

Insulin Increases The Central-To-Peripheral Arterial Stiffness Gradient in Response To Hyperglycemia in Healthy Humans: A Randomized Four-Way Crossover Study

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Abstract

Background: Increasing arterial stiffness is a physiological feature of vascular aging that is accelerated by conditions that enhance cardiovascular risk, including diabetes mellitus. Emerging evidence demonstrates that reversal of the normal central-to-peripheral arterial stiffness gradient predicts adverse cardiovascular consequences, including target organ damage. Preferential stiffening of central over peripheral arteries has been reported in type 2 diabetes, though mechanisms for this remain unclear.

Methods: We tested the effect of acutely increasing plasma glucose, plasma insulin, or both on central arterial stiffness (carotid-femoral pulse wave velocity) and peripheral arterial stiffness (radial artery augmentation index) in a randomized, four-way, crossover study of 19 healthy young adults. We also measured myocardial oxygen supply-demand (subendocardial viability ratio) and hemodynamic function.

Results: Carotid-femoral pulse wave velocity increased during hyperglycemic-hyperinsulinemia (+0.4 m/s; $p=0.02$) but not with euglycemia, hyperglycemia, or euglycemic-hyperinsulinemia. There were no significant changes in radial artery augmentation index within any protocol (all $p>0.05$), though this value trended lower with hyperglycemic-hyperinsulinemia (opposite of the observed effect on carotid-femoral pulse wave velocity). No changes were observed in subendocardial viability ratio within any protocol. Heart rate significantly increased only during hyperglycemic-hyperinsulinemia (+3.62 bpm; $p=0.02$). There was a significant inverse correlation between peripheral and arterial stiffness during hyperglycemic-hyperinsulinemia.

Conclusions: We conclude that combined hyperglycemia and hyperinsulinemia acutely increases aortic stiffness, diminishes the normal central-to-peripheral arterial stiffness gradient, and increases heart rate in healthy humans. (ClinicalTrials.gov number NCT03520569; registered 9 May 2018).

Clinical Trial Information: ClinicalTrials.gov identifier NCT03520569 (registered 9 May 2018).

Background

Arterial stiffness develops from dynamic interactions between structural and cellular elements of the vessel wall influenced by both hemodynamic forces and extrinsic factors (including hormones, salt, and glucose) [1]. Stiffness is not uniformly distributed throughout the arterial tree but is often patchy [2, 3], occurring in central elastic and conduit muscular arteries while sparing smaller arteries [1, 4, 5]. This pattern is due to marked differences in the expression of arterial wall components when moving from central-to-peripheral vessels, with elastin predominating in central elastic arteries and collagen fibers predominating in conduit muscular arteries [6].

Increasing arterial stiffness is a physiological feature of vascular aging that is accelerated by conditions with greater cardiovascular risk, including DM [7]. Vascular aging, even in the absence of atherosclerosis, leads to intimal and medial thickening (i.e., vascular remodeling) as well as gradual loss of arterial

elasticity [8]. Notably, arterial stiffness often antedates and is itself a strong risk factor for a spectrum of cardiovascular diseases (e.g., arterial hypertension, heart failure, myocardial infarction, etc.) [6, 7]. Data from numerous studies demonstrate that aortic stiffness (assessed by carotid-femoral pulse wave velocity (cfPWV)) is an important, independent determinant of cardiovascular risk in multiple populations [9–15] and may explain some of the notable residual cardiovascular risk associated with even well-controlled hypertension [16].

In healthy young adults, the central aorta is quite elastic, while peripheral, muscular arteries are inherently stiffer [6]. However, studies of the general population [17, 18] and hypertensive persons [4] demonstrate that age-related increases in peripheral artery stiffness are less rapid than in the central arteries [6]. This differential rate of stiffening results in aortic stiffness equaling or exceeding peripheral stiffness in the majority of older individuals [6]. This change of the central-to-peripheral arterial stiffness gradient is associated with a number of adverse cardiovascular consequences, including target organ damage to heart, brain, and kidney [6, 19–21].

