Adults With Type 2 Diabetes Mellitus Exhibit a Greater Exercise-Induced Increase in Arterial Stiffness and Vessel Hemodynamics

Adults with Type 2 Diabetes Exhibit a Greater Exercise-Induced Increase in Arterial Stiffness and Vessel Hemodynamics

Running title: Post-exercise arterial stiffness in diabetes

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ABSTRACT

Individuals with type 2 diabetes mellitus (T2DM) have a greater blood pressure (BP) response to acute maximal exercise compared to those without T2DM; however, whether they exhibit a different arterial stiffness (AS) response to maximal exercise has yet to be explored. Adults with (n=66) and without T2DM (n=61) underwent an 'arterial stress test': at rest and immediately post-exercise, carotid-femoral pulse wave velocity (cfPWV), the gold-standard measure of AS, brachial BP, heart rate (HR) and other hemodynamic measurements were assessed. Linear regression models were used to evaluate between-group differences at rest, and the response to exercise (post-exercise value), adjusting for covariates including BP and HR when relevant, and the corresponding baseline value of each parameter. All participants (mean±SD: age 59.3±10.6 years; BMI 31.2±3.9 kg/m^2) had hypertension (mean BP 130±14/80±9 mmHg). At rest, participants with T2DM had significantly higher cfPWV (10.3±2.7 vs. 9.1±1.9 m/s), HR (69±11 vs. 66±10 beats/min), and lower DBP (79±9 vs. 83±9 mmHg), but SBP (129±15 vs. 131±13 mmHg) was similar. In response to exercise, participants with T2DM showed greater increases in cfPWV (1.6, 95%CI 0.4, 2.9 m/s), and SBP (9, 95% CI 1, 17 mmHg) than participants without T2DM. A greater proportion of participants with T2DM had a hypertensive response to exercise compared to participants without T2DM (n=23, 35% vs. n=11, 18%) (P=0.033). By incorporating exercise as a vascular stressor, we provide evidence of a greater increase in AS in individuals with T2DM, independently of resting AS, and the BP post-exercise.

Keywords: arterial stiffness, hypertension, type 2 diabetes mellitus, exercise, blood pressure, exercise testing

INTRODUCTION

Type 2 diabetes mellitus (T2DM) increases arterial stiffness through pathological changes in the vasculature, including reduced nitric oxide bioavailability, increased oxidative stress and inflammation, as well as structural changes within the arterial wall[1]. As a result, for many individuals with T2DM, their vascular "age" surpasses their chronological age[2]. Furthermore, during maximal exercise, individuals with T2DM are more likely to experience an exaggerated blood pressure (BP) response[3]; this is defined as a rise in systolic BP (SBP) exceeding 210 mmHg in men and 190 mmHg in women, and is associated with higher cardiovascular disease (CVD) risk and mortality[4]. The physiological changes underlying this altered response have not been fully elucidated, but underlying vascular abnormalities are thought to play a pivotal role[5]. However, whether individuals with T2DM have a different arterial stiffness response to exercise, independent of the resting value, has yet to be explored. In this context, increased demands associated with acute exercise might exaggerate vascular abnormalities present in these individuals.

The 'gold standard' metric for assessing arterial stiffness non-invasively is carotid-femoral pulse wave velocity (cfPWV), a measure of the speed of the pressure pulse wave in the central elastic arteries[6]. Higher values of cfPWV indicate greater arterial stiffness, which is associated with a greater risk of CVD events and mortality[7, 8].

With increased metabolic demands during acute exercise, the vascular system plays an important role in the redistribution of blood flow to ensure adequate perfusion of the exercising muscle[9]. This leads to a transient increase in mean arterial pressure, sympathetic activity, and vascular tone, as well as central arterial stiffness[9]. During the recovery period, arterial stiffness has been shown to decrease to a level at, or below resting values[9]. While the initial increase in

arterial stiffness is recognized as a normal adaptation to acute exercise, the extent of the increase in arterial stiffness and recovery trajectory may reflect the ability of the arteries to respond to increased demands.

In the present study, we aimed to examine the acute response of arterial stiffness and hemodynamic parameters to maximal exercise in adults with and without T2DM. We hypothesized that individuals with T2DM would have a higher arterial stiffness in response to exercise, independently of the resting values and BP.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The study was approved by the ethics review board of McGill University Faculty of Medicine. Written informed consent was obtained from all participants.

Study Cohort

Participants were recruited through McGill-affiliated clinics for the SMARTER trial, a one-year randomized controlled trial examining the impact of step count prescriptions on arterial health[10]. All participants of the trial were overweight or obese (body mass index 25-40 kg/m^2), had T2DM and/or hypertension, and did not have any gait abnormalities preventing exercise. Hypertension and T2DM were diagnosed by the referring physician following Canadian guidelines[11, 12]. The analyses herein were conducted in hypertensive participants with and without T2DM who underwent the 'arterial stress test' at the baseline evaluation.

Exercise Testing

All participants underwent a maximal exercise test to exhaustion on a treadmill following a modified Bruce protocol[13]. Peak oxygen consumption ($VO_{2\ peak}$) was obtained using a metabolic cart (Medisoft's Ergocard, Sorinne, Belgium). To ensure all participants had achieved exhaustion, participants who did not attain age-based cutoffs for the respiratory exchange ratio (RER) were excluded (aged 20-49: RER≥1.10; aged 50-64: RER≥1.05; aged ≥65: RER≥1.00)[14]. Peak heart rate (HR) was obtained using the 3-lead electrocardiogram (ECG) connected to the metabolic cart but was not used as a criterion to establish maximal effort due to the influence of β-blockers on the HR response to exercise.

