart is the
11 perivascular adipose tissue (PVAT) surrounding the vasculature, which volume
12 increases proportionally to elevated visceral adipose tissue.⁷⁷ In obesity, PVAT has
13 been shown to shift from an anti-inflammatory and vasodilatory profile towards a
14 proinflammatory and impaired vasodilation status favouring the progression of
15 vascular disease.^{78, 79}

16 Finally, a prominent role for atrial and B-type natriuretic peptides (ANP and BNP,
17 respectively) has been proposed in the crosstalk between the heart and the adipose
18 tissue.⁸⁰ As such, the induction of lipolysis by natriuretic peptides secreted by the
19 damaged heart has been suggested to counteract obesity, with a disproportionately
20 greater role in reducing visceral adipose tissue than subcutaneous adipose tissue.⁸¹

21 On the other hand, increased release of adipocyte FA may contribute to cardiac
22 steatosis and cardiac cachexia.⁸²

23 Further analysis of the crosstalk between adipose tissue and the heart may identify
24 new treatment options such as targeting lipolysis and cardiac lipid metabolism in
25 diabetic CM to avoid its progression towards HFpEF.

1 ▪ **Liver**

2 Several studies support the bidirectional crosstalk between the heart and liver and
3 the consequences of simultaneous development of hepatic metabolic diseases,
4 diabetic CM, and HF. A better understanding of this hepato-cardiac axis is required to
5 ensure an effective management of T2DM patients with heart or liver diseases in
6 order to improve overall prognosis.

7 Whilst T2DM and metabolic diseases (obesity and non-alcoholic fatty liver disease)
8 are important risk factors to induce cardiac dysfunction,^{31, 83} a growing body of
9 evidence suggests that the dysfunctional heart per se could affect both systemic
10 metabolism and liver function, and thus, create a vicious injurious cycle between
11 heart and liver. The close association among cardiac and metabolic diseases
12 suggests a common pathophysiological basis. Notably, in metabolic diseases, the
13 heart and liver share similar intracellular defects such as mitochondria dysfunction,
14 ER stress, lipotoxicity and disrupted calcium homeostasis. Interestingly, mitochondria
15 and endoplasmic reticulum interact at contact sites called mitochondria-associated
16 membranes (MAMs) to exchange phospholipid and calcium and regulate metabolic
17 homeostasis and signalling.⁸⁴ Of note, reduction of ER-mitochondria communication
18 was observed in both heart³⁹ and liver⁸⁵ of HFD mice. In the heart, decreased ER-
19 mitochondria communication caused mitochondrial dysfunction leading to diabetic
20 CM, whereas in the liver, disrupted ER-mitochondria interactions alters hepatic
21 metabolic flexibility and insulin sensitivity. Therefore, targeting MAMs could be a new
22 strategy to concomitantly improve both heart and liver function in T2DM.

23 In addition, the heart secretes proteins referred to as cardiokines, which go beyond
24 local cardiac effects, and mediate changes in extracardiac tissues, including liver
25 function. For example, the cardiac atrial natriuretic peptide attenuates glycolysis and

1 increases gluconeogenesis in rat liver.⁸⁶ Other studies showed that the heart controls
2 systemic metabolism via the cardiac-specific microRNA-208a and the mediator
3 complex subunit 13 (MED13) signalling in rodent cardiomyocytes.⁸⁷ Overexpression
4 of MED13 or inhibition of miR-208a in cardiac tissue of transgenic mice enhanced
5 lipid uptake, β -oxidation, mitochondrial content, and other genes involved in FA
6 utilization in adipose tissue and liver,⁸⁸ thus supporting the existence of a functionally
7 relevant, metabolic crosstalk between the heart and liver.

