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Circadian and sex differences in post-ischemic vasodilation and reactive hyperemia in young individuals and elderly with and without type 2 diabetes

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Abstract

Objective: Cardiovascular events show morning preference and sex differences, and are related to aging and type 2 diabetes. We assessed circadian variations and sex differences in vascular conductance (VC) and blood flow (BF) regulations following a brief bout of forearm ischemia.

Methods: Young healthy individuals (H18-30) and elderly without (H50-80) and with type 2 diabetes (T2DM50-80) of both sexes were included. Forearm VC and BF, and mean arterial pressure (MAP) at baseline and following circulatory reperfusion were measured at 6 a.m. and 9 p.m.

Results: In the morning compared to evening, following reperfusion, the VC and BF increments were similar in H18-30 ($p>.71$), but lower in H50-80 ($p<.001$) and T2DM50-80 ($p<.01$). VC and BF following circulatory reperfusion were higher in men than women in H18-30 ($p<.001$), but similar between sexes in the older groups ($p>.23$).

Conclusions: Forearm vasodilation following reperfusion is attenuated in the morning in the elderly, impairing BF towards an ischemic area. Diabetes does not affect the circadian regulation of VC and BF, but that of MAP. There are sex differences in VC and BF at baseline and after circulatory reperfusion at a young age, being greater in men, which disappear with aging without being affected by diabetes.

KEYWORDS
aging, cardiovascular disease, circadian changes, diabetes mellitus, risk factors, sex differences, sympathetic activation

1 | INTRODUCTION

Cardiovascular events occur more frequently in the morning compared to the evening.[1–3] This fact has led to a growing interest in the investigation of the circadian variations of physiological variables potentially related to cardiovascular risk. The brachial artery flow-mediated vasodilation (FMD) following a period of ischemia is attenuated in the morning compared to the evening, suggesting blunted endothelial function and a greater risk of coronary artery disease in the morning.[4,5] However, artery FMD represents the percent

Abbreviations: BF, blood flow; FMD, flow-mediated vasodilation; H18-30, healthy individuals between 18 and 30 years of age; H50-80, healthy elderly between 50 and 80 years of age; MAP, mean arterial pressure; T2DM, type 2 diabetes; T2DM50-80, elderly with type 2 diabetes between 50 and 80 years of age; VC, vascular conductance.

change in diameter of a single conduit segment.[6,7] This variable does not provide information on the entire limb vasodilation, including changes at the microvascular level, or on the precise neurovascular control of limb blood flow (BF).[6,7] Conversely, the sudden increase in limb vascular conductance (VC) following circulatory reperfusion reflects vasodilation of the whole limb vascular tissues and is a key regulator of limb BF along with the mean arterial pressure (MAP).[7] The sudden increase in BF following circulatory reperfusion, named reactive hyperemia, also provides information on cardiovascular risk.[8–10] Attenuated reactive hyperemia has been related to several risk factors, such as obesity, total/HDL cholesterol ratio, type 2 diabetes (T2DM), smoking, and dyslipidemia.[8–10] VC at baseline is lower in the morning compared to the evening, suggesting a greater constriction of the vascular tissue in the morning.[3] However, it is still unknown whether the limb vasodilation capacity following circulatory reperfusion is likewise attenuated in the morning and whether any circadian restriction of vasodilation impairs subsequent reactive hyperemia towards a downstream ischemic area.

Aging is linked to an increase in cardiovascular events.[1–3] The presence of T2DM further increases such an occurrence.[11,12] There are also overt sex differences in the incidence of cardiovascular events.[6,13] After menopause, women start being at a greater cardiovascular risk compared to men due to the presence of a greater number of risk factors, as well as to the lack of the protective effects of estrogen on cardiovascular health.[13] These sex differences are more evident in individuals with T2DM.[14,15] Women with T2DM show earlier and more deleterious pathophysiological changes in cardiovascular risk-related variables compared to men, as well as greater cardiac risk.[14,15] Considering the different prevalence of cardiovascular events between morning and evening, as well as the impact of sex on cardiovascular risk, this study primarily aims to assess circadian variations of VC and reactive hyperemia following circulatory reperfusion in young healthy individuals, healthy elderly, and elderly with T2DM of both sexes. It is hypothesized that the VC increment following circulatory reperfusion be blunted in the morning compared to the evening within any of these groups, and that any restriction of vasodilation impairs BF towards the ischemic area. It is also hypothesized that any sex differences in VC and BF increments following reperfusion be more marked in individuals with T2DM compared to healthy individuals. Since the endothelial function is attenuated at 6 a.m. compared to at 9 p.m.,[4] evaluations will be performed according to such a timing schedule.

2 | METHODS

Thirty healthy individuals from 18 to 30 years old (H18-30), 30 healthy individuals from 50 to 80 years old (H50-80), and 30 individuals with T2DM from 50 to 80 years old (T2DM50-80) were recruited for this study (details in Table 1). Each group included 15 men and 15 women. All participants met common inclusion (>18 years old) and exclusion criteria (chronic hypertension, pacemaker-dependent, use of beta-blockers and ACE-inhibitors,

TABLE 1 Characteristics of the subjects ($N = 15$ men and $N = 15$ women within each group)