105 **Arterial Stiffness and Hemodynamics**

106 All measurements were performed in the morning to avoid circadian rhythm variations[15, 16].
107 Participants fasted for 12 hours prior to the assessment, and abstained from caffeine, alcohol, and
108 smoking. Participants were offered a small healthy snack after the blood draw and prior to the
109 'arterial stress test' to prevent hypoglycemia and because a fasted state could have prevented
110 participants from exerting themselves fully. Participants avoided exercise for 24 hours prior to
111 the assessment. All usual medications, except anti-hyperglycemic agents, were taken the
112 morning of assessment.

113 Brachial BP was measured using an automated oscillometric BP monitor (BpTRU, Medical
114 Devices Ltd, BC, Canada) in a seated position at rest[12], as well as in a supine position at rest and
115 after exercise (at 3, 5, 10, 15 and 20 minutes), following the cfPWV measurement. MAP was
116 calculated as: brachial diastolic BP (DBP) + 1/3(brachial SBP-DBP)[17]. Due to the impact of
117 body position on BP, brachial BP was assessed in the supine position in order to calibrate the
118 central hemodynamic measures obtained in a supine position. Standing measurements of brachial
119 BP were obtained manually using the auscultatory method immediately before and after exercise
120 (0 minutes). This measure was used to evaluate whether participants experienced a hypertensive
121 response to exercise, which was defined as a brachial SBP >210 mmHg in men and >190 mmHg
122 in women[4].

123 Arterial stiffness, central BP, and augmentation index (AIx) were measured using
124 applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia) in a supine position
125 before and immediately after exercise following a standardized protocol in a controlled
126 environment at the Vascular Health Unit at the McGill University Health Centre. Baseline
127 measurements were obtained after a 10-minute rest period. Following exercise completion,

participants returned to a supine position for the measurement of cfPWV (at 3, 5, 10, 15, and 20 minutes) and carotid-radial PWV (crPWV), central BP and AIx (at 5, 10, 15, and 20 minutes). As per SphygmoCor recommendations, the radial pressure waveforms were calibrated using brachial SBP and DBP. As calibration with MAP and DBP has been increasingly suggested[18], we also performed this analysis. HR was acquired at the same time as the cfPWV measurement using the built-in 3-lead ECG. To account for the influence of HR on wave reflection, AIx was corrected for a HR of 75 beats/ minute (AIx75). Path length was estimated using the subtraction method, whereby the distance between the carotid artery site and the sternal notch was subtracted from the distance between the sternal notch and the femoral artery site[6]. At rest, measurements were repeated until two PWV measurements were within 0.5 m/s, and two augmentation pressures were within 4%. PWA measurements with an operator index <80 and PWV measurements with a pulse transit time standard deviation >13% or HR difference >5 beats/min between sites were deemed poor quality and not considered. Due to time restrictions post-exercise, only one good quality measurement was collected. Non-invasively recorded central waveforms (derived from the radial artery) have been validated against invasively recorded central waveforms at rest, as well as during and after cycling exercise[19]. Furthermore, good test-retest reproducibility has been demonstrated for cfPWV, central BP and AIx acquired during and after exercise[20, 21].

We also evaluated the BP-independent changes in arterial stiffness by calculating an index of stiffness that is considered equivalent to the intrinsic stiffness index β_0, where β_0 is the exponent of the pressure (P)-diameter (D) relationship within the vessel[22]:

$$P = P_{\mathrm{ref}} e^{\beta_0\left(\frac{D}{D_{\mathrm{ref}}}-1\right)}.$$

P_{ref} is a reference pressure and D_{ref} is the diameter of the artery at the reference pressure. Using cfPWV, the corresponding brachial DBP (P_d), and estimated blood mass density (ρ=1.050 kg/L), and P_{ref}=100 mmHg, aortic stiffness index β_0 was determined[23] as

$$\beta_0 = \frac{\mathrm{cfPWV}^2 \cdot 2\rho}{P_\mathrm{d}} - \ln\left(\frac{P_\mathrm{d}}{P_\mathrm{ref}}\right).$$

The left ventricular ejection duration was derived from the central pressure waveform and calculated as the time from the foot of the waveform to the incisura.

The timing of the measurements is summarized in Figure 1. Due to a short time window post-exercise, we prioritized the measurement of brachial BP and cfPWV at the 3-minute time point. From 5 minutes onwards, all parameters were measured, in the same order for all participants.

Blood Collection

Fasting venous blood samples were obtained for the quantification of glucose and insulin levels following standard laboratory methods. In participants not taking insulin, fasting glucose and insulin values were used to compute the Homeostatic Model Assessment-Insulin Resistance (HOMA-IR).

Analysis

Demographic factors and resting parameters were compared between groups using the Student's T-test or Mann-Whitney test, as appropriate. Categorical variables were assessed using the chi-square test for independence. Linear regression models were used to evaluate between-group differences in hemodynamic parameters post-exercise. In evaluating the response to exercise, models were consistently adjusted for the baseline parameter, age, sex, as well as waist:hip ratio and angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) use to account for group differences in these variables. ACEis/ARBs are known to influence the cardiovascular response to exercise. We further evaluated models with and

without statin use due to group differences, but it should be noted that statin use was strongly correlated with T2DM status, given that clinical guidelines recommend statin therapy in patients with T2DM. Further, all measurements were adjusted for HR at the time of measurement.

To correct for the BP dependence of cfPWV, brachial DBP at the time of the measure was included as a covariate in our statistical models. DBP was chosen given that the SphygmoCor system uses the diastolic foot of the proximal and distal waveforms for the estimation of transit time, and therefore, provides a velocity measure that is dependent on DBP. However, we also assessed differences adjusting for mean arterial pressure (MAP) since we acknowledge that the brachial BP differs from central BP, and this difference may be amplified during exercise[24]. Lastly, we also evaluated two separate models, where 1) both SBP and DBP were included, and 2) SBP replaced DBP.