8 ▪ **Skeletal muscle**

9 Sarcopenia is characterized by a loss of skeletal muscle strength due to reduction in
10 the quality and quantity of muscle mass, replacement of muscle fibres with fat,
11 changes in muscle metabolism, oxidative stress, degeneration of neuromuscular
12 junctions and increased fibrosis. Whilst sarcopenia has been described in elderly
13 individuals, mounting evidence suggests a higher prevalence in T2DM patients.
14 Sarcopenia in T2DM patients may be caused by different mechanisms, such as
15 impaired insulin sensitivity, chronic hyperglycaemia, advanced glycosylation end
16 products, subclinical inflammation, microvascular and macrovascular complications.⁸⁹
17 It seems that the opposite also applies; patients with sarcopenia are at increased risk
18 to develop T2DM.^{89, 90} In addition, sarcopenia is associated with CVD^{91, 92} and both
19 share common risk factors, such as altered glucose metabolism, insulin resistance,
20 inflammation, and metabolic syndrome.⁹³ For instance, T2DM patients with chronic
21 HF exhibit severe skeletal muscle fibre atrophy, capillary remodelling and impaired
22 mitochondrial function, characterized by mitochondrial complex I dysfunction, lower
23 transcript levels of complex I and ROS overproduction.⁹⁴

24 ▪ **Brain**

1 Recent studies shed light on the relationship between the brain and CV system, and
2 how the brain-heart axis regulates T2DM.⁹⁵ Cohort studies highlight the link between
3 Alzheimer's disease and T2DM,⁹⁶ whilst drugs that are currently approved for the
4 treatment of T2DM, such as metformin, have shown promising results in improving
5 cognitive function, and even preventing the development of Alzheimer's disease in
6 diabetic patients.⁹⁷

7 **Investigating diabetic cardiomyopathy in preclinical models: the role of cellular** 8 **crosstalk within the heart:**

9 The same inter organ signalling also works in a paracrine and autocrine fashion,
10 where a given cell type can detrimentally affect neighbouring cells, leading to a
11 vicious cycle and subsequent cardiac dysfunction. In addition to its signature
12 parenchymal cells, the contracting cardiomyocytes, the heart contains many other
13 cells, including fibroblasts, smooth muscle cells, endothelial cells, and resident
14 macrophages. Crosstalk between these different cells ensures myocardial
15 homeostasis but a pathologically altered cell-cell communication may initiate and
16 propagate adverse cardiac remodelling and play a crucial role in the development of
17 diabetic CM.^{58, 98}

18 **▪ Fibroblasts / cardiomyocytes**

19 Cardiac fibroblasts play a crucial role in extracellular matrix (ECM) turnover, as they
20 are involved in both synthesis and degradation of ECM components through matrix
21 metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).
22 Fibroblasts adhere to ECM proteins through integrins that are critical mediators of cell
23 attachment, adhesive signalling, and remodelling of collagen fibrils. Excessive
24 cardiac ECM deposition is a key feature of the remodelling response in diabetic CM

1 and promotes myocardial stiffness and cardiac dysfunction in experimental rodent
2 models of diabetes.⁹⁹ Experimentally, high glucose levels induce cardiac fibroblasts
3 into a state of increased proliferation,¹⁰⁰ with increased DNA and collagen synthesis
4 as well as fibronectin and TGF-beta-1 gene expression.^{101, 102} Genetic inhibition of
5 $\alpha 11\beta 1$ integrin in STZ diabetic mice prevents the progression of fibrosis and
6 abnormal cardiomyocyte growth, indicating that this specific integrin plays a critical
7 role in modifying fibroblast-cardiomyocyte-ECM interactions.¹⁰³ Crosstalk between
8 cardiomyocytes and fibroblasts is also associated with cardiomyocyte switching to a
9 fibrogenic phenotype, characterized by increased synthesis and release of cytokines
10 that induce fibroblast proliferation and activation, as well as proinflammatory
11 molecules that trigger fibrosis through activation of immune cells.⁹⁹

12 ▪ **Endothelial cells**

13 Diabetic CM is associated with coronary microvascular dysfunction, which impairs
14 coronary blood flow and myocardial perfusion.¹⁰⁴ Abnormalities in the coronary
15 microcirculation result from endothelial cell dysfunction, which is considered a central
16 mechanism in HFpEF pathophysiology.^{7, 79} Indeed, endothelial cells have altered
17 paracrine signalling to cardiomyocytes by reducing the bioavailable vasodilator
18 molecules, nitric oxide (NO) and endothelium-derived hyperpolarising factors
19 (EDHFs), thereby limiting blood flow and promoting leukocyte infiltration in the
20 myocardium.¹⁰⁵ The latter leads to activation of myofibroblasts and interstitial
21 collagen deposition. As part of T2DM-associated glucotoxicity and lipotoxicity,
22 endothelial cells generate ROS and reactive nitrogen species (RNS) that uncouple
23 endothelial NO synthase (eNOS) activity (by oxidising eNOS cofactor
24 tetrahydrobiopterin) leading to decreased NO bioavailability.¹⁰⁶ This effect together
25 with insulin resistance converges on and minimizes the activity of guanylate cyclase

