Peripheral Oxygen Extraction and Exercise Limitation in Asymptomatic Patients with Diabetes Mellitus

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Abstract

Background: Patients with diabetes mellitus (DM) have a higher prevalence of subclinical heart failure and reduced exercise capacity. However, the extent to which peripheral oxygen extraction relates to exercise capacity has not yet been explored in asymptomatic patients with DM.

Methods: Asymptomatic patients with type-2 DM were prospectively enrolled and compared with age, sex and body mass index matched normoglycemic controls. Integrated cardiopulmonary exercise testing (CPX) including resting and stress echocardiography was performed using a ramp treadmill protocol. Exercise response was assessed by CPX using peak oxygen uptake (peak VO2) and ventilatory efficiency was measured using the slope of the relationship between minute ventilation and carbon dioxide production (VE/VCO2). Peripheral oxygen extraction was calculated as the ratio of VO2 to cardiac output. Cardiac function was evaluated by echocardiography using left ventricular longitudinal strain (LVLS), E/e' ratio, and relative wall thickness (RWT).

Results: Among 98 patients with DM analyzed (mean age of 59±11 years and 56% male sex), 26 patients (27%) presented reduced ppVO2 (<80%) and 18 patients (18%) presented abnormal VE/VCO2 slope (>34). There was no significant difference in peak heart rate or stroke volume between patients with DM and normoglycemic controls; peripheral oxygen extraction was lower in patients with DM compared to controls. Seventy-two patients (73%) presented with at least one cardiac abnormality and higher peak E/e' (beta=-0.24, p=0.004) was associated with lower peak VO2 along with age, male sex and BMI (R^2=0.53). A network correlation map revealed the connectivity of peak VO2 as a central feature and cluster analysis found LVLS, E/e', RWT and peak VO2 in different clusters.

Conclusions: Impaired peripheral oxygen extraction may contribute to reduced peak VO2 in asymptomatic patients with DM. Furthermore, cluster analysis suggests that CPX and echocardiography may be complementary for defining subclinical HF in patients with DM.

Introduction

Cardiopulmonary exercise testing (CPX) has gained a pivotal role to assess the degree of exertional impairment, help determine the mechanisms underlying symptoms of exercise intolerance and estimate risk in patients with HF [1]. Among CPX parameters, oxygen uptake at peak exercise (peak VO2) and the slope of the relationship between minute ventilation and carbon dioxide production (VE/VCO2 slope) have been validated as predictors of cardiovascular and overall mortality in patients with heart failure (HF) [2,3]. Recently, peripheral oxygen extraction was reported to be a major determinant of exercise capacity in patients with heart failure with preserved ejection fraction (HFpEF) [4]. Peripheral oxygen extraction is assessed as arteriovenous-mixed oxygen content difference, which widens in response to exercise [5]. A lower metabolic capacity of the muscle cell would change the demand for oxygen and thus lower oxygen extraction and increase the cardiac output relative to VO2 [6,7]. Hence, the disruption of peripheral oxygen extraction is emerging as an important pathologic limitation of oxidative metabolic function.

An abnormal exercise response is observed in patients with type-2 diabetes mellitus (DM) and is associated with cardiac mortality [8,9]. However, the association of peripheral oxygen extraction with reduced exercise capacity in asymptomatic patients with DM has not been well explored. Therefore, in this study, we first sought to compare CPX parameters as well as cardiac function between controls and asymptomatic patients with DM. We then sought to investigate the extent to which peripheral oxygen extraction contributes to impaired exercise response. Finally, we explored the relationship between CPX and cardiac function parameters and assessed their complementarity to detect subclinical HF in asymptomatic patients with DM.

Methods

Study population

We prospectively recruited patients with type-2 DM participating in clinical trials from Stanford Diabetes Research Center who agreed to participate in an early heart failure detection sub-study where they completed a comprehensive resting echocardiogram followed by combined exercise stress echocardiography cardiopulmonary test (ESE-CPX). Age, sex and body mass index (BMI) matched non-diabetic controls were also recruited as part of the National Institute of Health integrated Human Microbiome Project 2 (iHMP) [10]. Patients were excluded if a diagnosis of chronic kidney disease, neuropathy, diagnosed liver disease, or active malignancy were present. The absence of DM was determined by fasting plasma glucose (FPG) and HbA1c levels measured within 3 months of the exercise date (FPG < 126 mg/dL and HbA1C < 6.5%). The study was approved by the Stanford Institutional Review Board and all participants gave written informed consent.