To evaluate the impact of T2DM on overall vascular function after physical stress, area under the curve (AUC) values were calculated for vessel hemodynamic parameters measured at baseline, 3, 5, 10, 15, and 20 minutes. In order to compare the AUC irrespective of the baseline value, a 'baseline AUC' was determined using the pre-exercise value and subtracted from the total AUC (Figure S1). Differences in the AUC were assessed using linear regression, adjusting for age, sex, waist:hip ratio, and ACEi/ARB use.

Mean differences between groups were computed with 95% confidence intervals (CIs). SAS V9.3 was used.

RESULTS

Overall, 266 participants completed the exercise test. We excluded 1) participants with T2DM who did not have hypertension (n=30), 2) participants who did not meet criteria for exhaustion (n=80), and 3) participants who were missing the 3-minute post-exercise arterial stiffness measures (n=26) (Figure 2). We further identified two participants with T2DM who were significant outliers when we evaluated the post-exercise cfPWV, and whose inclusion likely exaggerated between-group differences (Table S1). Excluded participants who did not reach exhaustion during the exercise test exercised for a shorter duration, and had a lower VO_{2peak} and peak HR, but were otherwise comparable to those who were included in the final analysis (Table S2). Our main analyses compared participants with (n=66) and without T2DM (n=61).

In our main analysis, participants with T2DM had a greater waist:hip ratio, but body mass index was similar. A comparable proportion of participants with and without T2DM were treated for hypertension; however, a greater proportion with T2DM were taking ACEi/ARBs, in accordance with clinical practice guidelines (Table 1)[12]. There were differences in the lipid profile, and statins were taken by 79% of participants with T2DM versus 33% without T2DM. Fasting glucose and HOMA-IR levels were higher in those with T2DM, who had a mean hemoglobin A1c of 7.9±1.3%.

At rest, participants with T2DM had higher cfPWV and aortic stiffness β_0, and lower central and brachial DBP, but no significant differences in SBP or other hemodynamic measures were noted (Table 1).

Response to Exercise

Unadjusted values of all parameters post-exercise are presented in the online supplement (Table S3). In adjusted analyses, no differences were observed between subjects with and

without T2DM for the duration of exercise, exercise capacity (VO_{2peak}), or maximal HR (Table 2). A higher proportion of participants with T2DM had a hypertensive response to exercise compared to participants without T2DM [n=23 (35%) vs. n=11 (18%); difference 17% (95% CI 2, 32 %)]. However, the peak exercise BP (0 minutes) was not significantly different between groups in adjusted analyses. Table 2 also presents the arterial stiffness and hemodynamic parameters according to their first available measurement post-exercise (3 or 5 minutes) to demonstrate the initial response to exercise. Immediately after exercise (at 3 minutes), we observed significantly greater brachial SBP by 8.9 mmHg (95% CI 0.9, 16.9 mmHg) in participants with T2DM, but no differences in DBP or peak HR.

Interestingly, participants with T2DM had a greater increase in cfPWV and aortic stiffness β_0, as well as pulse pressure. The differences in cfPWV persisted in models adjusting for brachial DBP at the time of measurement (Table 2), MAP, and both SBP and DBP (Table S4). The increase in cfPWV was not significant when adjusting for only brachial SBP post-exercise (Table S4). In addition, it is noteworthy that the elevated SBP at 3 minutes post-exercise in T2DM was no longer significant when additionally adjusting for the corresponding post-exercise cfPWV [6.1 (95% CI -2.1, 14.2 mmHg)]. A significant between-group difference in aortic stiffness β_0 remained when SBP was included (7.70, 95% CI 0.05, 15.34). Univariate, partially adjusted, and fully adjusted models for aortic stiffness β_0 are presented in Table S5.

No significant differences in central BP, crPWV, AIx75, or left ventricular ejection duration were observed. Calibration of central BP with brachial MAP and DBP instead of SBP and DBP did not change the results (Table S6).

Participants with T2DM exhibited a greater AUC for cfPWV, aortic stiffness β_0, and brachial SBP and DBP than participants without T2DM (Table 3). There were no differences between

238 subjects with and without T2DM beyond 3 minutes for brachial SBP (Figure 3). While the
239 overall AUC was different between groups for brachial DBP, there were no differences at 3
240 minutes, or at other points during the recovery. cfPWV and aortic stiffness β_0 were both
241 significantly different at 3, 5, 10 and 20 minutes in unadjusted analyses, and only at 3 and 10
242 minutes in adjusted analyses, accounting for the pre-exercise value, age, sex, waist:hip ratio,
243 ACEi/ARB use, and DBP (cfPWV only) and HR at the time of measurement. Between-group
244 differences for all parameters during recovery (5, 10, 15 and 20 minutes) are presented in Table
245 S7.

DISCUSSION

By incorporating exercise as a vascular stressor, we provide evidence of a greater increase in cfPWV and aortic stiffness β_0 in individuals with T2DM, independently of resting arterial stiffness, and the brachial BP post-exercise. In a fully adjusted model, we observed a difference in cfPWV of 1.6 m/s between individuals with and without T2DM. A meta-analysis of 17 longitudinal studies (n=15,877 individuals) showed that a 1 m/s increase in resting aortic stiffness corresponds to a 14%, 15%, and 15% increased risk of CVD events, CVD mortality and all-cause mortality, respectively, adjusting for traditional CVD risk factors[7]. This robust association was confirmed in a more recent large individual participant meta-analysis in 17,635 individuals[8]. While the clinical significance of differences in cfPWV post-exercise has not been established, the magnitude of the difference in cfPWV observed in our study is not trivial.