Interestingly, preferential stiffening of central over peripheral arteries occurs in type 2 DM [22–24], though mechanisms responsible for this finding are unclear. A recent editorial encouraged investigation of healthy cohorts to understand mechanisms contributing to accelerated vascular aging [25]. In this study, we sought to quantify the independent effects of elevated circulating concentrations of insulin, glucose, and both on central and peripheral arterial stiffness in healthy humans. To isolate the effects of insulin and glucose from those of incretins and autonomic changes that occur with oral glucose, we used intravenous glucose and insulin infusions with co-administration of octreotide (OCT). We measured central arterial stiffness with carotid-femoral pulse wave velocity (cfPWV), peripheral arterial stiffness with radial artery augmentation index (AI), myocardial oxygen supply and demand with subendocardial viability ratio (SEVR), and hemodynamic changes during euglycemia, hyperglycemia, euglycemic-hyperinsulinemia, and hyperglycemic-hyperinsulinemia.

Materials And Methods

Recruitment and Study Population

Recruitment for this study was achieved by community advertisement and direct advertisement to healthcare clinics both within and outside the University of Virginia (UVA) Health System. Healthy young adults met inclusion criteria if they were ≥ 18 and ≤ 35 years old, had normal body mass index (18–25 kg/m²), did not have DM, and had fasting plasma glucose < 100 mg/dL and blood pressure $< 140/90$ mmHg at time of screening. Subjects were excluded if they were current smokers or quit smoking < 5 years ago, had a first-degree relative with type 2 DM, were taking vasoactive medications (e.g., anti-hypertensives, diuretics, statins, etc.), were pregnant (i.e., positive pregnancy test) or nursing, had history of allergy or prior adverse reaction to octreotide, or significant premorbid disease that could, in the investigator's opinion, affect outcome measures or subject safety.

Clinical Assessment and Initial Screening

All screening visits and infusion studies were conducted at the UVA Clinical Research Unit (CRU). Each subject gave written informed consent at their initial visit prior to being carefully screened to verify inclusion/exclusion criteria and certify overall good health. Screening included a detailed medical history and physical examination along with fasting measures of complete blood count, comprehensive metabolic panel, lipid panel, plasma glucose, and serum pregnancy test.

Experimental Protocols

Randomization was conducted by study personnel using a 1:1:1:1 allocation with a computer-generated sequence program [26]. After randomization, study personnel were blinded to subject and protocol when evaluating outcome measures. Subjects underwent four infusion protocols (Figure 1) designed to test the effects of euglycemia, hyperglycemia, euglycemic-hyperinsulinemia, and hyperglycemic-hyperinsulinemia on arterial stiffness. All protocols were approved by the UVA Institutional Review Board (#19948), with each protocol being performed ≥ 2 but ≤ 4 weeks apart for individual subjects. For each protocol, we measured cfPWV, AI, SEVR, systolic blood pressure, diastolic blood pressure, pulse pressure, mean arterial pressure, and heart rate immediately before (i.e., baseline) and at the end of the infusion period (Figure 1). Study participants were instructed to avoid alcohol, exercise, and caffeine for 24 hours and fast overnight prior to admission to the CRU. Infusion studies began with placement of intravenous catheters in the right wrist for blood sampling and in the right antecubital fossa for administration of insulin, glucose, and octreotide (OCT). Studies began with simultaneous infusion of regular insulin and OCT to maintain plasma insulin near basal levels. We did not replace glucagon or growth hormone, as there is currently no evidence that acutely suppressing basal levels of either hormone affects vascular function.

Protocol A (Euglycemia): A 90-minute saline infusion was initiated, with baseline vascular function measurements obtained during the final 30 minutes (Figure 1A). Then, OCT (30 ng/kg/min) with basal insulin replacement (0.15 mU/min/kg) was infused for 240 minutes. Blood glucose (BG) was sampled every 10 minutes and plasma insulin every 30 minutes. Euglycemia (EU) was maintained by a variable-rate glucose infusion using the negative feedback principle [27]. We then repeated vascular measurements over the final 30 minutes of study.

Protocol B (Hyperglycemia): Octreotide and basal insulin replacement were continuously infused for 90 minutes, with baseline vascular measurements obtained over the final 30 minutes (Figure 1B). Then, a primed, continuous variable-rate 20% dextrose infusion began to acutely raise and maintain BG at ~ 200 mg/dL using the hyperglycemic clamp method [27]. BG was sampled every 5 minutes and plasma insulin every 30 minutes, with repeat vascular measurements obtained over the final 30 minutes of hyperglycemia.