1 and cyclic guanosine monophosphate (cGMP)- protein kinase G (PKG) signalling that
2 results in deranged titin phosphorylation and increased cardiomyocyte hypertrophy
3 and stiffness in diabetic hearts.¹⁰⁷ Endothelial cells can also contribute to the
4 development of cardiac fibrosis through endothelial-to-mesenchymal transition to
5 myofibroblasts.¹⁰⁸

6 ▪ **Immune cells**

7 Numerous experimental and clinical studies have reported a role of adaptive
8 immunity in diabetic CM pathogenesis.¹⁰⁹⁻¹¹¹ T2DM is associated with chronic
9 systemic inflammation, which leads to leukocyte activation and recruitment to various
10 organs, further aggravating inflammatory cardiac tissue remodelling over time.¹¹⁰ This
11 results in cardiac fibrosis as resident fibroblasts become activated in response to
12 pathophysiologic conditions, which for the heart, leads to wall stiffening and
13 decreased contractility.⁹⁹ Although the role of B-cells is still unclear, T-cell derived
14 immune response has shown to contribute to the progression of diabetic CM.¹¹¹ As
15 such, in STZ-induced rodent models of diabetic CM, increased infiltration of T
16 lymphocytes into the myocardium is positively correlated with increased collagen
17 deposition and wall stiffness,¹¹⁰ whilst genetic depletion of CD4+ T cells protects
18 against cardiac fibrosis and impairment in LV function.^{111, 112} Yet, recent studies have
19 further delineated the contribution of each T-lymphocyte subset in the context of
20 diabetic CM. Pro-inflammatory T helper cells Th1, Th17 and Th22 subtypes are
21 increased in diabetic CM,¹¹³ whereas the activation of anti-inflammatory Th2 and
22 Foxp3+ Treg subtypes is delayed or impaired,¹¹⁴ overall promoting chronic
23 inflammatory tissue damage. Increased neutrophil/lymphocyte ratio (an indicator of
24 systemic inflammation) is associated with the occurrence of subclinical diabetic
25 CM.¹¹⁵ As per potential mechanisms involved, the sphingosine-1-phosphate

1 (S1P)/S1P-receptor signalling axis has been shown to regulate T-cell trafficking,
2 activation, and polarization.¹¹⁶ Indeed, targeted deletion of T-cell S1P-R or
3 administration of fingolimod (an analogue of S1P) both reduce myocardial fibrosis
4 and improve cardiac function in STZ-induced diabetic CM mice.¹¹⁷

5 Macrophages also play a key role in regulating inflammatory responses and
6 homeostatic maintenance of the myocardium. Normally in injured tissue, efferocytosis
7 allows macrophages to engulf apoptotic cells and cellular debris to reduce
8 inflammation.¹¹⁸ Efferocytosis is regulated by many processes in the context of high
9 glucose. In particular, the metalloproteinase disintegrin and metalloproteinase
10 domain-containing protein 9 (ADAM-9) were shown to be upregulated in
11 macrophages, secondary to a downregulation of miR-126, which increased MER
12 proto-oncogene, tyrosine kinase (MerTK) cleavage with a net effect of reduced
13 efferocytosis.¹¹⁹ Interestingly, human diabetic hearts display the same molecular
14 signatures in terms of miR-126, ADAM9, and cleaved MerTK expression, suggesting
15 that this chain of events may be involved in regulating human diabetic CM
16 progression. Recently, cardiac-resident MHCII-high macrophages showed a
17 pathogenic role in cardiac remodelling through production of IL-10. The profibrotic
18 effect of IL-10 autocrine loop promotes macrophages to secrete osteopontin and
19 TGF β , which activate cardiac fibroblasts to produce collagen that results in cardiac
20 fibrosis and increased cardiac stiffness.¹²⁰ Therefore, a new understanding of
21 communication between cardiac resident macrophages and fibroblasts could lead to
22 novel therapeutic strategies for diabetic CM and its progression towards HF.

