Exercise regulates microRNAs to preserve coronary and cardiac function in the diabetic heart

Diabetes is associated with numerous long-term health complications, in particular cardiovascular disease. The onset of heart disease in diabetes begins at an early stage with impaired coronary blood flow as a precursor for heart failure [1]. Unfortunately, patients in the early stages of diabetes remain asymptomatic to any cardiac dysfunction till the later stage of the disease, thereby restricting its effective therapeutic management. An effective method for the early diagnosis of diabetic heart disease is critical for implementing effective therapeutic strategies [2].

MicroRNAs (miRNAs) are key players in various cardiovascular events through the regulation of cardiac gene expression. Indeed, circulating miRNA's serve as potential diagnostic biomarkers for cardiovascular disease [2]. Their potential role as biomarkers for the early onset of diabetic heart disease has not yet been addressed.

Exercise is generally viewed as an effective prophylactic strategy for combating diabetes because of the benefits on weight management and insulin sensitivity [3]. Studies have shown '*high intensity*' exercise as an effective means of reversing diabetic heart disease, because exercise ameliorates vascular dysfunction. However, once heart disease is well established, this high intensity of exercise required to impede the progression of heart disease is often unsustainable for most patients.

This study [4] proposed that initiation of a '*moderate*' and sustainable exercise regime in the early stages of diabetes, *before* cardiac dysfunction begins, could prevent diabetic coronary artery and heart disease. Yet, only '*high intensity*' exercise could ameliorate cardiac dysfunction in diabetes if the exercise was started *after* the cardiac dysfunction had already become established. Finally, we used miRNA's as a non-invasive biomarker to predict and identify changes in coronary function during the progression of diabetic heart disease.

All experiments were performed at SPring-8 **BL28B2**. We used the *db/db* mouse model, which progressively develop diabetic heart disease. Subgroups of '*Early cardiac dysfunction* (Early CD)' and '*Late cardiac dysfunction* (Late CD)' diabetic mice (DM) (n = 8/group) were subjected to either no exercise, moderate intensity (MIE) or high intensity exercise (HIE) for 1-hour per day over eight weeks. Blood samples were extracted every two weeks to measure miRNA-126. Echocardiography was used for repeated measurements of cardiac structure and function. Age matched nondiabetic mice (ND) served as controls.

At the end of the exercise regimes, we imaged and assessed the functional capacity of the coronary vessels using Synchrotron Radiation (SR) microangiography, as previously described [5]. Coronary angiograms were imaged at baseline and then in response to i) acetylcholine (ACh - endothelial-dependent vasodilation) and ii) sodium nitroprusside (SNP – endothelial-independent vasodilation).

Diabetes: Microangiography data revealed that diabetes caused coronary vessel rarefaction (loss of coronary vessels) (Fig. 1). Diabetes impaired the magnitude of endothelial-dependent vasodilatory response to ACh of small coronary microvessels (Fig. 1). Moreover, echocardiographic evidence revealed that both Early-CD and Late-CD DM mice had clear hallmarks of cardiac damage and dysfunction (Fig. 2).

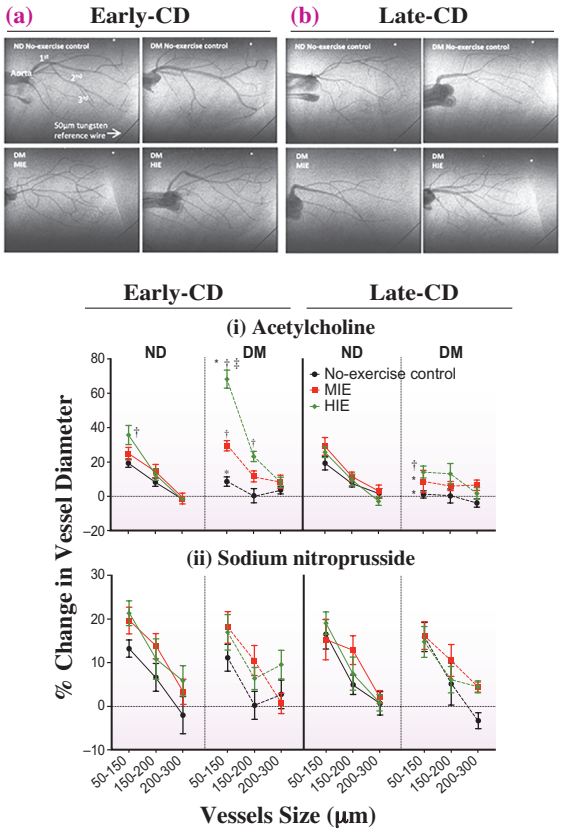

Fig. 1. Representative angiograms and quantitative line graphs showing the change in vessel diameter in response to (i) acetylcholine and (ii) sodium nitroprusside in a) Early-CD and b) Late-CD nondiabetic (ND) and diabetic (DM) mice, after eight weeks of no exercise, moderate intensity exercise (MIE) or high intensity exercise (HIE). *Significantly different to the ND, $p < 0.05$; †Significantly different to the no-exercise, $p < 0.05$.