Protocol C (Euglycemic-Hyperinsulinemia): Euglycemia was maintained throughout this protocol by a variable-rate 20% dextrose infusion using the negative feedback principle [27]. Baseline arterial stiffness measurements were obtained during the final 30 minutes of an OCT (30 ng/kg/min) plus basal insulin

(0.15 mU/min/kg) infusion (Figure 1C). Then, hyperinsulinemia was initiated with a primed (2 mU/kg/min x 10 min), continuous (1 mU/kg/min x 110 min) infusion and OCT continued for 120 minutes. Blood glucose (BG) was sampled every 5 minutes and plasma insulin every 30 minutes, with repeat arterial stiffness, SEVR, and hemodynamic measurements obtained during the final 30 minutes of the insulin clamp.

Protocol D (Hyperglycemic-Hyperinsulinemia): As in Protocol C, a variable-rate 20% dextrose infusion maintained euglycemia while OCT (30 ng/kg/min) and basal insulin (0.15 mU/min/kg) were simultaneously infused for the first 90 minutes of this study (Figure 1D). Then, a primed, variable-rate 20% dextrose infusion began to acutely raise and subsequently maintain BG at ~200 mg/dL using the hyperglycemic clamp method [27]. BG was then sampled every 5 minutes and plasma insulin every 30 minutes, with baseline arterial stiffness measurements obtained over the final 30 minutes of the 120-minute hyperglycemic period (Figure 1B). Subsequently, hyperinsulinemia was initiated with a primed (2 mU/kg/min x 10 min), continuous (1 mU/kg/min x 110 min) infusion with OCT and hyperglycemia maintained for 120 minutes. BG was sampled every 5 minutes with plasma insulin every 30 minutes, and repeat arterial stiffness, SEVR, and hemodynamic measurements were again obtained during the final 30 minutes of the insulin clamp.

Hemodynamics: Clinical hemodynamic assessments were obtained at two time points during each protocol (Figure 1). Blood pressure, pulse pressure, mean arterial pressure, and heart rate were measured and/or calculated with a Sphygmacor tonometer (ATCOR USA; Napierville, IL).

Carotid-Femoral Pulse Wave Velocity (cfPWV): To assess central aortic stiffness, cfPWV was measured per expert recommendations [28] using a Sphygmacor tonometer by the same trained operator. To minimize the effects of sympathetic activity on cfPWV measurements, participants laid in the supine position in a temperature-controlled room for at least 15 minutes prior to measurement. We measured the distance from the suprasternal notch to the carotid pulse and from the suprasternal notch to the femoral pulse on the same side. For each cfPWV measure, 10 seconds of carotid and 10 seconds of femoral arterial waveforms were recorded. cfPWV measures were made in duplicate and the mean value was reported. Of note, the cfPWV data in this manuscript were included in a separate report examining macro- and microvascular functional responses to the two insulin clamp protocols [29].

Radial Artery Augmentation Index (AI): To assess peripheral arterial stiffness, we measured AI noninvasively with a Sphygmacor tonometer. As with cfPWV, radial AI measurements were obtained by the same trained operator after participants laid in the supine position in a temperature-controlled room for at least 15 minutes prior to measurement. Radial AI was calculated as the difference of the amplitude of the late systolic peak to the early systolic peak divided by the pulse pressure and expressed as a percentage. Radial AI values were determined for each pulse over a 30 second period and a mean value was calculated by the device for each patient and corrected for a heart rate of 75 beats per minute.

Subendocardial Viability Ratio (SEVR): Measurements used to calculate SEVR were obtained with a Sphygmacor tonometer. The area under the curve of the systolic and diastolic portions of the central

aortic pulse wave were measured using pulse wave analysis. In the present study, the tonometric SEVR was as provided by the manufacturer; specifically, it was approximated automatically using the following equation: tonometric SEVR=diastolic aortic area/systolic aortic area [30, 31].

Biochemical Analyses

Complete blood count, comprehensive metabolic panel, lipid panel, fasting plasma glucose, and serum pregnancy tests were assayed at the UVA Clinical Chemistry Laboratory. Plasma glucose was measured with the YSI 2700 Biochemistry Analyzer (Yellow Springs Instrument Company; Yellow Springs, OH). Plasma insulin was measured with the ALPCO Insulin ELISA (ALPCO; Salem, NH). Insulin assays were read on a Synergy 2 microplate reader (BioTek; Winooski, VT).

