β-Adrenergic Responsiveness in the Type 2 Diabetic Heart: Effects on Cardiac Reserve

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ABSTRACT

WILSON, G. A., L. C. WILSON, R. R. LAMBERTS, K. MAJEED, S. LAL, G. T. WILKINS, and J. C. BALDI. β-Adrenergic Responsiveness in the Type 2 Diabetic Heart: Effects on Cardiac Reserve. *Med. Sci. Sports Exerc.*, Vol. 49, No. 5, pp. 907–914, 2017. Type 2 diabetes (T2D) is associated with reduced cardiac reserve and aerobic capacity. Altered myocardial autonomic nervous regulation has been demonstrated in humans with diabetes (indirectly) and animal models (directly). **Purpose**: This study aimed to determine the chronotopic and inotropic response of the type 2 diabetic heart to β-adrenergic stimulation. **Methods**: Eight people with uncomplicated T2D and seven matched controls performed a dual-energy x-ray absorptiometry scan and $\dot{V}O_{2peak}$ test. Plasma catecholamines were determined at rest and during peak exercise. On a second visit, HR and left ventricular contractility were assessed using echocardiography during supine rest, parasympathetic blockade (atropine), and during incremental β-adrenergic stimulation (dobutamine). **Results**: $\dot{V}O_{2peak}$ and HR reserve were lower in T2D ($P < 0.05$) as expected. Both groups increased norepinephrine comparably ($P = 0.23$) during peak exercise; however, epinephrine increased less in the T2D group ($P < 0.05$). The dobutamine dose required to achieve 85% of age-predicted maximal HR was 36% higher in CON ($P < 0.05$). Resting HR was higher ($P < 0.01$) and stroke volume indexed to fat free mass was smaller ($P < 0.05$) in T2D. During dobutamine infusion the response (% change) in HR, end-diastolic $volume_{FFM}$, stroke volume, ejection fraction, and cardiac output were not different between the groups. However, HR was higher ($P < 0.01$) and end-diastolic volume indexed to fat free mass ($P < 0.01$), stroke $volume_{FFM}$ ($P < 0.01$), ejection fraction ($P < 0.05$), and stroke work ($P < 0.01$) were lower in T2D. **Conclusions**: Although the type 2 diabetic heart worked at smaller volumes, the HR and contractile response to β-adrenergic stimulation were unaffected by diabetes. The reduced cardiac reserve observed in uncomplicated T2D was not explained by impaired myocardial sympathetic responsiveness but may reflect changes in the loading conditions or function of the diabetic left ventricle.
Key Words: LEFT VENTRICLE, CARDIAC OUTPUT, NERVOUS REGULATION

BASIC SCIENCES

A frequent observation in people with uncomplicated type 2 diabetes (T2D) is reduced cardiac reserve and a 10%–20% reduction in maximal oxygen consumption ($\dot{V}O_{2max}$) (2–4,23,26). Peak exercise leads to a two- to sixfold increase in resting cardiac output (27), facilitated by parasympathetic withdrawal and increased sympathetic nervous activity. These "shifts" in autonomic balance increase HR (chronotropy), contractility (inotropy), and relaxation rate (lusitropy), allowing the left ventricle to increase cardiac output. People with T2D have elevated resting HR (15,30) and sometimes contractility (23) but an attenuated chronotropic and inotropic response to exercise (4,23), which may result from altered regulation of autonomic signals to the heart.

HR variability (HRV) is reduced (18,28) and resting HR is increased (15,30) in people with T2D, consistent with reduced resting parasympathetic input (14,39). However, the state of sympathetic input to the diabetic heart is less clear. Efferent cardiac sympathetic nerve activity is 45% greater in the hearts of obese diabetic Zucker rats, without any indication of sympathetic nerve atrophy or hypertrophy (32). Chronic elevations in cardiac sympathetic nervous activity result in a downregulation of β_1-adrenergic receptor expression (5), which has been observed in animals with diabetes (8,32). If these changes are also evident in humans, they might explain the attenuated cardiac response to exercise in people with diabetes. However, radio nucleotide (I^{123}-MIBG) studies suggest that humans with diabetes and autonomic dysfunction are characterized by *reduced* sympathetic innervation of the heart (29), and two studies examining the β_1-adrenergic receptor expression of diabetic human myocardium are equivocal (9). Therefore, although reduced cardiac reserve is well established in T2D (4,23), it is unclear what role, if any, cardiac autonomic dysregulation plays in this response.