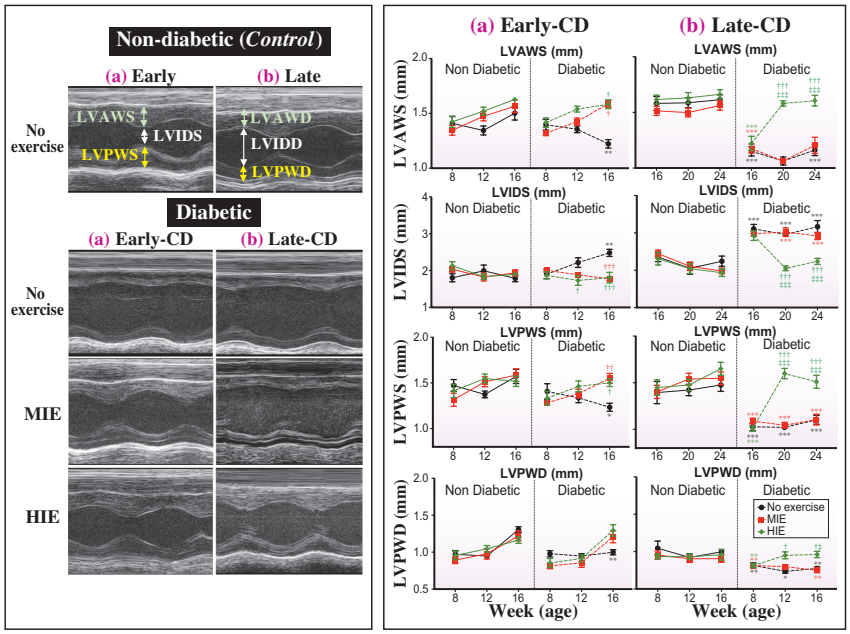

Fig. 2. Effect of exercise (MIE and HIE) on left ventricular (LV) structure in nondiabetic (ND) or diabetic (DM) mice. **Left panel**: Representative M-mode echocardiographic images. LVAWS & LVAWD - left ventricular anterior wall thickness at end systole and diastole; LVIDS & LVIDD – LV internal diameter at end systole and diastole; LVPWS & LVPWD – LV posterior wall thickness at end systole and diastole. **Right panel:** Line graphs showing the effect of exercise on left ventricular structure. *Significantly different from ND. †Significantly different from the no-exercise.

Diabetes + Exercise: Exercise, regardless of intensity (both MIE and HIE), prevented the diabetes-induced onset of coronary and cardiac damage and dysfunction when initiated from 8 weeks of age (Early-CD; Figs. 1 and 2). If, however, exercise was not initiated until 16 weeks of age, after cardiac dysfunction had already become well-established (i.e., Late-CD), then only HIE and not MIE could improve coronary vessel and heart function (Figs. 1 and 2).

Correlation with miRNA-126: Alterations in the circulating levels of pro-angiogenic miR-126 that were associated with diabetes, age and/or both MIE and HIE exercise regimes (Figs. 3(a,b)) were significantly and positively correlated with changes in coronary arteriole density (Fig. 3(d)) and capillary density (Fig. 3(c)).

Preventing the decline in coronary perfusion and myocardial remodeling that exacerbates cardiac dysfunction in advanced stages of diabetes remains a challenge. Our findings provide the first experimental evidence for the critical importance of early exercise intervention in ameliorating diabetic heart disease. Our results also suggest that the beneficial effects of exercise are mediated through the normalization of cardiovascular-enriched miRNA-126, which becomes dysregulated in diabetes.

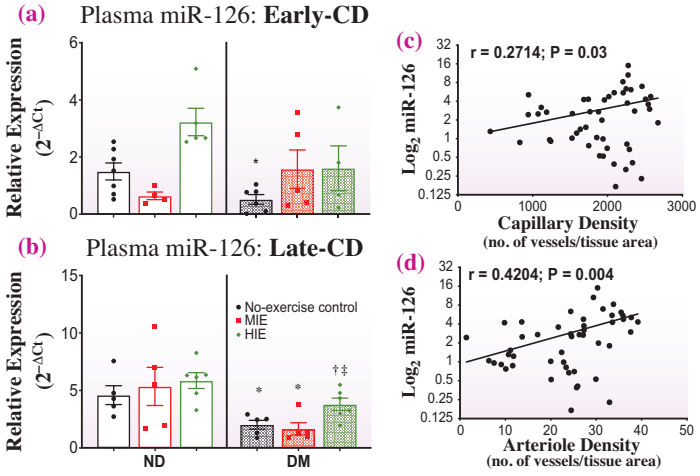

Fig. 3. Correlation between plasma miR-126 and coronary perfusion. Quantitative bar graphs showing plasma miR-126 following exercise in **(a)** Early-CD group and **(b)** Late-CD group. Right panel shows the correlation between plasma miR-126 expression and **(c)** total capillary density and **(d)** arteriole density.

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