Echocardiography

Echocardiography was performed using commercially available echocardiographic systems (EPIQ 7C; Philips Medical Imaging, Eindhoven, the Netherlands), according to the American Society of Echocardiography guideline recommendations [11]. Image analyses were performed on Xcelera workstation by trained cardiologists from the Biomarker and Imaging Core laboratory at Stanford Cardiovascular Institute. To evaluate the presence of subclinical HF, our study focused on three cardiac features; LV morphology, systolic function and diastolic function. LV morphology was assessed by relative wall thickness (RWT) and LV mass index. Relative wall thickness (RWT) was calculated from parasternal long axis view as (2*inferolateral wall thickness)/(LV internal dimension) and LV mass was obtained using linear method. Systolic function was assessed using LV longitudinal strain (LVLS) which was measured using Lagrangian strain by manual tracing from the apical views, computing the myocardial length in end-diastole (L_0) and end-systole (L_1) in the following formula: 100 x (L_1-L_0)/ L_0 as described before and presented in absolute value[12]. LV diastolic function was

assessed by the combination of pulsed-wave Doppler examination of mitral inflow and tissue Doppler imaging of mitral annulus. Transmitral pulsed-wave Doppler and tissue Doppler imaging were acquired from apical 4-chamber view to obtain early (E) and late (A) diastolic flow velocity as well as early diastolic (e') velocity of the mitral annulus at septal and lateral. E/e' ratio was obtained by the average of septal and lateral sites. Diastolic function was categorized according to the progression of diastolic dysfunction: normal ($0.75 < E/A < 1.5$ and $E/e' < 10$); mild defined as impaired relaxation without evidence of increased filling pressures ($E/A \leq 0.75$ and $E/e' < 10$); moderate defined as impaired relaxation associated with moderate elevation of filling pressures or pseudonormal filling ($0.75 < E/A < 1.5$ and $E/e' \geq 10$), and severe defined as advanced reduction in compliance or reversible or fixed restrictive filling ($E/A > 1.5$ and $E/e' \geq 10$) as previously described and validated [13,14]. E/e' ratio ≥ 10 as the threshold of diastolic stress was used because normal diastolic function allows adequate filling of the ventricles during rest and exercise without abnormal elevation of LV filling pressure [1516]. Diastolic function was assessed both at rest and stress as we recently demonstrated the importance of diastolic stress to improve the detection of subclinical HF in patients with DM [17].

In this study, subclinical HF was defined if at least one of cardiac features was abnormal. Abnormal morphology was present if patients present LV concentric remodeling (RWT>0.42 or LV hypertrophy defined as LV mass index (LVMI) >95g/m^2 for women and LVMI >115g/m^2 for men) [11]. Systolic dysfunction was defined as LVLS in absolute value < 16% [18]. Diastolic dysfunction is defined by the presence of elevation of filling pressure (E/e' ratio ≥ 10) [13,14].

CPX procedure

All subjects performed an EXE-CPX using a symptom-limited ramp protocol [19]. CPX tests were completed on a treadmill with an integrated metabolic cart (Quark CPET, CosMed USA Inc., Concord, CA, USA), using breath-by-breath data capture and analysis [19]. Peak VO2 was calculated as

the highest average VO2 over 30s during the last phase of exercise [20]. Minute ventilation (VE) and carbon dioxide output (VCO2) measured throughout the entire exercise bout (i.e. including every data point) were used to calculate the VE/VCO2 slope via least squares linear regression (y = mx + b, m = slope) as validated in previous studies [1,21]. The achievement of a respiratory exchange ratio (RER; VCO2/VO2) > 1.05 and rating of perceived exertion (6–20) of >16 were used to determine peak effort. The partial pressure of end-tidal CO2 (PETCO2) and the oxygen uptake efficiency slope (OUES) were calculated as previously described [22]. Peak age-predicted VO2 was obtained using the FRIEND equation [23] and percent peak age-predicted VO2 (ppVO2) was calculated as 100 x [measured peak VO2 / peak-predicted VO2]. An abnormal exercise response was defined as ppVO2<80% or VE/VCO2>34 [24] . Peak exercise capacity was assessed using peak VO2, VO2 ratio calculated as peak VO2 divided by standing VO2, VO2 reserve ratio calculated as the difference between peak and rest VO2 divided by standing VO2, and external work obtained from American College of Sports Medicine formula. Peak values vary depending on the individuals, thus the ratios to standing VO2 were used. Peripheral oxygen extraction was calculated as the ratio of VO2 to cardiac output [25], where cardiac output was obtained by echocardiography.