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The primary aim of the current study was to determine whether cardiac β-adrenergic responsiveness is reduced in people with uncomplicated T2D. To achieve this, the chronotropic and inotropic responses of the heart to incremental adrenergic (dobutamine) stimulation were investigated in healthy people with and without T2D. The parasympathetic nervous system (PSNS) can attenuate the cardiac sympathetic response (16); therefore, atropine (parasympathetic antagonist) was administered before incremental adrenergic agonism. A secondary aim of these experiments was to determine, using plasma catecholamines, whether an attenuated sympathetic response contributes to the blunted exercise capacity in T2D. We hypothesized that people with T2D 1) would require a higher dose of dobutamine to achieve 85% of their age-predicted maximum HR and 2) chronotropic (Δ HR) and inotropic (Δ ejection fraction, end-systolic volume, stroke work, and peak systolic annular velocity [S']) responses to controlled dobutamine infusion would be attenuated in people with T2D. Finally, in relation to our secondary aim, we predicted that plasma catecholamines would increase less during peak exercise in people with uncomplicated T2D.

RESEARCH DESIGN AND METHODS

Participants. Eight people with T2D and seven people without T2D (CON) who were matched for age, sex, and body mass index between the ages of 37 and 55 yr participated in this study. Participants had no history of cardiovascular disease (CVD) or respiratory disease. Participants were instructed to avoid alcohol, caffeine, and heavy exercise before testing. Informed consent was obtained from all participants before participating in the study. This study was approved by the Lower South ethics committee LRS/12/01/006 and complied with the Declaration of Helsinki.

HRV and cardiac autonomic screening. A series of orthostatic challenges were used to screen for cardiac autonomic neuropathy as described previously (35). On the first day of testing, participants were instructed to lie supine and were instrumented with the standard lead-II ECG, respiratory belt, and finger-photoplethysmography (finometer MIDI; verified against manual sphygmomanometer) to measure HR, breathing frequency, and beat-to-beat blood pressure (BP), respectively. These measures were continuously sampled using an analog-to-digital converter at 1 kHz (Powerlab/3508; ADInstruments, Dunedin, NZ) and stored for offline analysis.

Baseline and controlled breathing data (metronome guided at 15 breaths per minute) were collected for a 5-min period, from which HRV analysis was performed for the final 3 min using the fast Fourier transform method (HRV module v 1.4.2, ADInstruments) to quantify total power (0–0.4 Hz) in square millisecond. Autonomic function was tested by measuring HR and BP responses to a battery of tests (clinical autonomic reflex tests) described in detail elsewhere (31). These tests included deep breathing (6 breaths per minute for 1 min), Valsalva maneuver, and postural change (supine to stand). From these tests, the largest difference in HR during deep breathing, the R-R interval ratio (shortest R-R interval/longest R-R interval) within a minute after the Valsalva, the 30:15 ratio (longest R-R interval 20–40th beats/the shortest R-R interval 5–25th beats after standing), and the systolic BP during standing were quantified and compared with age-adjusted normative values to reflect an abnormal response (31,35).

Peak exercise test and catecholamines. Immediately after screening procedures, an indwelling catheter was inserted into an arm vein (usually antecubital) of the participants in a supine position. The participants then rested quietly for at least 20 min before a resting blood sample was collected, and a second sample was taken during peak exercise (within 60 s of exercise termination). The samples were collected, and plasma was extracted and stored at −80°C for later analysis of plasma catecholamines (10).

Peak oxygen consumption ($\dot{V}O_{2peak}$) was determined using a metabolic cart (COSMED Quark CPET, Italy) during an incremental protocol to exhaustion on an upright cycle ergometer. Initial workload was set at 25 or 50 W and was increased by 25- or 50-W increments (depending on the perceived level of fitness of the participant) with each 2-min stage until the termination of the test. Workloads were adjusted using the Borg scale of perceived exertion to assure test duration did not exceed 12 min. Breath-by-breath data were sampled at 10-s intervals and analyzed with O_2 and CO_2 analyzers, which were calibrated with room air and standardized gas containing 15% O_2 and 6% CO_2, and the gas turbine was calibrated by using a 3-L syringe. Brachial BP (by auscultation) and HR (by 12-lead ECG) were recorded at every increment and before and after exercise. The exercise test was terminated when the participant was unable to continue because of volitional exhaustion or discomfort. The highest three values were averaged to determine $\dot{V}O_{2peak}$, and the test was accepted if the participant reached an RER value (i.e., $\dot{V}CO_2/\dot{V}O_2$) ≥1.1. Peak HR was not considered a reliable index of peak exercise effort, as it is commonly reduced in T2D cohorts (4,15,30). The 12-lead ECG and BP responses during the exercise test were reviewed by a cardiologist for any evidence of CVD before continuation in the study.