23

24 **Investigating diabetic cardiomyopathy in preclinical models: identifying new**
25 **biomarkers and therapeutic targets**

▪ **Biomarkers**

Since T2DM patients at high risk of developing HF display altered metabolism in cardiomyocytes, with underlying changes in protein and metabolite profiles related to hyperglycaemia, lipotoxicity and oxidative stress, a systems biology approach may identify a specific signature of diabetic CM. To reduce disease burden, it is imperative to develop non-invasive biomarkers to detect and characterize diabetic CM processes at their early and possibly reversible stages in order to reveal new therapeutic targets and to follow disease progression. These last years, new methods have emerged, which offer a great potential to identify such biomarkers. Big datasets derived from in silico predictive models, imaging, and omics technologies (metabolomics, lipidomics, transcriptomics, proteomics) may be used for developing multiparametric datasets to assist improved diagnostic and therapeutic decisions.

Metabolic alterations and insulin resistance are early signs of future cardiac dysfunction and have a causative role in the development of the diabetic CM.¹²¹ Metabolomics using different analytical techniques such as magnetic resonance spectroscopy, mass spectrometry and chromatography¹²² are powerful approaches to follow simultaneous changes in multiple metabolite levels occurring in the diabetic heart. Indeed, cardiac energetic metabolism assessed by the PCr/ATP ratio, is reduced in some studies,¹²³ although some discrepancies exist depending on the models.^{124, 125} In parallel, lipid metabolism is altered with increased FA oxidation and lipid accumulation.⁶⁶

In silico predictive methods have the potential to reveal or to confirm effective biomarkers. Using this approach and exploiting meta-analysis of transcriptomic datasets, differential expression levels of lysyl oxidase like 2 (LOXL2) and electron transfer flavoprotein beta subunit (ETF β) in serum and heart tissue of 6–16-week-old

1 db/db mice correlated closely with a reduced LV diastolic dysfunction, supporting the
2 use of LOXL2 and ETF β as early predictive biomarkers for diabetic CM.¹²⁶

3 Moreover, systematic multiorgan biobanking of porcine models of diabetes and
4 obesity subjected to molecular profiling by transcriptomics, proteomics and
5 metabolomics has been proposed to better understand tissue specific pathogenic
6 mechanisms and organ crosstalk with the prospect of revealing novel molecular
7 targets.¹²⁷

8 In the field of imaging technologies, the development of machine learning algorithms
9 aims to provide more accurate biomarkers.¹²⁸ Thus, combining imaging, radiomics
10 and multi-omics data with machine learning will provide large datasets of parameters
11 to find pertinent biomarkers for early diagnosis of diabetic CM and predict its
12 progression.

13 ▪ **New therapeutic targets**

14 Drug development is time consuming and costly, urging the use of precision medicine
15 to replace the 'one size fits all' paradigm with more patient tailoring approaches.
16 Understanding T2DM-specific mechanisms shall lead to opportunities of developing
17 better therapies. Mechanistic studies have demonstrated dramatic glucotoxicity in the
18 heart, and linked it to accelerated sugar-related protein modifications, such as O-
19 GlcNAcylation¹²⁹ and AGE formation,¹³⁰ as well as from increased ROS formation.¹³¹
20 Yet, most interventional studies have focused on the reduction of plasma glucose in
21 T2DM patients and studies with DPP4 inhibitors and GLP-1 peptides produced
22 modest effects in terms of improving HF outcomes,¹³² whilst the recent benefits
23 obtained with SGLT2 inhibitor (SGLT2i) treatment for HF and CKD are partly
24 independent of their hypoglycaemic effects. Many potential mechanisms have been
25 proposed for SGLT2i.¹³³⁻¹³⁵ For example, studies suggest that natriuresis, diuresis

1 and decreased blood volume that reduced preload and afterload are possible
2 mechanisms. Reduced cardiac oxidative stress and fibrosis have also been observed
3 with SGLT2i treatment.¹³⁶ Occurrence of ketoacidosis prompted suggestions that
4 plasma ketone bodies serve as an alternative and efficient source of cardiac fuel.¹³⁷ It
5 has also been proposed that SGLT2 inhibitors have off-target pharmacology by
6 directly inhibiting cardiac NHE1 activity and protect the myocardium under ischemic
7 conditions.¹³⁸ Nevertheless, confirmation of specific SGLT-2 inhibitor cardioprotective
8 mechanisms remains elusive to date.