Statistical analysis

Categorical variables are presented as counts and percentages and continuous variables are expressed as mean and standard deviation whenever appropriate. Categorical variables were compared using Pearson's chi square test or Fisher's exact test, as appropriate. Normality of the continuous variables was confirmed with Shapiro-Wilk test. Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of the parameters to differentiate patients with DM from controls. Comparison between groups was performed using Student or Welch t-test or Mann-Whitney U test, as appropriate if two groups and Jonckhee-Terpstra test was used to evaluate the trend among three groups. Multivariable linear regression analysis to detect correlates of peak CO2, VE/VCO2 slope, cardiac output at peak and peripheral oxygen extraction at peak using covariates as age, sex, BMI, the

value of HbA1c, heart rate, systolic blood pressure, RWT, LVLS and E/e'. P values < 0.05 were considered statistically significant and analyses were performed using SPSS version 24 (SPSS Inc, Chicago, Illinois). Furthermore, pairwise Spearman's rank correlations were calculated using the R package 'Hmisc' (v4.4-0) and weighted, undirected networks were plotted with 'igraph' (v1.2.5). Correlations with Bonferroni adjusted P values below 0.05 were included and displayed via the fruchterman-reingold method. Edges were colored based on the direction of correlation and their thickness represented absolute value of correlation coefficient between the two nodes. Dendrogram was plotted using the R package 'amap' (v0.8-18) using Spearman correlation distance and complete correlation method.

Results

We prospectively enrolled 102 patients with DM and 31 controls without DM who performed EXE-CPX. Among them, one patient was excluded due to exercise induced wall motion abnormalities, one was excluded due to exercise-induced sustained ventricular tachycardia without hemodynamic compromise which was resolved during early recovery, and two were excluded due to technical difficulty to acquire images at peak. After these exclusions, a total of 98 patients with DM and 31 controls without DM were included in the analysis.

Patients with DM were matched based on age, sex and BMI. The prevalence of hypertension was higher and there were more Asians in patients with DM compared to controls (both p<0.001, Table1).

CPX parameters including Exercise capacity and Peripheral oxygen extraction

Resting VO2 was higher in patients with DM than controls (Table 2). At peak, patients with DM had lower peak VO2 and a hypertensive response during exercise was more common in patients with DM. Twenty-six patients (27%) presented reduced ppVO2 (<80%) and 18 patients (18%) presented

abnormal VE/VCO2 slope (>34), while no controls presented with reduced ppVO2 and six controls (19%) presented abnormal VE/VCO2 slope. Thirty-four (35%) patients with DM presented either abnormal peak VO2 or VE/VCO2 slope and five patients (5%) presented both abnormal peak VO2 and VE/VCO2.

Figure 1A shows the distribution of peak exercise capacity in controls and patients with DM assessed by measures of peak oxygen uptake as well as external work. We scaled peak VO2 to body weight as well as to resting VO2 (standing) to derive a metric of VO2 independent of body composition (as a simple peak to resting ratio or as a reserve ratio of (peak-resting)/resting). External work was estimated using treadmill speed and grade according to the ACSM equations. A value of 3.5 was used to divide peak VO2 (when scaled to kg) to derive an oxygen uptake based METs value. Patients with DM had lower peak VO2 (ml/kg/min), measured METs, VO2 ratio, VO2 reserve ratio, as well as estimated METs (Figure 1A). Cardiac efficiency, expressed as the external work divided by the peak oxygen uptake, or VE/VCO2 slope was comparable between controls and patients with DM ($p=0.07$ for efficiency and $p=0.56$ for VE/VCO2 slope). Figure 1B presents the odds ratio for DM by increasing oxygen uptake measures, external work, efficiency as well as peripheral oxygen extraction. There were no significant differences in peak heart rate, stroke volume, or cardiac output between patients with DM and controls, while peripheral oxygen extraction was lower in patients with DM compared to controls (Table 2) (Figure 2A). Peripheral oxygen extraction had a moderate correlation with peak VO2 and a weak correlation with VO2 ratio (Figure 2B).