Dobutamine stress protocol. On a second test day, no more than 7 d after the $\dot{V}O_{2peak}$ test, the participants underwent a dobutamine stress echocardiogram. Participants were fitted with an automatic BP cuff, a three-lead ECG, and an independent 12-lead ECG, which was regularly reviewed by a senior cardiology registrar. An indwelling catheter was inserted into the antecubital vein for a blood glucose sample and administration of atropine and dobutamine. Tests were rescheduled if T2D participants arrived at the laboratory with high (>15 $mmol \cdot L^{-1}$) or low (<3 $mmol \cdot L^{-1}$) blood glucose. Participants first underwent baseline echocardiographic examination (see next section for details), BP measurement, and 12-lead ECG. This was followed by a bolus infusion of atropine (muscarinic receptor antagonist = 0.04 $mg \cdot kg^{-1}$) to completely block the effects of the

parasympathetic system (13), and the measurements taken at baseline were repeated. After the PSNS blockade, an incremental stepped infusion of dobutamine was performed beginning with 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and continuing with steps of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ using a continuous infusion pump until 85% of age-predicted maximum HR (220 − age) was achieved. Echocardiographic images, ECG, and BP were obtained during each incremental dobutamine stage up to 85% of age-predicted HR after at least a 3-min stabilization period of HR (no more than five beats of variation). The HR response to each step (including baseline and PSNS blockade) was taken as the average HR for 3 min during which echocardiogram assessment was occurring.

Echocardiography. Echocardiographic images were obtained using a Vivid E9 (GE Medical Systems, Milwaukee, WI) ultrasound system by an unblinded clinical sonographer. All measurements were taken with the participant in the standard left lateral decubitus position. Left ventricular volumes at end diastole and end systole were obtained in the apical four- and two-chamber views. Diastolic filling velocities were obtained in the apical four-chamber view using pulsed wave Doppler with the sample volume placed between the mitral valve leaflets (20). Tissue velocities were obtained in the apical four-chamber view using tissue Doppler imaging (21) with the sample volume placed on the mitral valve annulus at the junction with the interventricular septum.

HR and contractile responses (end-systolic volume, ejection fraction, peak systolic mitral annular velocity, and stroke work) were measured at baseline and during each drug dosage/infusion rate. Three consecutive beats were stored digitally and analyzed with customized dedicated research software (EchoPAC Version 112.0.0, Advanced Analysis Technologies, GE Medical Systems). The same clinical sonographer performed all analyses. Left ventricular end-diastolic and end-systolic volumes were visually traced with papillary muscles excluded and calculated using the modified Simpson's biplane method in accordance with ASE guidelines (17) from which ejection fraction and stroke volume were derived. Volumes were indexed to participants' fat-free mass obtained from dual-energy x-ray absorptiometry (DEXA scan) because this has been identified as an appropriate scaling method for comparing people with different aerobic capacities (37). At HR > 120 bpm, images were taken at end expiration to improve the visual resolution. Stroke work was calculated as stroke volume × mean arterial BP × 0.0136 (12) to compare the work of the left ventricle between T2D and CON. Systemic vascular resistance was calculated as mean arterial BP/cardiac output.

Statistical analysis. Baseline and exercising dependent variables were compared between T2D and CON using an independent Student *t*-test. HRV data were not normally distributed; therefore, these data were log transformed for analysis. The HRV metrics (baseline and paced breathing conditions) and the response to atropine administration were examined using a two-way ANOVA. The chronotropic and inotropic responses to β-adrenergic stimulation were compared using linear mixed models where the 0.04-$mg \cdot kg^{-1}$ bolus of atropine was treated as the baseline. The significance for all two-tailed tests was defined as $P < 0.05$. All data are expressed as mean ± SD. Data were analyzed using SPSS statistical software (SPSS version 22.0.0; IDM Corporation, Armonk, NY).

RESULTS

Baseline comparisons. As shown in Table 1, the CON and the T2D were matched for sex ($P = 0.46$), age ($P = 0.72$), body mass index ($P = 0.12$), fat free mass ($P = 0.43$), and self-reported exercise ($P = 0.70$). One participant with diabetes smoked (~23 pack-years) and four were ex-smokers (two T2D and two CON). The mean duration of diabetes among T2D was 7 yr (range = 2–15 yr), with a mean HbA1c of 65 $mmol \cdot mol^{-1}$. Evidence for mild microalbuminuria was present in three T2D as indicated by their albumin/creatinine ratio (ranging from 7.2 to 8.7). No participants in either group were taking cardiac mediations. One participant in the T2D group was taking an ACE inhibitor (renal prophylaxis), and one T2D and one CON took statin. Diabetic management included metformin (6), glicazide (2), and insulin (2).

HRV and cardiac autonomic screening. One T2D participant had two abnormal clinical autonomic reflex test results (abnormal HR response to deep breathing and abnormal Valsalva ratio), and another T2D participant had one abnormal clinical autonomic reflex test result (abnormal HR response to deep breathing). None of the remaining T2D or CON participants had any abnormal responses to the clinical autonomic reflex tests. HRV total power was lower in T2D ($P = 0.04$), but no significant condition (breathing) effect ($P = 0.06$) or interaction effect (breathing condition × group) ($P = 0.40$) existed between T2D and CON.