Data Storage

Study data are stored in a Research Electronic Data Capture (REDCap) [32] project file repository hosted at UVA. The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Statistical Analyses

Sample Size: Our prior work has demonstrated that sample sizes of approximately 10-15 subjects were sufficient to identify significant within-study changes in macrovascular function under multiple metabolic conditions [33-36]. A crude sample size calculation using the Cohen's *d* effect size from a prior study of changes in cfPWV during euglycemic-hyperinsulinemia [35] indicated that a sample size of 10 subjects would have $\geq 95\%$ power to detect meaningful differences within each protocol.

Outcomes: The primary outcome for each protocol was change in cfPWV and secondary outcomes for each protocol included changes in AI, SEVR, systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse pressure, and plasma insulin.

Descriptive Summarization: Patient demographics were summarized using common descriptive statistics. The arithmetic mean and standard error of mean, standard deviation, median, and interquartile range were used to summarize continuous scaled outcome measures.

Statistical Analyses: Data are expressed as either mean \pm SEM or as change within protocol. Within-protocol changes were analyzed using paired, two-tailed t-test and two sample, unequal variance t-test where appropriate. Between-protocol changes were analyzed using mixed modeling for repeated measures. Spearman's correlation was used to evaluate the relationship between cfPWV and radial AI. All statistical analyses were performed with Excel (Microsoft; Redmond, WA) and GraphPad Prism 8 (GraphPad Software; San Diego, CA). In all cases a p-value of <0.05 was accepted as statistically significant.

Results

Baseline Subject Characteristics and Demographics

Table 1 details baseline demographics of the 19 total study participants. All had normal BMI, blood pressure, and fasting plasma glucose. Notably, 13 subjects completed Protocol A, 10 subjects completed Protocol B, 14 subjects completed Protocol C, and 12 subjects completed Protocol D. Nine subjects completed all four protocols.

Plasma Insulin and Glucose Concentrations

Figure 2 shows the time course for mean plasma glucose (upper panel), mean glucose infusion rate (middle panel), and mean plasma insulin levels throughout each protocol. Plasma glucose levels rose significantly from baseline within Protocols B and D, and plasma insulin concentrations rose significantly from baseline within Protocols C and D. These increases did not differ between respective protocol pairs.

Arterial Stiffness and Subendocardial Viability Ratio

Figure 3 shows the boxplots for pre- and post-intervention measures of cfPWV, AI, and SEVR in each protocol. cfPWV did not change during euglycemia, hyperglycemia, or euglycemic-hyperinsulinemia (all $p > 0.05$), but significantly increased after hyperinsulinemia was added to hyperglycemia (+0.4 m/s; $p = 0.02$) (Table 2). Radial AI trended downward within each protocol, but none of these changes reached statistical significance. Mean SEVR increased within each protocol, though none of these changes were statistically significant (all $p > 0.05$), indicating that the balance between coronary perfusion and arterial load did not acutely change (Table 2).

Relationship between Changes in cfPWV and Radial AI

We noted that central and peripheral stiffness trended in opposite directions during hyperglycemic-hyperinsulinemia (i.e., hyperglycemic-hyperinsulinemia significantly increased cfPWV and trended towards decrease in radial AI), thus we examined the relationship between change in cfPWV and change in radial AI within each protocol (Figure 4). A strong negative relationship ($r = -0.744$; $p = 0.011$) between radial AI and cfPWV was identified during hyperglycemic-hyperinsulinemia, indicating that radial AI decreased as cfPWV increased. No relationships were identified between these variables within any other protocol.

Hemodynamic Function

Table 3 details the within-protocol changes for all hemodynamic parameters. There were no significant changes of aortic or peripheral systolic, diastolic, mean arterial, or pulse pressure within any protocol (all $p > 0.05$). However, mean arterial pressure trended towards an increase during hyperglycemic-hyperinsulinemia (+4.14 mmHg; $p = 0.09$). Heart rate significantly increased during hyperglycemic-hyperinsulinemia only (+3.62 bpm; $p = 0.02$).