Calculating the AUC allowed us to generate a single variable that summarizes multiple longitudinal measurements, capturing the combined response and recovery of each parameter to maximal stress. Our results, indicating significant differences in the AUC for cfPWV and aortic stiffness β_0, support an overall difference in the response of arterial stiffness to exercise between individuals with and without T2DM. The AUC for brachial SBP was also higher in individuals with T2DM but this was mainly driven by differences between groups immediately post-exercise, given that both groups followed a similar trajectory afterwards, i.e., from 5 to 20 minutes post-exercise.

In subjects with T2DM, we observed a greater increase in brachial SBP at 3 minutes post-exercise, which is in line with findings by Scott and colleagues demonstrating an excessive rise in brachial SBP in response to maximal treadmill exercise in adults with T2DM compared to healthy controls[3]. While they also observed a significantly greater increase in central SBP

immediately post-exercise (<3 minutes), we only observed a trend for an increase, likely because central BP in our study was captured 5 minutes post-exercise, at which point values had returned to baseline.

To our knowledge, no prior studies have evaluated the arterial stiffness response immediately post-maximal exercise in adults with T2DM. A study of a hypertensive population demonstrated elevated cfPWV 40 minutes and 1 hour after maximal cycling exercise compared with baseline levels[25]. This increase post-exercise was not observed in normotensive controls; however, this analysis did not compare the post-exercise cfPWV between groups. Instead, we have demonstrated an elevated cfPWV response in individuals with T2DM and hypertension compared to subjects with hypertension alone. Climie and colleagues compared the arterial stiffness and hemodynamic response to a short bout of light-moderate cycling exercise between individuals with T2DM and healthy controls[26]. They measured cfPWV while still on the cycle ergometer, enabling more immediate cfPWV measurements. They observed a significantly higher cfPWV post-exercise in individuals with T2DM (unadjusted); however, this analysis did not account for differences in resting cfPWV or other covariates, as this was not the main interest of this paper.

The relationship between arterial stiffness and BP is bi-directional and complex[27]. Arterial stiffening increases the amplitude of the forward traveling pressure waves, as well as the speed of propagation of both the forward and backward waves[6]. Consequently, the reflected waves return earlier during the cardiac cycle and become superimposed on the systolic part of the forward wave, leading to elevated central SBP and a widened pulse pressure[6]. Interestingly, during light-moderate cycling exercise, the elevation in central SBP is mainly due to an increase in the amplitude of the forward travelling wave, rather than reflected waves[28]. Therefore, arterial

stiffness and forward wave amplitude both contribute to the BP change observed during exercise. Conversely, given the exponential relationship between artery diameter and pressure, there is a clear acute relationship between the arterial BP and stiffness, represented by the tangent slope[23]. Therefore, the intrinsic stiffness of the artery will depend on BP. This bi-directional relationship complicates the assessment of arterial stiffness independently of BP; however, different mechanisms for evaluating the BP-independent response of arterial stiffness have been proposed[23]. Most commonly, arterial stiffness is statistically adjusted for BP at the time of measurement. Adjusting for the MAP is often recommended[6]; however, adjusting for the DBP may be more relevant as this represents the pressure in the artery when the transit time is calculated[29]. We have performed analyses adjusting for brachial DBP as well as for MAP. Hermeling and colleagues have demonstrated that PWV changes dramatically over the cardiac cycle, reporting a mean difference of 2.4 m/s between the diastolic and systolic phase (range 0.8-4.4 m/s)[30]. In our study we have calculated transit time using the foot of the arterial pressure waveform, and therefore, elected to adjust analyses for the brachial DBP. Similarly, aortic stiffness β_0 is derived by inputting the DBP. Spronck and colleagues demonstrated that cardio-ankle vascular index (CAVI), which has been proposed to be a pressure-independent estimate of the intrinsic stiffness β, may show a residual acute BP dependence[23]. They provide a modified formula that theoretically removes the acute BP dependence, yielding $CAVI_0$. Our inclusion of cfPWV versus heart-to-ankle PWV in the case of $CAVI_0$ provides an estimate of the intrinsic stiffness β_0 in the central elastic arteries. In our study, statistical correction of cfPWV for DBP, and the aortic stiffness β_0 yielded comparable results. Similar to cfPWV, a significant aortic stiffness β_0 difference remained when adjusting for SBP. This observation strengthens our finding that the observed difference in arterial stiffness between groups is independent of the

intrinsic arterial stiffness dependence on DBP (as corrected for through calculation of aortic stiffness β_0), as well as independently of SBP. We also observed an elevated cfPWV response in models adjusting for MAP. A significant association between brachial SBP immediately post-exercise and the corresponding post-exercise cfPWV was also noted. Specifically, the elevated SBP response post-exercise in T2DM was no longer significant when adjusting for the corresponding post-exercise cfPWV. On the other hand, the higher cfPWV response in T2DM was independent of brachial SBP and DBP post-exercise. Taken together, these findings indicate that arterial stiffness may mediate the exaggerated SBP increase.

Participants with T2DM had elevated arterial stiffness at rest, which is likely a function of structural changes of the arteries. High levels of circulating glucose lead to the development of advanced glycation end products, whereby glucose forms cross-links with collagen proteins within the arteries, and therefore, may alter the important balance between elastin and collagen[1]. Hyperglycemia causes the activation of protein kinase C, which leads to the generation of reactive oxygen species, and inflammation, further altering the structural and functional integrity of vascular wall[1]. When assessing post-exercise values of cfPWV, we have adjusted for resting values of cfPWV. Furthermore, we have demonstrated that the increase in arterial stiffness after acute exercise occurs independently of BP at the time of measurement, suggesting that these changes are due to changes in intrinsic properties of the arterial wall. As structural changes in such time frame (minutes) are unlikely, we attribute differences in response to exercise mainly to functional changes. For example, individuals with T2DM have endothelial dysfunction; higher levels of endothelin-1 and reduced nitric oxide bioavailability may cause an impaired vasodilatory response and increased arterial stiffness post-exercise[1]. Additionally, excess sympathetic activity in individuals with T2DM may potentiate greater exercise-induced

vasoconstriction[1]. It is noteworthy that vasoconstriction does not always lead to a functional increase in stiffness; for example, in healthy subjects, vasoconstriction may shift pressure load bearing towards elastin, offloading the stiff collagen. However, in individuals with T2DM who have impaired arterial function, vasoconstriction presumably leads to increased functional stiffness[31].