Peak exercise test and catecholamines. Self-reported exercise was similar between groups (Table 1); however, $\dot{V}O_{2peak}$ was lower in T2D ($P < 0.05$). The combination of

TABLE 1. Participant baseline characteristics.

Variable	T2D ($n = 8$)	CON ($n = 7$)	*P*
Age (yr)	48 ± 6	47 ± 5	0.72
BMI ($kg \cdot m^{-2}$)	34 ± 8	29 ± 3	0.12
Fat-free mass (kg)	66 ± 13	61 ± 11	0.43
% female	25%	43%	0.46†
Diabetes duration (yr)	7 ± 4	N/A	N/A
Self-reported exercise ($h \cdot wk^{-1}$)	3.3 ± 2.1	3.0 ± 1.8	0.70
Blood biochemistry			
HbA_{1c} ($mmol \cdot mol^{-1}$)	65 ± 18	N/A	N/A
Renal function			
Creatinine ($\mu mol \cdot L^{-1}$)	76 ± 11	N/A	N/A
Albumin/creatinine ratio	≤1 (5):>1 (3)	N/A	N/A
Systolic BP (mm Hg)	128 ± 10	122 ± 12	0.29
Diastolic BP (mm Hg)	75 ± 10	77 ± 9	0.80
Mean arterial BP (mm Hg)	93 ± 8	92 ± 10	0.81
Resting HR (bpm)	71 ± 12	59 ± 8	0.05
Left ventricular mass (g)	172 ± 38	177 ± 50	0.85
Stroke volume ($mL \cdot kg_{FFM}^{-1}$)	0.93 ± 0.23	1.25 ± 0.31	0.04
Ejection fraction (%)	61 ± 3	65 ± 6	0.07
Cardiac output ($L \cdot min^{-1} \cdot kg_{FFM}^{-1}$)	0.07 ± 0.02	0.07 ± 0.01	0.45
Vascular systemic resistance ($mm\ Hg \cdot min^{-1} \cdot mL^{-1}$)	23.8 ± 9.2	21.8 ± 5.4	0.62

Values are presented as mean ± SD. BMI, body mass index.
†Chi-squared test used for significance testing.

TABLE 2. Exercise variables.

Variable	T2D	CON	P
$\dot{V}O_{2peak}$ (mL·min^{-1}·kg^{-1})	24 ± 4	34 ± 12	0.04
Peak exercising HR (bpm)	166 ± 8	172 ± 5	0.10
HR reserve (bpm)	95 ± 12	112 ± 11	0.01
ΔNorepinephrine (rest to peak exercise) (pmol·L^{-1})	14,246 ± 8945	19,609 ± 5528	0.23
ΔEpinephrine (rest to peak exercise) (pmol·L^{-1})	736 ± 607	2041 ± 1437	<0.05

Values are presented as mean ± SD.

increased resting HR ($P < 0.05$) and insignificantly lower peak HR ($P = 0.10$) contributed to the significant reduction in HR reserve in T2D (92 ± 12 bpm in T2D vs 111 ± 10 bpm in CONT, $P < 0.05$). At rest, norepinephrine was lower in T2D (1366 ± 570 pmol·L^{-1}) compared with controls (2339 ± 876 pmol·L^{-1}; $P < 0.05$), and epinephrine was not significantly different (137 ± 83 pmol·L^{-1} vs 108 ± 20 pmol·L^{-1} in T2D vs CON, respectively, $P = 0.38$). The change in plasma norepinephrine did not differ between the groups; however, the increase in plasma epinephrine from rest to exercise was significantly reduced in T2D. These data are summarized in Table 2.

Baseline and parasympathetic blockade echocardiogram. There were no significant differences in left ventricular mass (Table 1). HR was higher in T2D at rest ($P = 0.05$) and after PSNS blockade (atropine) ($P < 0.05$); however, the increase in HR during PSNS blockade was the same in both groups ($P > 0.05$) (Fig. 1A, *shaded region*). Mean arterial BP was not different at rest and did not change during PSNS blockade in either group ($P < 0.05$) (Fig. 1B, *shaded region*). End-diastolic volume$_{FFM}$ was not different at rest but was lower in T2D after PSNS blockade (Fig. 2A, *shaded region*). Stroke volume$_{FFM}$ was lower in T2D at rest (Table 1) and after PSNS blockade ($P < 0.05$) (see Supplementary Table, Supplemental Digital Content 1, Stroke volume, cardiac output, S', and systemic vascular resistance responses after parasympathetic blockade (atropine) and sympathetic stimulation (dobutamine), http://links.lww.com/MSS/A835); however, the percent change in stroke volume during PSNS blockade was not different between groups. There was no group difference in end-systolic volume$_{FFM}$ at rest ($P = 0.75$) or after PSNS blockade ($P = 0.65$), and end-systolic volume$_{FFM}$ decreased during PSNS blockade in both groups ($P < 0.05$) (Fig. 2A, shaded region). Ejection fraction was not different at rest ($P = 0.07$), and although ejection fraction did not change significantly in either group ($P = 0.20$), it was lower in T2D after PSNS blockade ($P < 0.05$) (Fig. 2B, *shaded region*). S' was not different at rest nor during PSNS blockade, and there were no differences in resting measures of diastolic filling (E/A) or diastolic and systolic function (E', A', and S') between groups (Table 3).