9 Research efforts need therefore to focus on finding therapeutic strategies to inhibit
10 these pathophysiological pathways, and thereby reduce the risk of diabetic CM. In
11 addition, the understanding of diabetic CM pathophysiology should generate
12 awareness regarding its multiorgan nature. Thus, holistic approaches taking the
13 complexity of myocardial damage induced by T2DM along with the functional
14 interplay between different key organs into account will advance our knowledge of
15 diabetic CM. This type of multidimensional approach will increase the likelihood of
16 early diagnosis and the translational success of new drugs in development. Currently,
17 there are no specific therapies for diabetic CM. Further refinement of diabetic CM
18 molecular signatures derived from improved preclinical models should provide new
19 mechanistic insights leading to specific targets, drugs, biomarkers, and effective
20 patient management in the future.

21

22 **Future Perspectives**

23 The lack of experimental models that reproduce all structural, functional, and
24 molecular alterations of human diabetic CM is one of the barriers to advance patient

1 care. Animal models have provided information about the initiation and progression of
2 diabetic CM. They have decoded a few molecular mechanisms of diabetic CM. In
3 addition, they are useful for testing new treatments, and identifying possible side-
4 effects.

5 From the results of the CARDIATEAM study involving cohorts of diabetic patients
6 with and without overt diabetic CM and control subjects, it is anticipated that many
7 pathways specifically involved in diabetic CM will be discovered by employing
8 unsupervised analysis and disease modelling of imaging and “omics” data. By
9 backtranslation of these clinical results, the aim of CARDIATEAM consortium is to
10 develop a preclinical model that recapitulates human diabetic CM. We postulate this
11 will prove a powerful approach in improving the diagnosis and treatment of diabetic
12 CM.

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4

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12 88.
- 13
- 14

1

2 **Table 1. Rodent models that recapitulate diabetic cardiomyopathy features**
 3 **found in humans.**

		Animal model	Cardiac functional and structural alterations	Ref.	
Mice	HFD	<i>C57/BL6 mice</i>	Early onset of metabolic alterations and cardiac LV dysfunction (5 weeks after starting HFD) and defects in glucose handling.	32, 139	
		<i>Old female C/57BL/6J mice + HFD + angiotensin II infusion.</i>	Model of HFpEF (LV hypertrophy and LV diastolic dysfunction; no change in LVEF)	140	
		<i>ob/ob Mouse Model</i>	LV diastolic dysfunction and features of lipotoxicity.	141, 142	
		<i>C57BL/6N mice + HFD+L-NAME administration (p.o)</i>	Model of HFpEF (Hypertrophic response, diastolic dysfunction, pulmonary congestion, reduction in contraction velocity and impaired relaxation)	143	
	Models of lipotoxicity		<i>Alteration in myosin heavy chain acyl-CoA synthetase (MHC-ACS mice)</i>	Lipotoxicity, abnormal cardiac metabolism, cardiac hypertrophy, LV dysfunction and premature death	144
			<i>GPI-anchored human lipoprotein lipase transgenic mice (hLpLGPI mice)</i>	Lipotoxicity, cardiac hypertrophy, abnormal cardiac metabolism, LV dysfunction, and cardiac fibrosis.	145
			<i>Myosin heavy chain-peroxisome proliferator-activated receptor α mice (MHC-PPARα mice)</i>	Lipotoxicity, cardiac hypertrophy, abnormal cardiac metabolism, LV dysfunction, and cardiac fibrosis.	146
			<i>Myosin heavy chain fatty acid transport protein mice (MHC-FATP mice)</i>	Lipotoxicity, LV diastolic dysfunction and prolonged QTc intervals.	147, 148
			<i>Adipose TG lipase knockout mice</i>	Lipotoxicity. LV dysfunction and premature death	149
T2DM		<i>Streptozotocin</i>	Reduction in heart rate, amplitude of contraction and of ventricular pressure, and prolongation on the rate of ventricular myocyte contraction and relaxation	150-156	
		- <i>Intraperitoneal route</i> - <i>Intravenous route</i>			
T1DM		<i>db/db Mouse Model</i>	Decreased systolic function, abnormal diastolic filling, and electrophysiological alterations	157	