The sample size of our study is relatively small; however, we demonstrated conclusive between-group differences in our main outcome, while adjusting for relevant covariates. This study constituted a secondary analysis of our SMARTER trial[10], and thus we did not carry out power calculations *a priori*. Due to time constraints post-exercise, we could only obtain single measurements at each time point and were only able to measure select indices of arterial stiffness (i.e., cfPWV) at the 3-minute time point. Thus, we were not able to capture differences in central hemodynamic parameters earlier, as these measurements were only obtained after 5 minutes post-exercise. To this end, because we did not have central DBP measures immediately after exercise wwe have included brachial DBP in our models. However, DBP is relatively stable, with little difference between peripheral and central values[6]. Pulse pressure amplification increases during exercise in healthy individuals[24]; however, a follow-up study by the same group demonstrated that the degree of amplification is reduced in older patients with hypercholesterolemia[32]. Moreover, the pulse pressure amplification is likely driven more by an increase in SBP. We examined central and peripheral BP at 5 minutes; although on average brachial SBP was 15 mmHg greater than central SBP, there was only a 2 mmHg average difference for DBP (data not shown). Therefore, while brachial DBP seems to closely estimate the central DBP, we still included analyses adjusting for MAP (mainly driven by DBP)[17]. Following guidelines, measurements of arterial stiffness and hemodynamics were performed in a

supine position pre- and post-exercise; however, we were not able to control for the possible postural influence of lying down after treadmill exercise on vessel hemodynamics. Since we aimed to provoke maximal changes in arterial stiffness and hemodynamics, a graded treadmill test was selected over supine cycling exercise. Lastly, since all participants included in our analysis were hypertensive, the results of this study may not be generalizable to younger, lower-risk individuals with T2DM.

PERSPECTIVES

Our study has demonstrated that evaluating the exercise-induced response of arterial stiffness provides additional information by capturing the effect of T2DM on the ability of the arteries to respond to increased demands during exercise. Central arterial stiffness directly influences BP and likely contributes to the exaggerated BP response in participants with T2DM. Increased central arterial stiffness has a number of clinical consequences; it imposes a greater load on the left ventricle, decreases coronary perfusion, and exposes the microcirculation and end-organs to increased pulsatile pressure. Given that we do not spend our lives at rest, and physical stress commonly occurs during daily activities, this altered arterial stiffness response to strenuous exercise may contribute to the increased risk for CVD events in these individuals. The 'arterial stress test' may serve as a useful model for evaluating vascular impairment and CVD risk in individuals with T2DM. Future studies are needed to confirm the clinical utility of this model.

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CONFLICTS OF INTEREST/DISCLOSURE

None.

SUPPLEMENTAL MATERIALS

Online Figure S1

Online Data Tables S1-S7

REFERENCES

1. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: Pathophysiology, clinical consequences, and medical therapy: Part i. *Eur Heart J*. 2013;34:2436-2443

2. Stone JA, Fitchett D, Grover S, Lewanczuk R, Lin P. Vascular protection in people with diabetes. *Can J Diabetes*. 2013;37 Suppl 1:S100-104

3. Scott JA, Coombes JS, Prins JB, Leano RL, Marwick TH, Sharman JE. Patients with type 2 diabetes have exaggerated brachial and central exercise blood pressure: Relation to left ventricular relative wall thickness. *Am J Hypertens*. 2008;21:715-721

4. Schultz MG, Otahal P, Cleland VJ, Blizzard L, Marwick TH, Sharman JE. Exercise-induced hypertension, cardiovascular events, and mortality in patients undergoing exercise stress testing: A systematic review and meta-analysis. *Am J Hypertens*. 2013;26:357-366

5. Schultz MG, Sharman JE. Exercise hypertension. *Pulse (Basel)*. 2014;1:161-176

6. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T, American Heart Association Council on H. Recommendations for improving and standardizing vascular research on arterial stiffness: A scientific statement from the american heart association. *Hypertension*. 2015;66:698-722

7. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: A systematic review and meta-analysis. *J Am Coll Cardiol*. 2010;55:1318-1327

8. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Boutouyrie P, Cameron J, Chen CH, Cruickshank JK, et al. Aortic pulse wave velocity improves

cardiovascular event prediction: An individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol*. 2014;63:636-646

9. Mutter AF, Cooke AB, Saleh O, Gomez YH, Daskalopoulou SS. A systematic review on the effect of acute aerobic exercise on arterial stiffness reveals a differential response in the upper and lower arterial segments. *Hypertens Res*. 2017;40:146-172

10. Dasgupta K, Rosenberg E, Joseph L, Cooke AB, Trudeau L, Bacon SL, Chan D, Sherman M, Rabasa-Lhoret R, Daskalopoulou SS. Physician step prescription and monitoring to improve arterial health (smarter): A randomized controlled trial in patients with type 2 diabetes and hypertension. *Diabetes Obes Metab*. 2017;19:695-704