Dobutamine infusion. After the dobutamine infusion, all but one participant achieved 85% of their age-predicted HR. One CON participant became unwell and requested to stop the protocol achieving only 79% of their target rate at 30 μg·kg^{-1}·min^{-1}. This participant was excluded from the peak dose and echocardiographic measures. The peak dose required to reach 85% of age-predicted HR was 36% higher in CON (11 ± 4 μg·kg^{-1}·min^{-1} in T2D vs 17 ± 5 μg·kg^{-1}·min^{-1} in CON, $P < 0.05$). HR was higher in T2D during the dobutamine infusion ($P < 0.01$); however, the change in HR (interaction) was not different between the groups (Fig. 1A). There was no group difference in mean arterial BP ($P = 0.39$) or systemic vascular resistance ($P = 0.12$), and neither mean arterial BP ($P = 0.12$) (Fig. 1B) or systemic vascular resistance ($P = 0.15$) (see Supplementary Table, Supplemental Digital Content 1, Stroke volume, cardiac output, S', and systemic vascular resistance responses after parasympathetic blockade (atropine) and sympathetic stimulation (dobutamine), http://links.lww.com/MSS/A835) changed during the infusion.

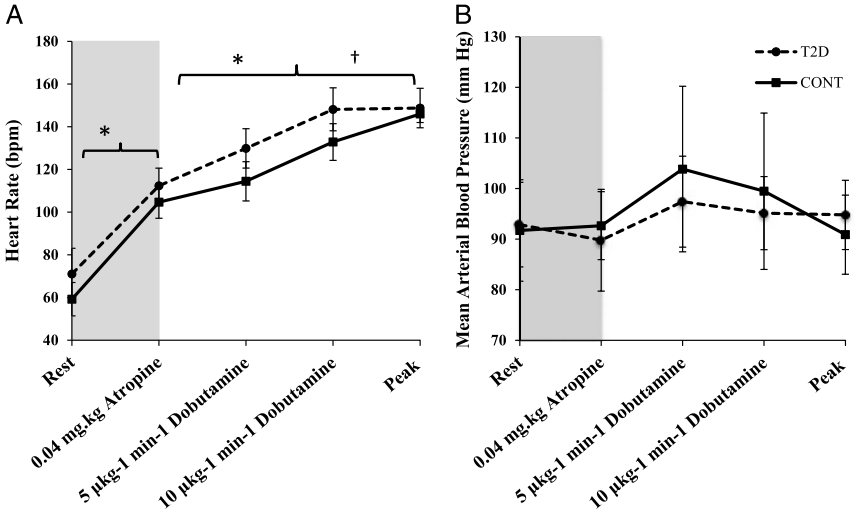

FIGURE 1—HR (A) and mean arterial BP (B) responses from rest to parasympathetic blockade (gray shade) and during incremental dobutamine infusion (no shade). Dotted line = T2D and solid line = CONT. *$P < 0.05$ significant difference between T2D and CONT. †$P < 0.05$ significant difference across conditions. Values are presented as mean ± SD.