		Age	Weight	Height	BMI	Systolic blood pressure[a]	Diastolic blood pressure[a]	Heart rate[a]	Fasting blood glucose level
	Measure unit	years	kg	m	kg/m^2	mmHg	mmHg	bpm	mg/dL
H18-30	Men	23.0 ± 4.0	73.6 ± 7.1	1.80 ± 0.05	22.6 ± 1.6	122.9 ± 7.4	68.4 ± 5.5	68.3 ± 7.6	76.3 ± 8.2
	Women	23.4 ± 3.1	57.2 ± 8.1*	1.67 ± 0.07*	20.5 ± 2.1*	107.9 ± 9.3*	67.2 ± 8.3	68.2 ± 11.2	71.1 ± 6.8
H50-80	Men	66.1 ± 7.1	76.5 ± 10.0	1.71 ± 0.05	26.2 ± 2.9	136.9 ± 9.4	81.0 ± 4.6	62.2 ± 8.5	88.1 ± 12.4
	Women	66.9 ± 7.4	70.3 ± 9.4	1.61 ± 0.05*	27.1 ± 4.2	128.6 ± 13.2	78.1 ± 6.9	59.6 ± 8.2	86.5 ± 13.9
T2DM50-80	Men	67.3 ± 7.5	86.1 ± 14.9	1.77 ± 0.2	27.6 ± 5.2	144.4 ± 13.7	82.4 ± 8.9	63.5 ± 7.2	138.9 ± 23.4
	Women	66.5 ± 8.1	79.4 ± 22.0	1.59 ± 0.07*	31.2 ± 7.7	140.2 ± 11.4	82.6 ± 8.9	68.0 ± 6.0	146.4 ± 26.0

Note: Data are reported as mean ± SD.
[a]Average value between morning and evening measure at rest.
*$p < .05$ in women compared to men.

pregnancy or presumed pregnancy).[16,17] Healthy subjects had to report fasting blood glucose lower than 100 mg/dL. Subjects with T2DM had to have been diagnosed with T2DM for at least 1 year, as well as to be free of severe autonomic neuropathy, pre-proliferative and proliferative retinopathy, and renal failure.[17] Subjects were instructed to avoid caffeine for 24 hours prior to testing, as well as to sleep at least 8 h the night before the experiments.[18] Tests were performed at 6 a.m. and 9 p.m., when the endothelial function is different,[4] at the Cardiovascular Physiology Laboratory, School of Sports Science, University of Verona. The laboratory temperature was controlled at 25°C. Participants reported to the laboratory twice for morning and evening measures, respectively. Within each group, 50% of the subjects performed the experiments in the morning-evening order, whereas the other 50% performed the experiments in the evening and the following morning. The primary endpoints of our study were the circadian and sex differences in VC and BF within each group. Because of the lack of specific studies, the sample size within each group was determined via software (GPower 3.1.9.7; Universität Düsseldorf, Germany) after having collected data from 10 participants in each group. Such an analysis suggested the need for (H18-30: $n = 14$; H50-80: $n = 18$; T2DM50-80: $n = 20$) subjects to reach a statistical power of 80% in the assessment of circadian changes in VC and BF, while the need for (H18-30: $n = 18$; H50-80: $n = 24$; T2DM50-80: $n = 26$; to be equally divided in men and women) subjects to reach a statistical power of 80% in the assessment of sex differences. The comparison of differences among groups has only an explorative role within this study. The study was approved by the Ethics Board of the University of Verona (3293CESC) and conducted following the declaration of Helsinki. Informed oral and written consent was obtained from all participants before starting any test.

2.1 | Experimental protocol

Both in the morning and evening sessions, participants lay supine on an ambulatory bed throughout the test with their right arm extended on a support for ultrasound measures. Subjects were instructed to stay relaxed, breathe regularly, and not to speak throughout the experiment. Participants were suited with a beat-by-beat finger arterial pressure monitoring system (Portapres; Finapres Medical System BV) on the third medial phalanx of the left hand recording the MAP. The finger arterial pressure monitoring system was calibrated on brachial artery arterial pressure in each subject. Brachial artery arterial pressure was measured manually with the Riva-Rocci method. Participants were also suited with the 3-lead electrocardiograph (ECG) of the Ultrasound Device (LOGIQ S7 pro, GE). Subjects were suited with a pressure cuff around the right forearm and distal to the imaged artery. A rapid cuff inflator (Hokanson) was used to inflate the cuff >50 mmHg above systolic arterial pressure and to deflate the cuff within approximately 300 ms. While subjects were recommended to stay relaxed and breathe regularly, scanning of the right brachial artery via pulsed Doppler ultrasonography was started. Ultrasound data consisted of the concurrent measure of mean blood velocity and brachial artery diameter. The brachial artery was scanned above the antecubital fossa. The probe location was marked to evaluate the same artery section in the morning and the evening. Data were measured with a 4.4 MHz probe and a 60° angle of insonation. The ultrasound gate was regulated to scan the whole artery width. The sample volume was aligned and regulated according to vessel size. Ultrasound measures were performed by an expert sonographer with >500 h of experience. Once these procedures were completed, the main experiment started as follow. Participants performed 15 min of complete rest. Participants were steady during the resting time. The forearm cuff on the right arm was thus inflated for 5 min and then suddenly released. Ultrasound and Portapres data were collected during a 3-min baseline before cuff inflation, during cuff inflation, and during the 90 s after cuff release. Data were synchronized throughout the test by using signal markers.