11. Punthakee Z, Goldenberg R, Katz P. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes*. 2018;42 Suppl 1:S10-s15

12. Nerenberg KA, Zarnke KB, Leung AA, Dasgupta K, Butalia S, McBrien K, Harris KC, Nakhla M, Cloutier L, Gelfer M, et al. Hypertension canada's 2018 guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults and children. *Canadian Journal of Cardiology*. 2018;34:506-525

13. Bruce RA. Exercise testing of patients with coronary heart disease. Principles and normal standards for evaluation. *Annals of clinical research*. 1971;3:323-332

14. Edvardsen E, Hem E, Anderssen SA. End criteria for reaching maximal oxygen uptake must be strict and adjusted to sex and age: A cross-sectional study. *PLoS One*. 2014;9:e85276

15. Papaioannou TG, Karatzis EN, Papamichael CM, Karatzi KN, Zakopoulos NA, Lekakis JP, Mavrikakis M, Stefanadis C. Circadian variation of arterial pressure wave reflections. *American Journal of Hypertension*. 2006;19:259-263

16. Kollias GE, Stamatelopoulos KS, Papaioannou TG, Zakopoulos NA, Alevizaki M, Alexopoulos GP, Kontoyannis DA, Karga H, Koroboki E, Lekakis JP, Papamichael CM. Diurnal variation of endothelial function and arterial stiffness in hypertension. *J Hum Hypertens*. 2009;23:597-604

17. Salvi P. *Pulse waves*. Italy: Springer-Verlag; 2012.

18. Papaioannou TG, Karageorgopoulou TD, Sergentanis TN, Protogerou AD, Psaltopoulou T, Sharman JE, Weber T, Blacher J, Daskalopoulou SS, Wassertheurer S, et al. Accuracy of commercial devices and methods for noninvasive estimation of aortic systolic blood pressure a systematic review and meta-analysis of invasive validation studies. *J Hypertens*. 2016;34:1237-1248

19. Sharman JE, Lim R, Qasem AM, Coombes JS, Burgess MI, Franco J, Garrahy P, Wilkinson IB, Marwick TH. Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension*. 2006;47:1203-1208

20. Keith LJ, Rattigan S, Keske MA, Jose M, Sharman JE. Exercise aortic stiffness: Reproducibility and relation to end-organ damage in men. *J Hum Hypertens*. 2013;27:516-522

21. Holland DJ, Sacre JW, McFarlane SJ, Coombes JS, Sharman JE. Pulse wave analysis is a reproducible technique for measuring central blood pressure during hemodynamic perturbations induced by exercise. *Am J Hypertens*. 2008;21:1100-1106

22. Hayashi K, Handa H, Nagasawa S, Okumura A, Moritake K. Stiffness and elastic behavior of human intracranial and extracranial arteries. *J Biomech*. 1980;13:175-184

23. Spronck B, Avolio AP, Tan I, Butlin M, Reesink KD, Delhaas T. Arterial stiffness index beta and cardio-ankle vascular index inherently depend on blood pressure but can be readily corrected. *J Hypertens*. 2017;35:98-104

24. Sharman JE, McEniery CM, Campbell RI, Coombes JS, Wilkinson IB, Cockcroft JR. The effect of exercise on large artery haemodynamics in healthy young men. *Eur J Clin Invest*. 2005;35:738-744

25. Attina TM, Drummond ID, Malatino LS, Maxwell SR, Webb DJ. Phosphodiesterase type 5 inhibition improves arterial stiffness after exercise but not exercise capacity in hypertensive men. *Am J Hypertens*. 2013;26:342-350

26. Climie RE, Srikanth V, Keith LJ, Davies JE, Sharman JE. Exercise excess pressure and exercise-induced albuminuria in patients with type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol*. 2015;308:H1136-1142

27. Mitchell GF. Arterial stiffness and hypertension: Chicken or egg? *Hypertension*. 2014;64:210-214

28. Schultz MG, Davies JE, Roberts-Thomson P, Black JA, Hughes AD, Sharman JE. Exercise central (aortic) blood pressure is predominantly driven by forward traveling waves, not wave reflection. *Hypertension*. 2013;62:175-182

29. Spronck B, Delhaas T, Butlin M, Reesink KD, Avolio AP. Options for dealing with pressure dependence of pulse wave velocity as a measure of arterial stiffness: An update of cardio-ankle vascular index (cavi) and cavi0. *Pulse (Basel)*. 2018;5:106-114

30. Hermeling E, Vermeersch SJ, Rietzschel ER, de Buyzere ML, Gillebert TC, van de Laar RJ, Ferreira I, Hoeks AP, van Bortel LM, Reneman RS, Segers P, Reesink KD. The change in arterial stiffness over the cardiac cycle rather than diastolic stiffness is independently associated with left ventricular mass index in healthy middle-aged individuals. *J Hypertens*. 2012;30:396-402

31. Lacolley P, Regnault V, Segers P, Laurent S. Vascular smooth muscle cells and arterial stiffening: Relevance in development, aging, and disease. *Physiol Rev*. 2017;97:1555-1617

32. Sharman JE, McEniery CM, Dhakam ZR, Coombes JS, Wilkinson IB, Cockcroft JR. Pulse pressure amplification during exercise is significantly reduced with age and hypercholesterolemia. *J Hypertens*. 2007;25:1249-1254

NOVELTY AND SIGNIFICANCE

What is new?

- Our study is the first to examine the acute response of arterial stiffness and hemodynamic parameters to acute maximal exercise in individuals with hypertension and with and without type 2 diabetes mellitus (T2DM)
- We provide evidence of a greater increase in carotid-femoral pulse wave velocity, the 'gold standard' measure of central arterial stiffness, in individuals with T2DM in response to acute maximal exercise, independently of resting arterial stiffness and the blood pressure (BP) post-exercise
- A significantly higher post-exercise response of aortic stiffness β_0, a novel BP independent measure of arterial stiffness, was observed in individuals with T2DM versus without T2DM

What is relevant?