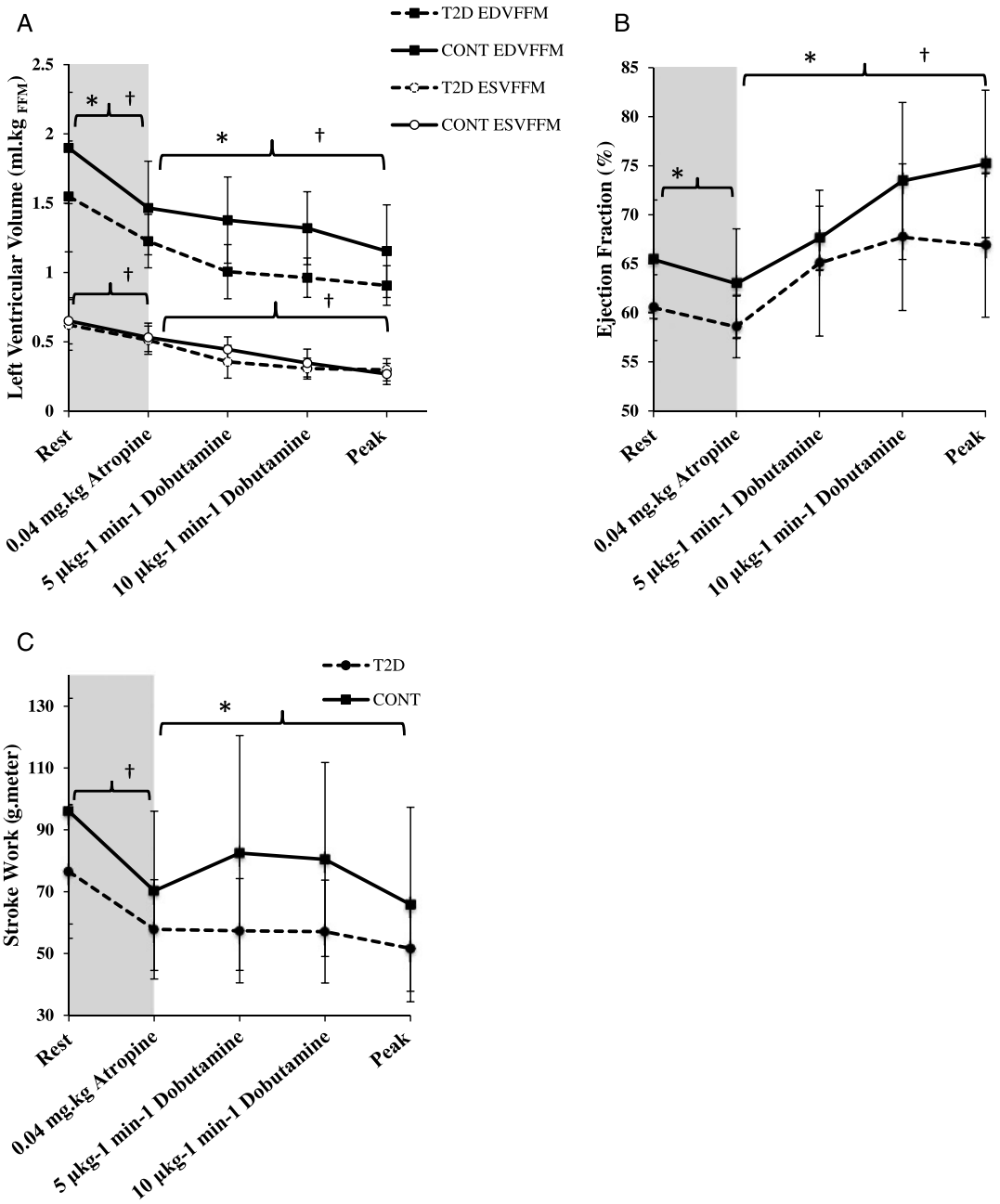

FIGURE 2—Left ventricular volumetric (A), ejection fraction (B), and stroke work (C) responses from rest to parasympathetic blockade (*gray shade*) and during incremental dobutamine infusion (*no shade*). *Open circles* represent ESV; *solid squares* represent EDV. *Dotted line* = T2D and *solid line* = CONTs. **P* < 0.05 difference between T2D and CONT. †*P* < 0.05 difference across conditions. Values are presented as mean ± SD.

End-diastolic volume$_{FFM}$ remained lower in T2D ($P < 0.01$) during incremental dobutamine infusion, and the decrease in end-diastolic volume$_{FFM}$ was the same in both groups ($P = 0.85$) (Fig. 2A). End-systolic volume$_{FFM}$ was not different between groups, and the change in end-systolic volume$_{FFM}$ during dobutamine infusion was not different between groups ($P = 0.23$) (Fig. 2A). Ejection fraction increased during the infusion ($P < 0.05$, Fig. 2B) in both groups but remained lower in T2D ($P < 0.05$). Stroke volume ($P < 0.01$), stroke work ($P = 0.01$) (Fig. 2C), and cardiac index$_{FFM}$ ($P < 0.01$) were lower in T2D vs CON but were unaffected by dobutamine infusion ($P = 0.89$, $P = 0.67$, and $P = 0.07$, respectively) (see Supplementary Table, Supplemental Digital Content 1, Stroke volume, cardiac output, S', and systemic vascular resistance responses after parasympathetic blockade (atropine) and sympathetic stimulation (dobutamine), http://links.lww.com/MSS/A835). S' was not different at rest and increased throughout the dobutamine infusion in both groups ($P < 0.05$) [see Table, Supplemental Digital Content 1, Stroke volume, cardiac output, S', and systemic vascular resistance responses after

TABLE 3. Baseline measurements of diastolic function and septal tissue velocities.