Discussion

To our knowledge, this study is the first to investigate the acute effects of hyperglycemia and hyperinsulinemia on central and peripheral arterial stiffness in the same subjects, with several significant and novel observations warranting discussion. First, it is the combination of both hyperglycemia and hyperinsulinemia that increases cfPWV, while isolated hyperglycemia or hyperinsulinemia alone do not. Second, hyperglycemic-hyperinsulinemia preferentially stiffens the central aorta and increases the central-to-peripheral arterial stiffness gradient, changes that are typically seen in vascular aging [6]. Finally, hyperglycemic-hyperinsulinemia acutely increases heart rate but does not alter aortic or radial systolic, diastolic, or pulse pressure.

Prior work from Puzantian et al. found that acute hyperglycemia (using pancreatic clamping methodology in healthy subjects) did not alter cfPWV, but they noted that further studies were needed to determine the independent and combined roles of glucose and insulin on cfPWV [37]. To our knowledge, the current study is the first to investigate this question. Infusion of OCT allowed us to isolate the effects of insulin and glucose and provide the first evidence that moderate hyperglycemia unmasks an action of physiologic hyperinsulinemia to increase central aortic stiffness in healthy humans. Interestingly, cfPWV increased during hyperglycemic-hyperinsulinemia without a concomitant increase in blood or pulse pressure. We note that the only variable to change during this protocol was heart rate. A positive association between heart rate and cfPWV has been demonstrated in recent studies [38, 39]. However, this effect is quite small and on the order of 0.02 m/s per 1 bpm change [38, 39]. Given that mean heart rate increased during hyperglycemic-hyperinsulinemia by 3.62 bpm, we would theoretically expect a ~ 0.07 m/s increase in mean cfPWV. Our results showed that mean cfPWV increased by 0.4 m/s during hyperglycemic-hyperinsulinemia, indicating that increased heart rate alone cannot explain the change observed in cfPWV. This result is hypothesis-generating and raises an important question: if blood pressure and heart rate cannot fully explain the increased cfPWV, what does? We hypothesize that two unmeasured factors, namely sympathetic tone and vascular smooth muscle cell (VSMC) tone, are responsible for the observed increase in cfPWV. Norepinephrine causes vasoconstriction in most arteries and also transiently increases heart rate, resulting in increased sympathetic tone [40]. The primary source of circulating norepinephrine is spillover from sympathetic nerves innervating blood vessels, and recent work has shown that hyperglycemic-hyperinsulinemia significantly increases circulating norepinephrine (but not epinephrine) in healthy humans [41]. In contrast, insulin has vasodilatory effects, with insulin-mediated vasodilation and glucose uptake being functionally linked in humans [42]. Moreover, a prior study of healthy humans utilizing the perfused forearm model demonstrated that local hyperinsulinemia caused a rightward shift of the vasoconstrictive dose-response curve to norepinephrine [43]. VSMCs are also gaining increasing attention for their role in aortic stiffness [44], with VSMC contraction and relaxation recognized as a critical regulator of aortic compliance [45]. VSMC take up and utilize norepinephrine for protein modification, and this modification plays an important role in how norepinephrine directly stimulates VSMC contraction [46, 47]. Intriguingly, one study has shown that the combination of glucose and insulin has additive effects (beyond either factor alone) on infrageniculate VSMC growth [48]. Our study was not designed to establish a mechanistic basis for how glucose, insulin,

and potentially norepinephrine work in concert to increase aortic stiffness, but future work could focus on investigating this question.