2.2 | Data analysis

Video analysis software (Medical Imaging Applications LLC) was used to detect the brachial artery diameter and mean blood velocity from the ultrasound video clips. Brachial artery diameter was measured at the onset of each R-wave of the ECG. Arterial pressure data were exported from the proprietary software of Portapres (BeatScope 1.1; Finapres Medical System BV). The mean values of brachial artery diameter, mean blood velocity, and MAP were calculated at baseline as well as during the 0–30 s, 30–60 s, and 60–90 s after cuff release. Mean values of brachial artery diameter and mean blood velocity were used to calculate the mean values of BF as follows: mean blood velocity (cm*s − 1)*π*r^2*60 (mL*min − 1), where r is the mean brachial artery radius. Similarly, mean values of VC at baseline and at different time points after circulatory reperfusion were calculated by dividing the mean values of BF by the mean values of MAP recorded at baseline and during the 0–30 s, 30–60 s, and 60–90 s after cuff release. The peak systolic velocities (peak on the first ascending tract of the Doppler blood velocity waveforms) in the Doppler Ultrasound recordings were measured over the first five heartbeats after cuff release and then averaged.

2.3 | Statistics

Data passed the normality test. Within each group, circadian variations in VC, BF, and MAP at baseline and over the 0–30 s, 0–60 s, and 0–90 s after circulatory reperfusion were identified via two-way repeated-measures ANOVA and Sidak post-hoc test. The average values between morning and evening values of VC, BF, and MAP were used to assess differences between sexes. Within each group,

sex differences in VC, BF, and MAP at baseline and over the 0–30 s, 0–60 s, and 0–90 s after circulatory reperfusion were identified via two-way repeated-measures ANOVA and Sidak post-hoc test. The average values between morning and evening values of VC, BF, and MAP were also used to identify differences among groups through one-way ANOVA test and Sidak post-hoc test. In this regard, one-way ANOVA was singularly performed on data at baseline and over the 0–30 s, 0–60 s, and 0–90 s after cuff release. Change-scores (average value over the 0–90 s after cuff release minus preceding baseline value) in VC, BF, and MAP after circulatory reperfusion were calculated in the three groups. Within each group, change-scores in VC, BF, and MAP were compared in men versus women and in the morning versus evening via ANCOVA, using the baseline value as covariate. Sidak post-hoc test was then used to assess differences across the data. Within each group, circadian variations in brachial artery diameter and mean blood velocity at baseline and over the 30 s, 60 s, and 90 s after circulatory reperfusion were identified via two-way repeated-measures ANOVA and Sidak post-hoc test. Circadian variations and sex differences in the peak systolic velocity within each group were identified by paired and un-paired *t*-tests, respectively, comparing the data collected in the morning versus evening and men versus women. Statistical significance was set at $p < .05$. GraphPad Prism 8 (GraphPad Software) was used to perform statistical analysis and graphs. Results are reported as mean ± standard deviations.

3 | RESULTS

As shown in Figure 1, baseline VC was higher in H18-30 compared to the other groups ($p < .03$) and similar between H50-80 and T2DM50-80 ($p = .87$). However, no differences in VC were noted after circulatory reperfusion among groups ($p > .13$). Baseline BF did not differ among groups ($p > .84$), but it was greater in H50-80 compared to H18-30 ($p < .01$) after cuff release. MAP was greater in H50-80 compared to H18-30 ($p < .01$), as well as in T2DM50-80 compared to H50-80 ($p < .01$), both at baseline and after cuff release. Comparisons among groups of brachial artery diameter, mean blood velocity, and peak systolic velocity data among groups are reported in the Table S1.

3.1 | Circadian variations

As shown in Figure 2, values of VC, BF, and MAP at baseline and after circulatory reperfusion were similar in the morning compared to the evening in H18-30 ($p > .71$). Values of VC and BF at baseline and after circulatory reperfusion were lower in the morning compared to the evening in H50-80 ($p < .001$) and T2DM50-80 ($p < .01$). Values of MAP were similar in the morning compared to the evening in H50-80 ($p > .53$) but higher in T2DM50-80 ($p < .01$). Brachial artery diameter was statistically lower ($p < .001$) in the morning compared to the evening at each time point and in each group (Table S2). Mean blood velocity was similar ($p > .52$) in the morning compared to the evening at every time point and in each group (Table S3). The peak systolic velocity scored similarly ($p > .60$) in the morning compared to the evening in each group (Table S4).

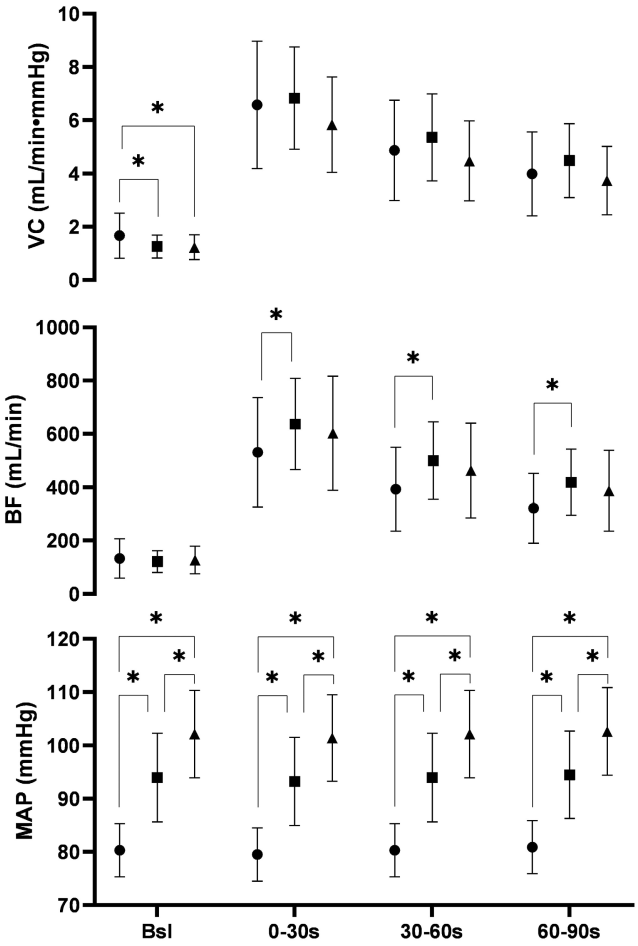

FIGURE 1 Differences in vascular conductance (VC), blood flow (BF), and mean arterial pressure (MAP) at baseline and after circulatory reperfusion across the three groups (●H18-30; ■H50-80; ▲T2DM50-80). (*$p < .05$; mean value between morning and evening data provided by each individual, in $N = 15$ men and $N = 15$ women within each group; data are reported as mean ± SD.)