- Our study confirmed an exaggerated BP response in individuals with T2DM, which has been previously associated with higher cardiovascular disease risk and mortality
- Our findings demonstrating a greater arterial stiffness response in individuals with T2DM help unravel the physiological mechanisms of the elevated BP response to exercise observed in this population

Summary

- By incorporating acute maximal exercise as a vascular stressor, we provide evidence of a greater increase in arterial stiffness post-exercise in individuals with hypertension and T2DM compared to individuals with hypertension alone

FIGURE LEGENDS

Figure 1. Timing of procedures included in the 'arterial stress test' protocol.

AIx75, augmentation index corrected for a HR of 75 beats/minute; BP, blood pressure; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; HR, heart rate; RER, respiratory exchange ratio, VO_2, oxygen consumption.

Figure 2. Participant flowchart outlining the number of participants excluded from the final analysis.

cfPWV, carotid-femoral pulse wave velocity; RER, respiratory exchange ratio; T2DM, type 2 diabetes mellitus.

Figure 3. Trajectory of unadjusted A) cfPWV, B) aortic stiffness β_0, C) systolic blood pressure and D) diastolic blood pressure changes from rest to post-exercise at 3, 5, 10, 15, and 20 minutes. Error bars represent 95% confidence intervals. Linear regression models were used. *Indicates a significant between-group difference in unadjusted analyses, and ^ indicates a significant difference in adjusted analyses (described in Table 2).

cfPWV, carotid-femoral pulse wave velocity; T2DM, type 2 diabetes mellitus.

TABLES

Table 1: Baseline characteristics

Variable	Without T2DM (n=61)	With T2DM (n=66)	P- value
Demographic factors			
Age (years)	59.0±10.4	59.6±10.9	0.749
Women, no (%)	35 (57.4)	28 (42.4)	0.092
Body mass index (kg/m^2)	31.7±3.9	30.7±3.8	0.132
Waist circumference (cm)	101.7±9.5	103.4±10.1	0.353
Hip circumference (cm)	111.8±8.9	107.2±7.7	**0.002**
Waist:hip ratio	0.91±0.07	0.96±0.07	**<0.001**
Smoking history, no (%)			
Past Smoker	21 (34.4)	23 (35.4)	0.910
Current Smoker	2 (3.3)	5 (7.6)	0.269
Type 2 Diabetes			
Duration (years)		10.5±7.5	
Medications, no (%)			
Anti-hypertensive agents	58 (95.1)	65 (98.5)	0.273
ACEi or ARBs	39 (63.9)	62 (93.9)	**<0.001**
Calcium channel blockers	18 (29.5)	14 (21.2)	0.282
Diuretics	29 (47.5)	28 (42.4)	0.562
Beta-blockers	18 (29.5)	15 (22.7)	0.384
Statins	20 (32.8)	52 (78.8)	**<0.001**

Insulin		22 (33.3)	
Metformin		57 (86.4)	
Sulfonylureas		22 (33.3)	
Laboratory Parameters			
Fasting glucose (mmol/L)*	5.5 [5.0-6.1]	7.9 [6.5-8.8]	**<0.001**
Fasting insulin (pmol/L)*	65.0 [44.1-92.9]	55.8 [43.1-87.7]	0.698
Hemoglobin A1c (%)		7.6 [7.0-8,4]	
HOMA-IR	2.7 [1.7-3.6]	3.2 [2.3-4.6]	**0.043**
HDL (mmol/L)	1.3±0.3	1.2±0.3	**0.035**
LDL (mmol/L)	3.0±1.0	2.1±0.6	**<0.001**
Triglycerides (mmol/L)	1.3 [1.0-2.0]	1.5 [1.1-2.2]	0.326
Total cholesterol (mmol/L)	5.1±1.2	4.1±0.8	**<0.001**
Arterial Stiffness and Hemodynamics (measured supine)			
cfPWV (m/s)	9.2±1.9	10.3±2.7	**0.009**
Aortic stiffness β_0	15.1 [12.3-19.8]	19.8 [15.0-25.8]	**0.003**
crPWV (m/s)	8.6±1.1	8.9±1.3	0.184
Brachial SBP (mmHg)	131±13	129±15	0.630
Brachial DBP (mmHg)	82±9	78±9	**0.030**
Brachial PP (mmHg)	49±10	51±13	0.284
Central SBP (mmHg)	121±12	119±14	0.454
Central DBP (mmHg)	83±9	79±9	**0.030**
Central PP (mmHg)	38±10	40±13	0.421
MAP (mmHg)	99±10	97±10	0.120

AIx75 (%)	22.8±10.8	23.2±8.7	0.836
Pulse Pressure Amplification	1.3±0.2	1.3±0.2	0.991
Resting HR (beats/minute)	66.1±9.8	68.5±11.1	0.205
Left ventricular ejection duration (ms)	323.8±26.8	321.0±31.6	0.594
Blood Pressure (measured seated)			
Brachial SBP (mmHg)	125±12	125±16	0.983
Brachial DBP (mmHg)	79±9	76±11	0.079

Values expressed as mean±standard deviation, median [interquartile range], or number (%) as appropriate.

*Not measured in participants with T2DM on insulin therapy (n=34).