We also found that hyperglycemic-hyperinsulinemia changes the normal central-to-peripheral arterial stiffness gradient. In healthy young individuals, the aorta is highly elastic and expands to accommodate stroke volume during systole, then recoils due to stored energy during diastole [6]. This continuous cycle of expansion and recoil is advantageous because it keeps the systolic pressure low during arterial expansion but maintains diastolic pressure during the recoil phase, allowing adequate perfusion of the coronary circulation during diastole [6]. In individuals with compliant aortas, peripheral muscular artery stiffness exceeds central elastic artery stiffness [49]. With aging, central arterial stiffness increases with little change in peripheral stiffness, resulting in a reversal of the normal stiffness gradient [6, 49]. This decreased compliance of the central vasculature subsequently alters arterial pressure and flow dynamics and impacts cardiac performance and coronary perfusion [1]. Indeed, reversal of the normal central-to-peripheral arterial stiffness gradient is associated with a number of adverse cardiovascular consequences, including transmission of excessive pressure pulsatility into the microcirculation and target organ damage [6]. Among older adults, DM is associated with greater central than peripheral arterial stiffness, with the magnitude of the effect of DM on central stiffness equating to ~ 6 years of arterial aging [50]. In this study, we identified an inverse relationship for change in central (cfPWV) and peripheral (radial AI) stiffness during hyperglycemic-hyperinsulinemia. Hyperglycemic-hyperinsulinemia significantly increased cfPWV and trended towards decrease in radial AI, while a significant negative correlation was identified in change of these variables (i.e., AI decreased as cfPWV increased). Our findings align with epidemiological reports demonstrating preferential stiffening of central over peripheral arteries in both T2DM [22–24, 50] and T2DM with ischemic heart disease [51] (i.e., conditions characterized by hyperglycemia and hyperinsulinemia). Our prior work has also shown that insulin has opposing effects on peripheral and arterial stiffness in various metabolic conditions. Specifically, insulin (with euglycemia) acutely reduced radial AI in both healthy and metabolic syndrome subjects, but increased cfPWV in metabolic syndrome subjects only [35]. In that study, metabolic syndrome subjects were insulin-resistant and had chronically higher fasting plasma glucose and insulin concentrations (i.e., the milieu of metabolic syndrome), contributing to reversal of the normal central-to-peripheral arterial stiffness gradient during the euglycemic insulin infusion.

A third point is that hyperglycemic-hyperinsulinemia acutely increased heart rate but not central or peripheral blood pressure. Recent work has shown that arterial stiffness precedes any increase in systolic blood pressure [52, 53]. Given the short duration of our study period, it is unsurprising that blood pressure trended up but did not significantly change. Oral glucose administration has also been shown to increase plasma norepinephrine levels [54], sympathetic nervous system activity [54, 55], and heart rate [54–56] in healthy humans. Acute hyperglycemia [57] and norepinephrine [58] are established inducers of insulin resistance, and epidemiological studies indicate that insulin resistance is associated with higher resting heart rate in healthy populations [59, 60]. Thus, the increase in heart rate seen during hyperglycemic-hyperinsulinemia in this study mimics the increased sympathetic nervous system activity observed in metabolic syndrome [61].

Taken together, our findings suggest a potential mechanism for how DM increases aortic stiffness, alters the normal central-to-peripheral arterial stiffness gradient, and ultimately contributes to the development of cardiovascular disease. Preclinical studies of human aortic endothelial cells have shown that both insulin [62] and hyperglycemia [63] independently increase expression of endothelial nitric oxide synthase and production of nitric oxide, while hyperglycemia in the presence of insulin specifically inhibits insulin-stimulated nitric oxide synthesis and downregulates some aspects of insulin signaling (including the Akt and CAP/Cbl signaling pathways) [57, 64, 65]. Insulin, at physiological concentrations, has acute vasodilatory effects that increase arterial (especially aortic) distensibility; however, these beneficial effects are blunted in insulin-resistant states characterized by hyperglycemia (including obesity, metabolic syndrome, and types 1 and 2 DM [66, 67]). Srinivasan et al. studied 20 persons with type 1 DM during either euglycemic-hyperinsulinemia or hyperglycemic-hyperinsulinemia and found that acute hyperglycemia has a deleterious effect on myocardial vasodilator function that outweighs the beneficial effects of hyperinsulinemia [68]. Insulin resistance in type 2 DM has been shown to limit insulin's ability to decrease central aortic pressure, which may predispose to development of systolic hypertension [69]. Similarly, Henry et al. reported a greater impact of type 2 DM on central arteries, emphasizing that the increase in arterial stiffness begins from the stage of impaired glucose tolerance, with underlying mechanisms including hyperglycemia and glucotoxicity, advanced glycation end products, hyperinsulinemia and insulin resistance, endothelial dysfunction, and oxidative stress ultimately leading to arterial wall remodeling [70]. In the current study, we build upon these findings and note that acute hyperglycemic-hyperinsulinemia (for merely two hours in lean, healthy subjects) increases central aortic stiffness and heart rate when compared to euglycemia, hyperglycemia, and euglycemic-hyperinsulinemia. These results have implications for future research given that the phenotype of type 2 DM includes both hyperglycemia and hyperinsulinemia, and that persons with type 1 DM experience frequent and wide hyperglycemic excursions in the setting of hyperinsulinemia due to mismatched timing of insulin administration and meal intake.