Variable	T2D	CONT	P
E ($m \cdot s^{-1}$)	0.73 ± 0.20	0.78 ± 0.19	0.62
E/A ratio	1.18 ± 0.28	1.41 ± 0.43	0.26
A ($m \cdot s^{-1}$)	0.63 ± 0.13	0.56 ± 0.06	0.30
E' ($m \cdot s^{-1}$)	0.09 ± 0.02	0.10 ± 0.03	0.53
A' ($m \cdot s^{-1}$)	0.10 ± 0.01	0.09 ± 0.01	0.51
S' ($m \cdot s^{-1}$)	0.08 ± 0.01	0.09 ± 0.02	0.32
Deceleration time (ms)	206 ± 38	213 ± 47	0.76

Values are presented as mean ± SD.

parasympathetic blockade (atropine) and sympathetic stimulation (dobutamine), http://links.lww.com/MSS/A835].

DISCUSSION

Our data show that adults with T2D have reduced HR reserve, increased resting HR and reduced HRV consistent with resting cardiac autonomic dysregulation (34). HR remained higher after blockade of the PSNS, but ejection fraction, stroke volume, and stroke work were lower in T2D and remained lower during subsequent β-adrenergic stimulation. In contrast to our hypothesis, T2D achieved their target HR with less β-adrenergic stimulation than nondiabetic controls, and relative left ventricular responses (% change) to incremental β-adrenergic stimulation paralleled (at lower values) the response of nondiabetic controls. These findings are consistent with a previously reported derangement of resting cardiac autonomic regulation (14,36) and a basal reduction in left ventricular systolic and diastolic function. However, the chronotropic and inotropic responses to adrenergic stimulation appear to be unchanged in people with uncomplicated T2D.

There are reports that the diabetic heart receives increased sympathetic nervous activity (29), has reduced β_1-adrenergic receptor expression (8), and has a well-documented reduction in cardiac reserve (2,4,26). For these reasons, we hypothesized that people with T2D would have a reduced contractile response to β-adrenergic stimulation compared with their nondiabetic counterparts. In contrast to our hypothesis, we found that ejection fraction, stroke volume$_{FFM}$, cardiac index$_{FFM}$, and stroke work were lower in T2D during dobutamine infusion, but the relative responses did not differ between the groups. Moreover, the dobutamine dose required to achieve 85% of maximal HR was 36% higher in CON. Consequently, we conclude that, in people with uncomplicated T2D, the reduction in cardiac reserve is not caused by an impaired response of the diabetic heart to sympathetic stimulation but may reflect changes in the loading conditions or function of the diabetic left ventricle.

Our data suggest that reduced diastolic left ventricular filling may have affected the systolic response of the diabetic heart. Consistent with previous studies (15,23,25), we found that left ventricular end-diastolic volume was smaller in the T2D group. Pinto et al. (23) suggested that smaller resting end-diastolic volume was effectively "compensated" by increased resting ejection fraction (reduced end-systolic volume) in adolescents with T2D. During exercise, they showed that stroke volume became smaller in adolescents with T2D (but not controls) because of an inability to maintain end-diastolic volume and an attenuated reduction in end-systolic volume (23). By contrast, we showed that adults with T2D had smaller end-diastolic volume$_{FFM}$ but had similar resting end-systolic volume and reduced resting ejection fraction compared with their nondiabetic controls. The adults with T2D responded to β-adrenergic stimulation with a "normal" increase in ejection fraction (not different from nondiabetic response), albeit at consistently lower values. The discrepancies between these studies may describe a difference between adolescents, who had diabetes for < 3 yr, and adults who had diabetes for ~7 yr. The difference in stimulus (e.g., left ventricular preload/peripheral resistance) provided by dynamic exercise in the adolescents versus supine dobutamine infusion in the adults in this study may have also influenced these findings. Regardless of these differences, the diabetic heart appears to be limited by factors unrelated to sympathetic nervous regulation during stress. Increased myocardial stiffness (25), impaired myocardial relaxation (3), and reduced total blood volume (15) contribute to reduced ventricular volumes among people with T2D; however, these variables do not appear to be differentially influenced by sympathetic stimulation.

Patterson and Starling (22) showed, through a series of *ex vivo* experiments, that the heart responds to an increase in preload (venous inflow) with greater mechanical energy and that this is a result of the volume of the ventricles at the beginning of a contraction rather than the pressure within the ventricle. With increasing end-diastolic volume (filling), the sarcomere stretches to an "optimal" length, creating more potential for cross-bridges to form and generate force (1). Our T2D cohort had smaller end-diastolic volumes which may indicate that their left ventricles worked at suboptimal sarcomere lengths as a result of reduced filling, thus limiting the potential for cross bridge formation and force production.