3.2 | Sex differences

As shown in Figure 3, values of VC, BF, and MAP at baseline and after circulatory reperfusion were higher in men compared to women in H18-30 ($p < .001$), while no sex differences in such variables were noticed in the older groups ($p > .23$). Men reported higher ($p < .03$) values of brachial artery diameter at every time point and in each group (Table S2). Mean blood velocity was similar ($p > .25$) in young men compared to young women at every time point (Table S3). However, mean blood velocity was higher ($p < .02$) in women compared to men in H50-80. Women in T2DM50-80 reported higher ($p = .02$) values of mean blood velocity compared to men at rest, but similar ($p > .16$) values after cuff release. The peak systolic velocity scored similarly ($p > .63$) in men compared to women in each group (Table S4).

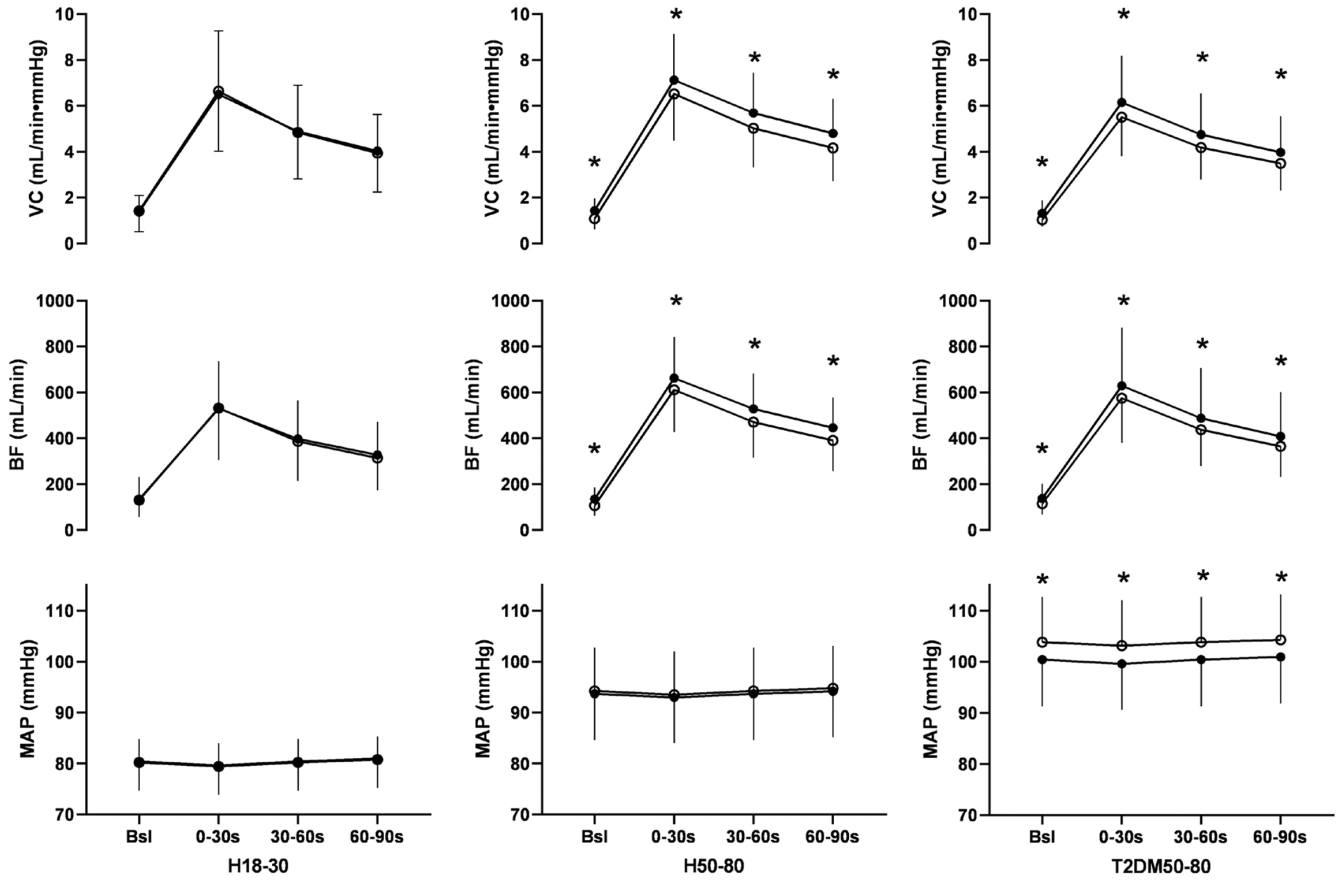

FIGURE 2 Changes in vascular conductance (VC), blood flow (BF), and mean arterial pressure (MAP) at baseline (Bsl) and 30 s, 60 s, 90 s after circulatory reperfusion in the morning (○) compared to the evening (●) within each group. (*$p < .05$; morning values vs. evening values collected in $N = 15$ men and $N = 15$ women within each group; data are reported as mean ± SD.)