There are several limitations to the current study. First, we studied a small number of healthy young adults and the study was powered to detect within-protocol responses to glucose and insulin. Thus, we identified no between-protocol response differences, likely due to insufficient statistical power. Second, persons with DM or those who are older and/or less healthy might respond differently. Finally, we cannot rule out that OCT has in some unknown manner skewed the vascular responses and recognize that this possibility cannot be discounted. We do note, however, that no vasoactive effects have been identified in previous studies using a similar dose of OCT [41, 71–73] and that OCT infusion does not alter the hemodynamic effects of acute hyperglycemia [74].

Conclusions

We conclude that the combination of hyperglycemia with hyperinsulinemia increases aortic stiffness, changes the normal central-to-peripheral arterial stiffness gradient, and increases heart rate in healthy humans. These changes, if sustained chronically, may contribute to the development of cardiovascular disease.

Abbreviations

cfPWV = carotid-femoral pulse wave velocity; DM = diabetes mellitus; OCT = octreotide; AI = augmentation index; SEVR = subendocardial viability ratio; UVA = University of Virginia; CRU = clinical research unit; BG = blood glucose; VSMC = vascular smooth muscle cell.

Declarations

Ethics Approval and Consent to Participate: All protocols were approved by the University of Virginia Institutional Review Board (#19948). Each subject gave written informed consent at their initial screening visit prior to study participation.

Consent for Publication: Not Applicable

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no competing interests.

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Author Contributions: WBH and EJB conceived and designed the study. WBH, LAJ, LMH, KWA, JTP, and EJB acquired, analyzed, and interpreted data. WBH drafted the manuscript. WBH, LAJ, LMH, KWA, JTP, and EJB revised the manuscript. All authors approved the final version of the manuscript before submission.

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Tables

Table 1
Baseline Subject Characteristics and Demographics.

Variable	Mean ± SEM
Sex	12 Female; 7 Male
Age (years)	24.4 ± 1.0
Body Mass Index (kg/m ²)	22.4 ± 0.4
Systolic Blood Pressure (mmHg)	114.9 ± 2.6
Diastolic Blood Pressure (mmHg)	66.5 ± 1.9
Fasting Blood Glucose (mg/dL)	87.8 ± 1.4
Total Cholesterol (mg/dL)	162.4 ± 5.4
LDL Cholesterol (mg/dL)	92.1 ± 5.1
HDL Cholesterol (mg/dL)	59.9 ± 2.9
Triglycerides (mg/dL)	63.6 ± 5.0
SEM = standard error of mean	

Table 2. Summary statistics for pre- and post-intervention cfPWV, radial AI, and SEVR. (cfPWV= carotid-femoral pulse wave velocity; AI= augmentation index; SEVR= subendocardial viability ratio; EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia; SEM= standard error of mean).

Vascular Parameter	Protocol	Assessment	n	Mean ± SEM	P-value	
cfPWV	EG	Pre	13	5.25 ± 0.13	0.74	
		Post	13	5.21 ± 0.18		
	HG	Pre	10	4.91 ± 0.23	0.88	
		Post	10	4.79 ± 0.26		
	EH	Pre	14	5.19 ± 0.25	0.40	
		Post	14	5.06 ± 0.20		
	HH	Pre	12	4.67 ± 0.12	0.02	
		Post	12	5.07 ± 0.20		
	Radial AI	EG	Pre	13	0.08 ± 3.56	0.07
			Post	13	-4.15 ± 3.25	
HG		Pre	10	-2.80 ± 4.22	0.13	
		Post	10	-4.80 ± 3.83		
EH		Pre	14	-0.79 ± 2.37	0.10	
		Post	14	-2.23 ± 3.27		
HH		Pre	12	-4.25 ± 2.67	0.12	
		Post	12	-8.45 ± 2.76		
SEVR		EG	Pre	13	167.77 ± 9.76	0.09
			Post	13	178.92 ± 9.98	
	HG	Pre	10	195.60 ± 12.80	0.51	
		Post	10	201.90 ± 9.97		
	EH	Pre	13	184.00 ± 9.53	0.40	
		Post	13	194.15 ± 11.75		
	HH	Pre	12	173.08 ± 6.85	0.64	
		Post	12	183.18 ± 16.19		

Table 3. Summary statistics for pre- and post-intervention hemodynamic parameters. (sBP= systolic blood pressure; dBP= diastolic blood pressure; PP= pulse pressure; MAP= mean arterial pressure; HR= heart rate; EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia; SEM= standard error of mean).