4

		Animal model	Cardiac functional and structural alterations that recapitulate human features of Diabetic Cardiomyopathy	Ref.
Rat	HFD	<i>Sprague-Dawley rats</i>	Lipotoxicity, cardiac fibrosis and hypertrophy	158, 159
	Obesity	<i>Obese Zucker rat (fa/fa)</i>	Lipotoxicity and increased LV end-diastolic volume and stroke volume	160
		<i>DahlS.Z-Lepr(fa)/Lepr(fa) (DS/obese) rat</i>	LV diastolic dysfunction, LV hypertrophy, and cardiac fibrosis.	161
	T1DM	<i>Streptozotocin</i>	LV systolic and diastolic dysfunction and fibrosis.	162-
		- <i>Intraperitoneal route</i>		164
		- <i>Intravenous route</i>		165-167
	T2DM	<i>Zucker diabetic fatty rat (ZDF)</i>	Increased heart and LV weights, presence of fibrosis, depressed RV and LV systolic function	168, 169
<i>Goto-Kakizaki rat (GK)</i>		Cardiac hypertrophy, increased extracellular matrix deposition and increased heart size	170, 171	

1

2 Abbreviations: HFD: high fat diet. LV: left ventricle; LVEF: left ventricle ejection fraction; T1DM: Type 1

3 diabetes mellitus; T2DM: Type 2 diabetes mellitus

4

1

2 **Figures Legends**

3 **Figure 1: Mechanisms contributing to cardiomyocyte dysfunction in diabetic**
4 **cardiomyopathy.** Hormonal and metabolic alterations may result in hyperglycemia,
5 insulin resistance and lipid overload which cause through specific signalling pathways
6 subcellular component abnormalities. This includes mitochondrial dysfunction,
7 impaired metabolic flexibility, Ca²⁺ dysregulation and activation of gene transcription
8 program involved in cardiac remodelling and senescence. These molecular and
9 cellular events contribute to diastolic and systolic dysfunction. GPCR, G protein-
10 coupled receptor; IR, insulin receptor; ROS, reactive oxygen species; RyR, ryanodine
11 receptor.

12

13 **Figure 2:** Diabetic heart is at the cross-road of environmental factors, organ-crosstalk
14 and paracrine cellular-crosstalk between cardiomyocytes, fibroblasts, endothelial
15 cells and immune cells in the myocardium.