3.3 | Circadian variations in men compared to women

As shown in Figure 4, change-score analysis confirms significant differences in VC and BF after cuff release between young men and women that are independent of different baseline values, as well as that these variables change similarly between morning and evening in both sexes. In contrast, change-scores in MAP are not statistically different between young men and women when different baseline MAP values are accounted for, although change-scores in MAP remain similar between morning and evening. In H50-80 and T2DM50-80, change-scores in VC and BF were not statistically different between morning and evening, as well as between men and women. There are no significant differences in the change scores in MAP in the older groups.

4 | DISCUSSION

We investigated circadian and sex differences in VC and BF following circulatory reperfusion in young individuals and elderly with and without type 2 diabetes. Measures were performed at 6 a.m. compared to at 9 pm, when the endothelial function is attenuated in the morning compared to the evening.[4] Rapid vasodilation after circulatory reperfusion is due to the sudden release of local vasodilatory agents produced by the vascular endothelium or the muscle itself, which quickly (<2 s) relax the vascular smooth muscle to allow sudden reactive hyperemia.[6,19] The changes in VC and BF after cuff release consist of a first phase of approximately 20–30 s in which maximal vasodilatory and hyperemic response occurs, followed by a second phase reflecting the persistence of vasodilatory effects over time in which VC and BF progressively return to the previous baseline values.[6,19] To verify whether any differences in the changes of VC and BF were present throughout the reperfusion phase or only at specific moments (i.e., immediately or at delayed times only) after circulatory reperfusion, we calculated the mean values of VC and BF over the 0–30 s, 30–60 s, 60–90 s after circulatory reperfusion. Blood velocity profile over time in Doppler ultrasound tracings exhibits an initial sharp systolic rise up to peak systole. Previous studies have reported a link between peak systolic velocity and risk of cardiovascular disease, being greater in patients with hypertension[20] and congestive heart failure[21] compared to control individuals.

As shown in Figure 1, VC at baseline was higher in young individuals compared to the older ones, consistent with the notion of an increase in vascular peripheral resistance with aging. Augmented

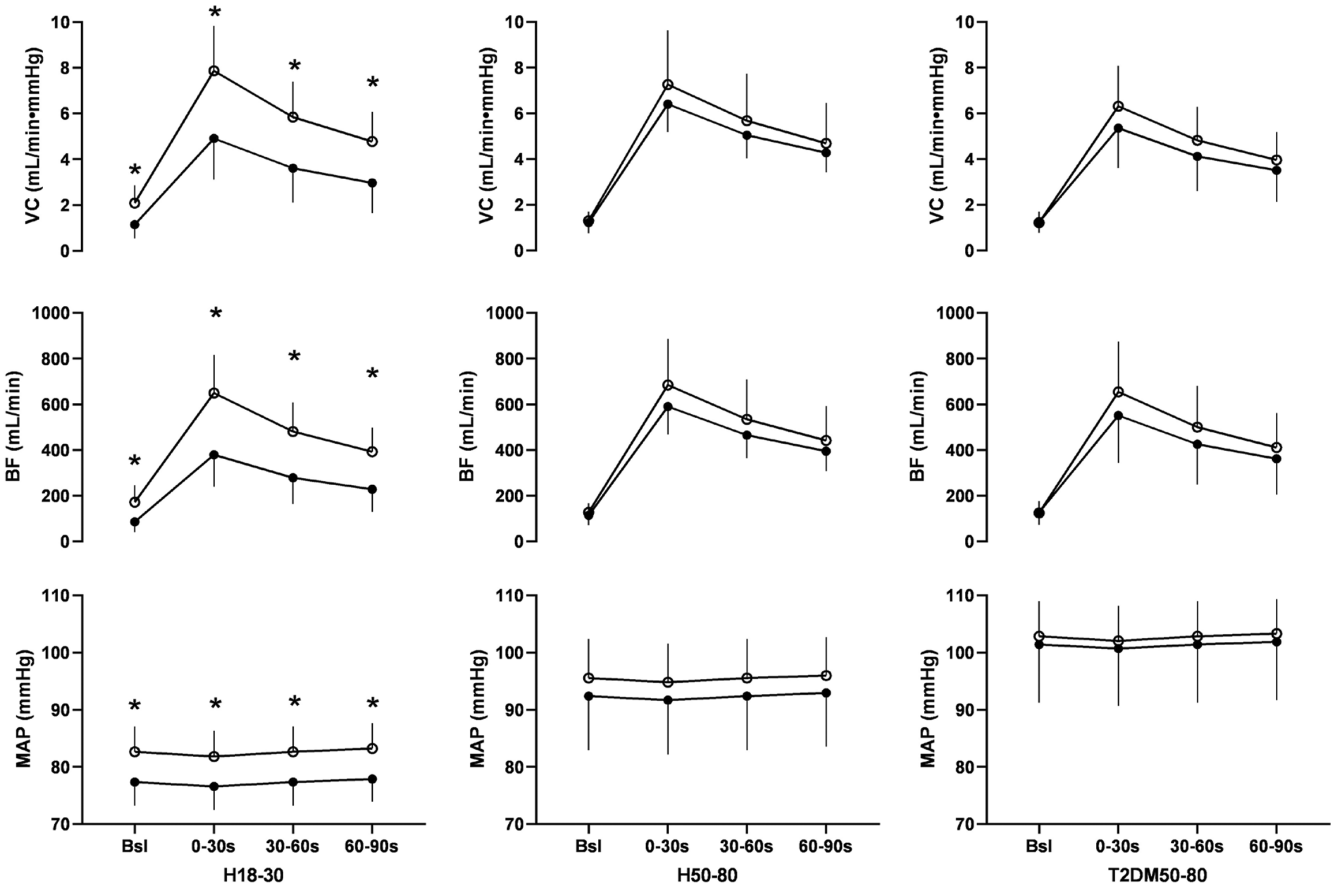

FIGURE 3 Changes in vascular conductance (VC), blood flow (BF), and mean arterial pressure (MAP) at baseline (Bsl) and 30s, 60s, 90s after circulatory reperfusion in men (○) compared to women (●) within each group. (*$p<.05$; mean value between morning and evening data provided by each individual, in $N=15$ men vs. $N=15$ women within each group; data are reported as mean ± SD).