ACEi, angiotensin-converting enzyme inhibitor; Aix75, augmentation index corrected for a heart rate of 75 beats/minute; ARB, angiotensin receptor blocker; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; DBP, diastolic blood pressure; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; HR, heart rate; LDL, low density lipoprotein; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

554 **Table 2. Between-group differences in arterial stiffness and hemodynamics in initial**

555 **response to exercise each parameter (3 or 5 minutes)**

Variable	Without T2DM (n=61)	With T2DM (n=66)	Mean difference (with-without T2DM) (95% CI)
Immediately Post-Exercise			
Exercise time (minutes)	14.8 (14.3, 15.3)	15.0 (14.5, 15.5)	0.2 (-0.6, 1.0)
VO_{2peak} (mL/kg/min)	24.3 (23.1, 25.5)	24.0 (22.9, 25.2)	-0.3 (-2.0, 1.5)
Max HR (beats/min)	154.0 (148.8, 159.2)	153.1 (148.2, 158.0)	-0.9 (-8.5, 6.7)
Peak SBP (mmHg)	173.1 (166.2, 180.0)	182.8 (176.3, 189.4)	9.7 (-0.4, 19.8)
Peak DBP (mmHg)	78.0 (74.1, 81.9)	74.6 (70.8, 78.4)	-3.4 (-9.2, 2.4)
3 minutes			
Brachial SBP (mmHg)	164.0 (158.6, 169.5)	173.0 (167.8, 178.2)	**8.9 (0.9, 16.9)**
Brachial DBP (mmHg)	82.7 (80.7, 84.8)	84.1 (82.1, 86.1)	1.4 (-1.7, 4.5)
Brachial PP (mmHg)	81.4 (76.886.1)	88.8 (84.4, 93.2)	**7.4 (0.6, 14.2)**
cfPWV (m/s)	12.8 (12.0, 13.7)	14.5 (13.7, 15.3)	**1.6 (0.4, 2.9)**
Aortic stiffness β_0	35.0 (29.7, 40.2)	43.6 (38.7, 48.6)	**8.7 (1.0, 16.4)**
HR (beats/min)	98.3 (94.7, 101.8)	98.6 (95.3, 102.0)	0.4 (-4.8, 5.6)

5 minutes			
crPWV (m/s)	8.7 (8.3, 9.0)	8.9 (8.6, 9.2)	-0.3 (-0.2, 0.7)
Central SBP (mmHg)	118.2 (115.0, 121.4)	121.1 (118.0, 124.2)	2.9 (-1.7, 7.7)
Central DBP (mmHg)	79.6 (77.6, 81.6)	81.2 (79.3, 83.1)	1.6 (-1.4, 4.6)
Central PP (mmHg)	38.6 (36.2, 41.0)	40.0 (37.7, 42.2)	1.36 (-2.1, 5.0)
AIx75 (%)	26.0 (24.3, 27.7)	24.4 (22.8, 26.0)	-1.6 (-4.1, 1.0)
Ejection duration (ms)	302.6 (296.1, 309.1)	304.8 (298.6, 311.0)	2.2 (-7.3, 11.7)

AIx75, augmentation index corrected for a heart rate of 75 beats/minute; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; DBP, diastolic blood pressure; PP, pulse pressure; SBP, systolic blood pressure; VO_{2peak}; peak oxygen consumption.

Adjusted means (95% CI) are presented.

Exercise time, VO_{2peak}, maximal HR, ejection duration, HR and AIx75 are adjusted for age, sex, waist:hip ratio and ACEi/ARB use.

cfPWV and crPWV are adjusted for the pre-exercise value, age, sex, waist:hip ratio, ACEi/ARB use, as well as HR and MAP at the time of measurement.

Aortic stiffness β_0 and BP is adjusted for for the pre-exercise value, age, sex, waist:hip ratio, ACEi/ARB use, and HR at the time of measurement.

Table 3: Between-group differences in the area under the curve for arterial stiffness and hemodynamics in response to exercise

Area Under the Curve Variable	Without T2DM (n=61)	With T2DM (n=66)	Mean difference (with-without T2DM) (95% CI)
Brachial SBP (mmHg·min)	-6.6 (-64.6, 51.4)	79.9 (25.5, 134.3)	**86.5 (2.2, 170.7)**
Brachial DBP (mmHg·min)	-42.7 (-73.7, -11.7)	9.4 (-19.7, 38,4)	**52.1 (7.1, 97.1)**
Brachial PP (mmHg·min)	36.2 (-8.4, 80.7)	70.5 (28.7, 112.4)	34.4 (-30.4, 99.2)
cfPWV (m/s·min)	20.7 (12.9, 28.6)	36.3 (28.6, 44.0)	**15.5 (4.0, 27.1)**
Aortic stiffness β_0	105.3 (66.1, 144.5)	175.6 (137.5, 213.6)	**70.2 (12.6, 127.8)**
crPWV (m/s·min)	-2.3 (-7.7, 3.1)	-0.7 (-6.0, 4.7)	1.6 (-6.4, 9.7)
Central SBP (mmHg·min)	-134.1 (-184.4, -83.9)	-79.1 (-126.3, -31.8)	55.1 (-18.4, 128.6)
Central DBP (mmHg·min)	-33.3 (-64.5, -2.2)	0.7 (-28.6, 30.0)	34.0 (-11.6, 79.6)
MAP (mmHg·min)	-63.3 (-97.6, -28.9)	-26.4 (-58.6, 5.9)	36.9 (-13.3, 87.1)
Central PP (mmHg·min)	-100.8 (-135.7, -65.9)	-79.8 (-112.5, -47.0)	21.0 (-30.0, 72.1)

AIx75 (%·min)	-9.0 (-31.3, 13.3)	-33.7 (-55.0, -12.3)	-24.7 (-57.5, 8.2)

All analyses were adjusted for age, sex, waist:hip ratio, and ACEi/ARB use. Adjusted means (95% CI) are presented.

AIx75, augmentation index corrected for a heart rate of 75 beats/minute; BP, blood pressure; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; T2DM, type 2 diabetes mellitus.