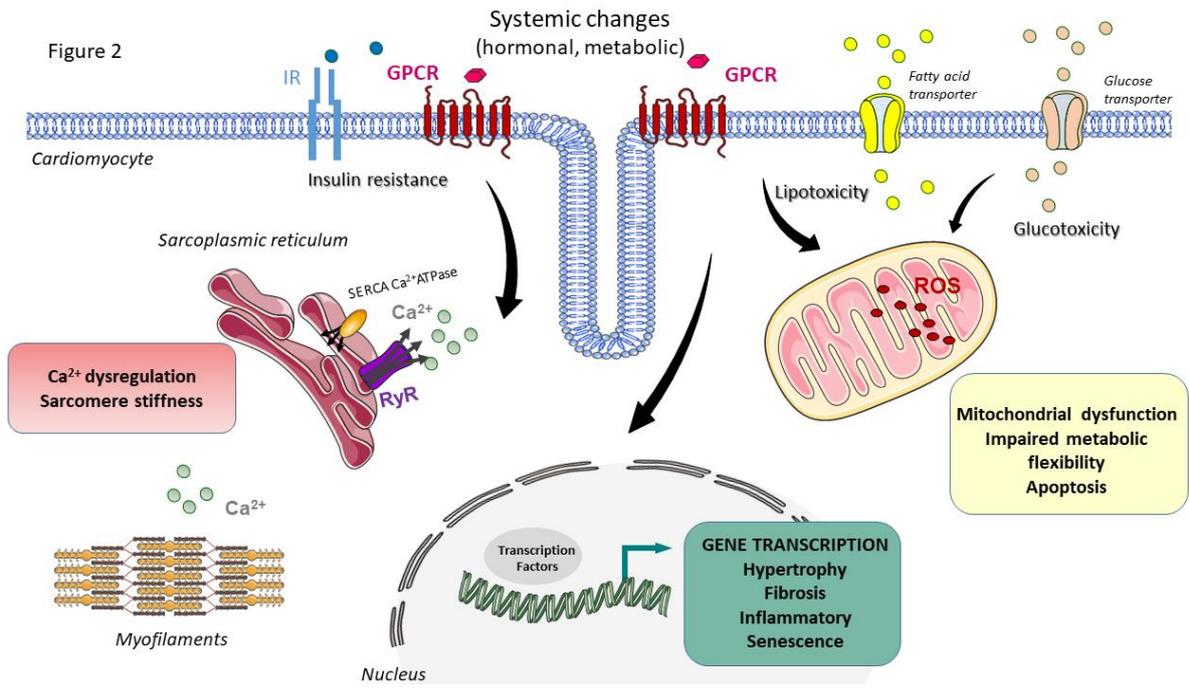
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17 **Graphical Abstract: Rodent models should recapitulate the clinical phenotype**
18 **to better mimic the progression of diabetic cardiomyopathy towards heart**
19 **failure**

20

1

2 **Figure 1:**



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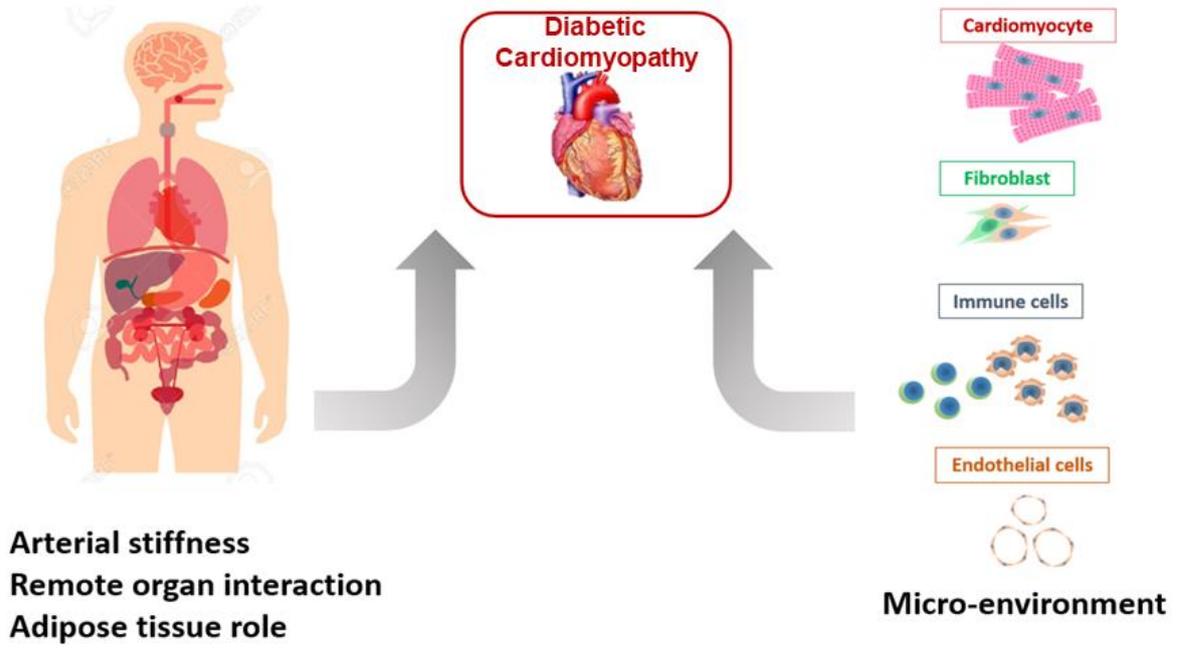
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2 **Figure 2:**