baseline levels of sympathetic outflow along with diminished nitric oxide bioavailability have been suggested to account for the increase in peripheral resistance with aging.[19,22–25] Additionally, there are further aging-related factors that may be responsible for such a reduction in baseline VC, including changes in the vascular intrinsic myogenic response, as well as in circulating hormones such as catecholamines, cortisol, and estrogen.[26–29] However, VC after circulatory reperfusion was similar among groups at each time point. In this regard, the dynamic regulation of VC is based on the interaction between vasodilation and vasoconstriction forces.[7] A possible explanation for our findings might be that the sudden release of vasodilator agents following circulatory reperfusion be effective in counteracting any aging-induced restriction in VC in the older groups.[6] MAP at baseline and after circulatory reperfusion was greater in healthy elderly compared to healthy young individuals, as well as in elderly with T2DM compared to healthy elderly. Arterial pressure increases with aging due to several factors, including an increase in sympathetic tone.[19,22–25] The sympathetic outflow further increases in the presence of T2DM, which may explain the greater MAP in individuals with T2DM compared to healthy age-matched individuals.[30–32] Tissue BF is precisely determined by the interaction between tissue VC and MAP.[7] Thus, similar baseline values of BF in old versus young individuals could be explained by higher values of MAP along with lower values of VC. The increase in BF after circulatory reperfusion was greater in healthy old individuals than in healthy young individuals, probably due to the greater MAP. However, the presence of T2DM abolishes such a difference, despite this condition further augments MAP. Overall, these findings show that differences or similarities in VC and BF among groups identified at baseline can change after cuff release.

Interestingly, differences in brachial artery diameter are consistent with differences in BF among groups, but not with those in VC. Brachial artery diameter is just one of the determinants of BF since the section of a vessel is inversely related to the resistance that the blood encounters while flowing, in accordance with Poiseuille's law.[33] However, changes in brachial artery diameter do not reveal the contribution of MAP in BF regulation. In contrast, VC is a physiological parameter that conceptually expresses the ease at which blood flows at a given pressure difference through the entire vascular tissue, considering both the contribution of flow resistance and that of perfusion pressure (MAP),[7] as well as changes at microvascular level. Despite changes in brachial artery BF and MAP after cuff release were found among the three groups, no differences in the average peak systolic velocity were identified.