It is possible that group differences in intrinsic HR affected our findings. Resting HR was elevated in T2D despite the fact that plasma NE levels were reduced. Parasympathetic withdrawal resulted in similar increases in HR between groups (e.g., similar parasympathetic response), suggesting that the higher resting HR in the T2D group was explained by increased intrinsic HR or increased β-adrenergic sensitivity (or both). We are not aware of any study in humans that has compared the intrinsic rate of diabetic versus nondiabetic hearts; however, Thaung et al. (32) found that the denervated HR of the fatty diabetic Zucker rat was 45% lower than that of the nondiabetic rat hearts. Interestingly, resting *in situ* (e.g., innervated) HR were the same due to an increase in cardiac sympathetic nerve activity. Our data appear to contradict these findings because resting HR was elevated in T2D and plasma NE (our proxy for cardiac sympathetic nervous activity) was lower. Although it was not the aim of this study, it would be interesting to know how a complete autonomic blockade (sympathetic and parasympathetic) may have influenced the rates and contractility of the participants of this study.

The uncontrolled influence of the PSNS may explain why the response to adrenergic agonism in our study contradicted some previous reports (29,33). Parasympathetic stimulation alone does not elicit an inotropic response; however, an uninhibited PSNS attenuates the inotropic response to sympathetic activation (16). To negate this parasympathetic influence, we examined β-adrenergic responsiveness after a large bolus dose of atropine; thereby abolishing parasympathetic input to the heart (13). Previous studies of diabetic cohorts report blunted contractile responses (reduced S' and ejection fraction, respectively) during dobutamine stress echocardiography studies (29,33). However, these studies have not consistently controlled for parasympathetic nervous activity (e.g., atropine was administered only if target HR was not achieved with dobutamine alone). By contrast, ejection fraction and S' increased, and end-systolic volume$_{FFM}$ decreased comparably in the T2D and control groups in the present study when parasympathetic blockade (atropine) preceded incremental dobutamine.

The plasma catecholamine data in this study may indicate that the diabetic heart receives less sympathetic nervous activity. Plasma norepinephrine concentration was lower in T2D at rest and during peak exercise, but the relative increase from rest to peak exercise was not different between groups. Plasma samples from arm veins are comparable with the coronary sinus under resting conditions (19), which has been associated with the quantity of norepinephrine released at the heart (6,38). These data contradict findings in diabetic rats (32) but are consistent with reduced incorporation of I^{123}-MIBG, a labeled norepinephrine analog associated with sympathetic nervous activity (29). Arm vein plasma catecholamine samples underestimate the increase in coronary sinus catecholamines during exercise (19); therefore, we believe less can be gleaned from the exercising data. Nonetheless, the fact that both groups had comparable increases in norepinephrine and epinephrine increased less in the T2D group during exercise fails to support our hypothesis that "sympathetic responsiveness" is reduced in this cohort. If anything, the achievement of similar peak HR with lower catecholamine concentrations, combined with the finding that 36% less dobutamine was necessary to elicit 85% maximal HR, would argue that the T2D group had *greater* sympathetic responsiveness than their nondiabetic cohorts.

Limitations. An obvious limitation of this study is that our T2D cohort was relatively healthy, and therefore our findings may not be representative of people whose diabetes is associated with micro- or macrovascular comorbidity. The T2D participants in this study were normotensive, had no evidence of ischemic disease or history of CVD, and only one showed any evidence of autonomic dysfunction (an abnormal HR response to deep breathing) (31,35). Three participants had mild elevation in the albumin/creatinine ratio, and there was no evidence of diastolic dysfunction, a common early consequence of T2D (3,4,24). Ewing et al. (11) suggested that reduced parasympathetic nervous activity is an early consequence of diabetes, which progresses to sympathetic dysregulation and eventual denervation (7). This progression has been linked to resting tachycardia and exercise intolerance (34). Our cohort had higher resting HR (71 vs 59) and lower aerobic capacity (6.8 vs 10.0 METs) than their nondiabetic peers; however, neither of these values were clinically significant. It is possible that people with longer diabetes duration and/or cardiovascular comorbidity would also exhibit cardiac autonomic dysregulation.

It is also important to mention that supine echocardiographic studies of resting subjects exposed to high adrenergic stimulation are "nonphysiological" in that they do not represent increased venous return, or upright posture which often occurs during periods of high adrenergic activity. This may have been particularly important in relation to afterload, where T2D appeared to have a smaller increase in mean arterial BP ($P > 0.05$) than CON.

In conclusion, our data indicate that an autonomic imbalance alters resting cardiac performance in adults with uncomplicated T2D. However, the chronotropic and inotropic responses to incremental β-adrenergic stimulation were not impaired in these individuals. Instead, the diabetic left ventricle appeared to operate at reduced volumes, resulting in a reduction in stroke volume and cardiac output during stress. These findings suggest that people with uncomplicated diabetes have reduced left ventricular capacity that cannot be explained by differences in β-adrenergic sensitivity.

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