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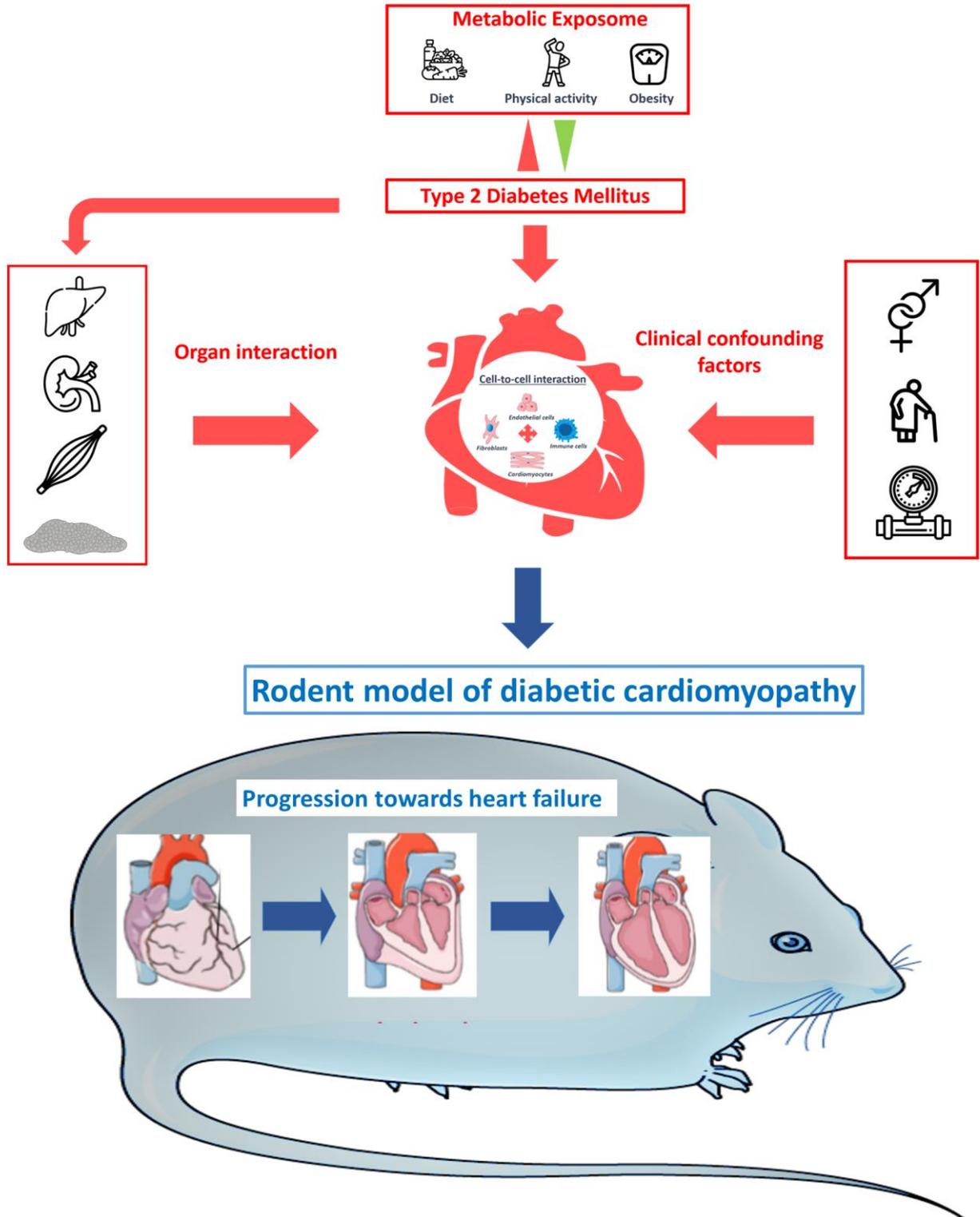
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2 **Graphical abstract:**

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