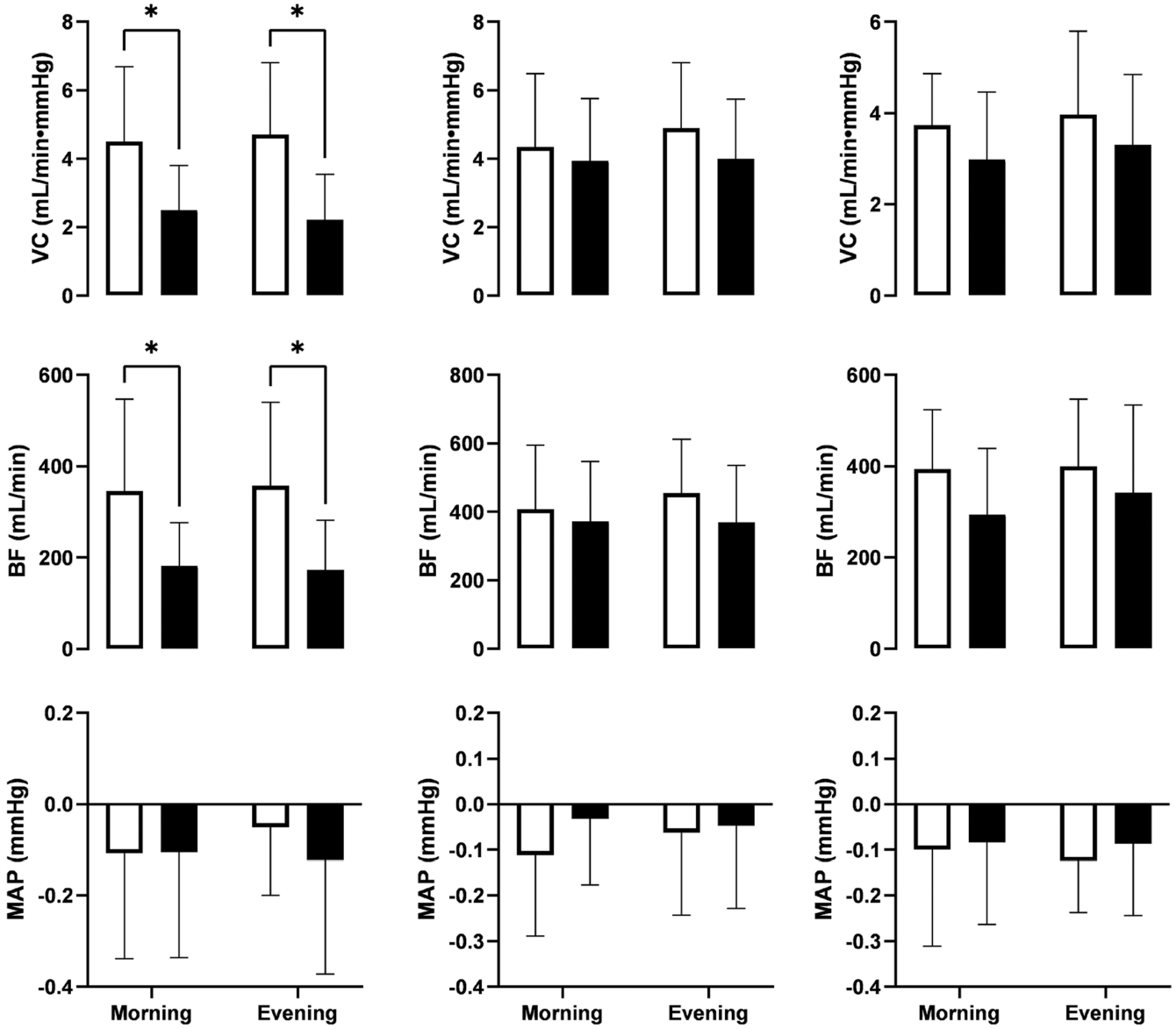

FIGURE 4 Change-scores from baseline in the average (0–90 s) vascular conductance (VC), blood flow (BF), and mean arterial pressure (MAP) after circulatory reperfusion in men (white bars) compared to women (black bars) within each group. (*$p<.05$; $N=15$ men vs. $N=15$ women within each group; data are reported as mean ± SD).

4.1 | Circadian variations

As shown in Figure 2, the mean values of BF at baseline and after circulatory release were similar in the morning compared to the evening in young healthy individuals. This finding is consistent with similar values of VC and MAP between morning and evening in young subjects. Interestingly, lower values of BF at baseline and after circulatory release in the morning compared to the evening were noticed in H50-80, which could only be explained by the lower increase in VC after cuff release since MAP values were similar. Similar responses were found in older individuals with T2DM, suggesting that T2DM does not change the normal circadian variation of VC and BF noticed in healthy older individuals. Figure 4 shows the change-scores in VC and BF from previous baseline values in the three groups. Interestingly, this analysis suggests that the changes in VC and BF from baseline after cuff release are similar between morning and evening in all groups. Therefore, this analysis would confirm that there are baseline reductions in VC and BF which then impact the absolute values of these variables after cuff release. While the presence of lower forearm VC at baseline in healthy elderly has already been reported,[3] our findings add to the literature that also the subsequent forearm vasodilation is attenuated in the morning in healthy elderly and that such an attenuated vasodilation capacity in the morning impairs BF towards an ischemic area. Since forearm rapid vasodilation is largely endothelium-mediated,[34] our finding of blunted VC increment following circulatory reperfusion agrees with previous studies reporting attenuated endothelial function at 6 a.m. compared to 9 p.m. assessed through brachial artery

FMD.[4] Lower VC values at baseline and after cuff release in the morning compared to the evening might also be consistent with increased sympathetic outflow levels at baseline in the morning,[3] but not with increased sympathetic vasoconstriction responsiveness in the morning. Indeed, we have previously shown that the peripheral vasoconstriction response triggered by acute activation of the sympathetic nervous system is similar between morning and evening in elderly individuals with and without T2DM of similar age to those recruited in the present study.[35] Our data also reveal a circadian variation of MAP in individuals with T2DM, but not in healthy age-matched individuals. Increases in sympathetic outflow as a consequence of T2DM have been suggested.[30–32] Such an increase in the sympathetic outflow caused by T2DM might be particularly evident in the morning, explaining the higher values of MAP in the morning in individuals with T2DM. Brachial artery diameter at baseline and at each time point after cuff release proved to be lower in the morning compared to the evening within each group. This finding is likewise consistent with a possible attenuated endothelial function along with augmented baseline sympathetic outflow in the morning compared to the evening. However, as mentioned above, changes in BF also depend on limb perfusion pressure (MAP) in addition to changes in flow resistance due to variations in the vessel section. Despite the circadian changes in VC and BF in the older groups were found, peak systolic velocity scored similar in the morning compared to the evening, suggesting that changes in BF may be independent of the velocity of ventricular ejection or viscoelastic properties of the arterial wall.[20,21] In support of this notion, we have previously shown that carotid-femoral pulse wave velocity, an index related to central artery stiffness, is similar at 6 a.m. compared to 9 p.m. in elderly individuals with and without T2DM of similar age to those recruited in the present study.[36]

4.2 | Sex differences

As shown in Figure 3, the mean values of BF at baseline and after circulatory release were augmented in men compared to women in young healthy individuals. This finding is consistent with higher values of VC and MAP in men compared to women in young subjects.[6] In contrast, values of BF, VC, and MAP were similar between men and women in the older groups both at baseline and after circulatory reperfusion. In this regard, Figure 4 shows that the changes in VC and BF from baseline after cuff release are greater in young men than in young women regardless of different baseline values, and that this sex difference disappears with aging, regardless of the presence of T2DM. Neurovascular regulation differs between sexes and over aging due to several reasons. Resting sympathetic nerve activity is lower in young women compared to young men.[6,37] There is no correlation between muscle sympathetic nerve activity and vascular resistance in young women, whereas it is present in young men, suggesting a blunted sympathetic vascular transduction in women.[37–40] Indeed, young women show blunted vasoconstriction in response to sympathetic stimulants.[6,37] A relaxing effect of oestrogens on vascular smooth muscle has also been suggested in young women.[41] Overall, such factors account for the lower values of VC and arterial pressure in young women compared to men. However, muscle sympathetic nerve activity increases with aging.[22–25] Following menopause, muscle sympathetic nerve activity in women can exceed that of men.[25,39] Moreover, there is a decline in oestrogens production in women after menopause.[13] Consequently, women show a steeper increase in arterial pressure with aging, especially after menopause, which may abolish sex differences in adulthood that are otherwise present at a young age.[42] No sex differences in peak systolic velocity after cuff release were found between men and women at any age, regardless of the presence of T2DM.