Echocardiographic parameters

As shown in Table 1, patients with DM had higher relative wall thickness, impaired LVLS as well as higher E/e' ratio. LA volume index did not differ between patients with DM and controls; however, LA reservoir strain was significantly reduced in patients with DM. Of all patients, 24 patients (24%) presented with abnormal morphology, 24 patients (24%) presented with impaired LVLS and 60

patients (61%) presented with diastolic dysfunction including 17 (17%) patients revealed after exercise. Subclinical HF based on one criterion was present in 39 (40%), by two criteria in 30 (31%) and by three criteria in 3 (3%), and 26 patients (27%) did not present any abnormalities (Figure 3A).

Subclinical HF profile from EXE-CPX

Table 3 shows the echocardiographic as well as CPX parameters according to the number of cardiac abnormalities. Increasing the number of cardiac abnormalities was associated with older age, while there was no significant difference in sex or prevalence of hypertension. Among resting echocardiographic or CPX parameters, RWT, LVLS or E/e' was worse with increasing number of cardiac abnormalities. Exercise capacity, including peak VO2, VO2 ratio and VO2 reserve ratio and peak external work, were also worse with advanced cardiac abnormalities. When analyzed according to the individual type of abnormality (morphology, diastolic dysfunction or strain), the proportion of patients with reduced ppVO2 was comparable between groups (Figure 3B).

As shown in the network in Figure 3C, the correlation network highlights the peak VO2 as a central exercise metric connected with the heart rate response, cardiac index, biometrics as well as diastolic stress. As highlighted by this network figure as well as the cluster dendrogram (Figure 3D), the resting echocardiographic features and the peak VO2 are part of different clusters highlighting their complementarity in defining subclinical heart failure.

Multiple linear regression models integrating cardiopulmonary and echocardiographic profiles

As shown in Table 4, multivariable analysis found that among echocardiographic parameters, peak E/e' (beta=-0.24, p=0.004) was an independent associate of peak VO2 (R^2=0.53) along with age (beta=-0.25, p=0.002), male sex (beta=0.24, p=0.004) and BMI (beta=-0.47, p<0.001). In contrast, no resting echocardiographic parameters were associated with VE/VCO2 slope. Peak E/e' (beta=-0.21, p=0.04) was also an independent associate with peripheral oxygen extraction along with female sex (beta=0.30, p=0.005) and BMI (beta=0.33, p=0.002) (R2=0.22). This is also highlighted in the supplemental table stratifying the patients according to the peripheral oxygen extraction tertiles.

Peak VO2 adjusted physiological differences

Since directly measured peak VO2 allows us to evaluate cardiac morphology and blood pressure response using physiological based scaling or adjustment, remodeling parameters were indexed by peak VO2. While there was no significant difference between controls and patients with DM in LVM indexed by BSA, indexed by peak VO2 revealed LVM and LAV differences in patients with DM compared to controls (Table 5). Furthermore, LVM indexed by peak VO2 ($r=0.37$, $p<0.001$) was correlated with RWT more than LVM indexed by BSA ($r=0.26$, $p=0.009$). Blood pressure response was also significantly different even after adjustment for peak VO2 (Table 5).

Discussion

The main findings of our study were that asymptomatic patients with DM have impaired peak oxygen uptake as well as decreased peripheral oxygen extraction. Peak VO2 was impaired when scaled by body weight as well as when normalized to resting standing oxygen uptake. Both our network and cluster analysis also highlight the complementary relationship between peak VO2 and the echocardiographic features often used to define subclinical diabetic cardiomyopathy, i.e. abnormalities in LV morphology, diastolic dysfunction and LV strain.

CPX has gained attention as an important tool to assess the degree of exertional impairment and help estimate risk in the general population as well as in patients with HF [2,3,26]. Among CPX parameters, peak VO2 and VE/VCO2 slope have been recognized as well established predictors of cardiovascular and overall mortality in patients with heart failure (HF) [2,3]. Previous studies have investigated the relationship between peak VO2 and outcomes in patients with DM. For example, Seyoum et al. investigated 468 patients with type-2 DM and found that peak VO2 predicted future cardiovascular events [27]. One of the issues in assessing exercise capacity using peak VO2 scaled to total body weight in patients with DM is that obesity may disportionately decrease VO2 compared to lean body mass based scaling. Furthermore, the percent predicted VO2 is often overcorrected in very

obese patients. This is well highligted in our study by showing that ppVO2 overestimated oxygen uptake in DM compared to the ratio of peak VO2 to resting VO2. This suggests that obtaining a stable resting VO2 value may be of physiological and clinical value in this patient population.