4.3 | Importance of the current findings in the context of microcirculation

Sudden vasodilation of a vascular tissue following a period of ischemia, and consequently the capacity to provide fast blood supply towards an area in need of oxygen, takes on particular importance in tissues where oxygen is vital.[6,43] In this regard, microcirculation plays a primary role in tissue homeostasis by matching local tissue perfusion with oxygen demand.[43] Microvascular dysfunction has indeed been proposed as a powerful predictor of cardiovascular events.[43] A crucial aspect that makes our findings possible is the focus at the microvascular level by globally assessing the changes that take place in the upper limb vascular tissue, rather than focusing on the diameter change of the brachial artery. The flow-mediated vasodilatation of a conduit artery is a consequence of the shear stress-mediated nitric oxide release caused by the BF directed towards the entire downstream vascular and microvascular tissues, which dilate to accommodate blood.[43,44] Previous studies have repeatedly shown that blunted brachial artery FMD may reflect blunted coronary function[45] and have strongly focused on this relationship to assess vascular function. However, as evidenced by the numerous inconsistencies between changes in brachial artery diameter and VC or BF reported in our data, the information provided by changes in a conduit artery diameter is not exhaustive to investigate changes in BF regulation as these do not consider the impact of the limb perfusion pressure (MAP). In contrast, the assessment of VC changes also provides a reasonable good picture of the changes that take place at the microvascular level. In the elderly, our findings show the presence of a greater state of vasoconstriction in the morning at baseline, which subsequently impacts the capacity of the microcirculation to dilate after a period of ischemia. Although this effect could have been expected, it had not yet been verified and could lead to new insights into cardiovascular prevention. Our data may suggest that this phenomenon takes place with aging and remains similar in the presence of T2DM despite the different vascular responses that individuals with T2DM may develop. Indeed, we have previously shown that individuals with T2DM seem to exhibit greater neurogenic vasodilation compared to similar age-matched individuals

without T2DM in response to sympathetic stress,[35] but this change does not appear to impact tissue vasodilation following a brief period of ischemia according to the present data. The greater morning vasoconstriction state at baseline could be determined by reduced endothelial function, greater sympathetic outflow, or both, and be a potential actor involved in the greater cardiovascular risk in the morning. Future studies are needed to score to what extent this reduction in vasodilation capacity may affect cardiovascular risk and determine whether this reduction in hyperemia critically affects tissue re-oxygenation. The literature also reports important sex differences in BF regulation, as well as on the impact of cardiovascular disease, which vary with aging and the presence of T2DM.[6,35,39,40] Women show a lower cardiovascular risk than men until menopause.[13] Thereafter, this trend starts reversing due to the decline in estrogen bioavailability and its cardioprotective effect.[13] The focus on VC has allowed for a fair comparison between sexes, which goes beyond different MAP values between men and women that these may report over the course of life and in the presence of T2DM. The change-scores confirm that there are sex differences in vascular regulation at a young age that are independent of different baseline values between men and women, and that these differences are abolished with aging.[36,39] The study of sex differences in vascular and microvascular regulation has led to new insights into cardiovascular medicine and prevention. The documentation of potential causes affecting vascular health in one sex has led to translational research and development of therapeutics to apply to the opposite sex, as well as to different treatments between sexes in targeting cardiovascular diseases.[46]

4.4 | Limitations of the study

A young population with diabetes was not included in the study. In this regard, the recruitment of a group of young individuals with T2DM is challenging. Although young individuals with type 1 diabetes are more common, the vascular effects of this condition differ from those of T2DM.

5 | CONCLUSIONS

Forearm vasodilation following circulatory reperfusion is attenuated in the morning compared to the evening in healthy elderly and such an attenuated vasodilation capacity impairs BF towards an ischemic area. Such impairment could be determined by a higher constriction state of the vascular tissue at baseline in the morning which also impacts the subsequent capacity to vasodilate. The presence of T2DM does not affect circadian responses in VC and BF compared to those observed in healthy individuals of similar age, but induces circadian variations of MAP, being greater in the morning. There are overt sex differences in VC, BF, and MAP at baseline and after circulatory reperfusion at a young age, being greater in men, which disappear with aging without being affected by T2DM.

6 | Perspective

The fact that, in the elderly, the attenuated vasodilation capacity in the morning impairs blood flow to an ischemic area could be relevant for organs and tissues where oxygen is vital. The higher mean arterial pressure induced by diabetes could be a functional mechanism to maintain adequate reactive hyperemia. Further studies are needed to verify these hypotheses.

AUTHOR CONTRIBUTIONS

AG was involved in study concept and design, subject recruitment, data acquisition, and analysis, formal analysis and investigation, and original draft preparation. AG, AC, PM, AVM, FS, and CT were involved in data interpretation and review and critical revision of the manuscript. All authors read and approved the final version of this manuscript.

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None declared.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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