One of the important findings of our study is that reduced peripheral oxygen extraction contributed to impaired exercise capacity in asymptomatic patients with DM, where peripheral oxygen extraction was calculated as VO2 divided by cardiac output, according to the Fick equation [25]. This was also highlighted more recently in patients with symptomatic heart failure. In 104 symptomatic patients with HF, Dhakal et al. demonstrated that peripheral oxygen extraction (arterio-venous oxygen difference) was a major determinant of exercise capacity in HF with preserved EF (HFpEF) [4], where they measured peripheral oxygen extraction directly. Previous studies have demonstrated that stroke volume and cardiac output in patients with HFpEF are impaired during exercise [28,29]. In contrast, cardiac output at peak exercise was comparable in our two groups even though systolic function assessed by LVLS was impaired at rest. These results suggest that reduced exercise capacity in early HF with DM might be caused by impaired peripheral oxygen extraction that occurred before systolic dysfunction. Altered metabolic status, such as hyperglycemia or insulin resistance, may perturb the balance between vasodilation and vasoconstriction signaling, resulting in abnormal vasomotor control [30]. The impaired microvascular function may hamper peripheral muscle tissue oxygen extraction.

Our study identified the four clusters which capture key physiological domains to define early heart failure in patients with DM. Peak VO2 and each of three cardiac features; RWT or hypertrophy, LVLS, and E/e' were classified into the different clusters, suggesting their complementary value for classification purposes. In fact, each of the parameters has been demonstrated to be of prognostic value in patients with HF [31,32 33 34,35]

Gas exchange analysis can also allow a better physiological assessment of cardiac remodeling as well as blood pressure response. Historically, cardiac scaling was considered using body surface area (BSA) based on the fact that BSA estimates the energetic requirement of the heart [36]. Peak VO2 measurements during exercise can provide a more direct estimation of energetics. For example, LVM in the athletic heart is demonstrated to relate to peak VO2 [37,38]. On the other hand, asymptomatic patients with DM presented comparable LVM and lower peak VO2 compared with controls in our study. This disproportionate ratio of LVM to peak VO2 would suggest pathological change. In fact, LVM indexed by peak VO2 is more strongly related to concentric remodeling than LVM indexed by BSA even without obvious hypertrophy. Similarly, even when adjusted for peak VO2, the blood pressure response in patients with DM was higher than in controls, suggesting impairment is vascular function or its regulation [39].

Our study has several limitations. First, the population was relatively small because patients were selected prospectively for participation in exercise programs. Thus, the sample may not be representative of a general population and they may have been more fit and motivated compared to general patients with DM. Second, the 2016 ASE/ESCVI recommendations use E/e', e' velocity, TR velocity and LAVI to evaluate the presence of diastolic dysfunction; however, optimal TR velocity was available in a few cases in our population. Also, to simplify the criteria to define diastolic dysfunction for screening, we used the combination of E/A and E/e' ratio for diagnosis. Third, with the revised and lower diagnostic criteria for hypertension and based on the fact that hypertension often co-exists with DM, we used a broader definition of DCM including hypertension in the absence of ischemic heart disease in this study. This explains why we preferentially used the term subclinical HF rather than DCM in this paper.

Conclusions

Asymptomatic patients with DM exhibited lower peak VO2 which may be in part explained by impaired peripheral oxygen extraction. Furthermore, exercise capacity and three echocardiographic features of LV morphology, diastolic function and ventricular strain complement one another in defining subclinical heart failure.

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Figure Legend

Figure 1.

(A) Distribution of peak exercise capacity assessed by different parameters in violin plots (B) A forest plot showing the hazard ratio (HR) and 95% confidence intervals (CI) in the univariable analyses with the presence of DM as the dependent variable. Squares represent the HR and the horizontal bars extend from the lower limit to the upper limit of the 95%CI. The values of HR and 95%CI are standardized by standard deviation of each parameter.

Figure 2.

(A) Distribution of cardiac output and peripheral oxygen extraction of controls and patients with DM (B) Scatter plots of peripheral oxygen extraction and peak VO2 or VO2 ratio

Figure 3.

(A) Venn diagram of early cardiac dysfunction (B) ratio of reduced VO2 in patients with each cardiac dysfunction feature (C) Pairwise spearman correlation network. Edges were colored based on the direction of correlation between parameters (red; positive correlation, blue; negative correlation) and

the thickness of the edges represents absolute value of correlation coefficient between two nodes. (D) Cluster dendrogram using Spearman correlation distance classified parameters into 4 clusters.

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