Hemodynamic Parameter	Protocol	Assessment	n	Mean \pm SEM	P-value	
Central sBP	EG	Pre	13	95.54 \pm 2.27	0.72	
		Post	13	95.00 \pm 2.66		
	HG	Pre	10	95.20 \pm 3.57	0.40	
		Post	10	93.80 \pm 2.99		
	EH	Pre	14	96.43 \pm 3.11	0.35	
		Post	14	98.57 \pm 2.13		
	HH	Pre	12	92.75 \pm 2.95	0.33	
		Post	12	95.64 \pm 2.48		
	Central dBP	EG	Pre	13	66.23 \pm 1.90	0.25
			Post	13	67.69 \pm 1.98	
HG		Pre	10	66.10 \pm 2.63	0.42	
		Post	10	64.40 \pm 2.55		
EH		Pre	14	67.29 \pm 3.06	0.13	
		Post	14	70.29 \pm 2.31		
HH		Pre	12	61.75 \pm 3.39	0.10	
		Post	12	65.91 \pm 2.62		
Central PP		EG	Pre	13	30.00 \pm 1.56	0.09
			Post	13	27.31 \pm 1.19	
	HG	Pre	10	29.10 \pm 1.54	0.81	
		Post	10	29.40 \pm 1.84		
	EH	Pre	13	29.14 \pm 1.83	0.70	
		Post	13	28.29 \pm 1.55		
	HH	Pre	12	31.00 \pm 1.50	0.38	
		Post	12	29.73 \pm 1.36		

MAP	EG	Pre	13	79.85 ± 1.89	0.71
		Post	13	80.31 ± 2.13	
	HG	Pre	10	78.90 ± 2.92	0.35
		Post	10	77.30 ± 2.74	
	EH	Pre	14	80.50 ± 3.00	0.14
		Post	14	83.21 ± 2.23	
	HH	Pre	12	75.50 ± 3.19	0.10
		Post	12	79.64 ± 2.52	
Peripheral sBP	EG	Pre	13	110.15 ± 2.54	0.53
		Post	13	111.23 ± 3.13	
	HG	Pre	10	110.10 ± 3.77	0.66
		Post	10	110.80 ± 3.58	
	EH	Pre	14	111.86 ± 2.96	0.13
		Post	14	116.00 ± 2.52	
	HH	Pre	12	110.25 ± 2.83	0.22
		Post	12	114.27 ± 2.80	
Peripheral dBP	EG	Pre	13	65.62 ± 1.89	0.35
		Post	13	66.85 ± 2.02	
	HG	Pre	10	65.40 ± 2.60	0.44
		Post	10	63.70 ± 2.52	
	EH	Pre	14	66.36 ± 2.96	0.13
		Post	14	69.29 ± 2.20	
	HH	Pre	12	61.00 ± 3.34	0.11
		Post	12	65.09 ± 2.67	

Peripheral PP	EG	Pre	13	46.46 ± 2.16	1.00
		Post	13	46.46 ± 2.42	
	HG	Pre	10	44.70 ± 2.09	0.25
		Post	10	47.10 ± 2.76	
	EH	Pre	14	45.50 ± 2.18	0.55
		Post	14	47.43 ± 2.64	
	HH	Pre	12	49.25 ± 1.73	0.53
		Post	12	47.55 ± 3.10	
HR	EG	Pre	12	61.92 ± 3.25	0.66
		Post	12	62.67 ± 2.98	
	HG	Pre	10	55.40 ± 2.74	0.84
		Post	10	55.10 ± 2.14	
	EH	Pre	14	59.64 ± 2.53	0.22
		Post	14	61.93 ± 3.77	
	HH	Pre	12	58.83 ± 2.10	0.02
		Post	12	62.45 ± 2.27	

Figures

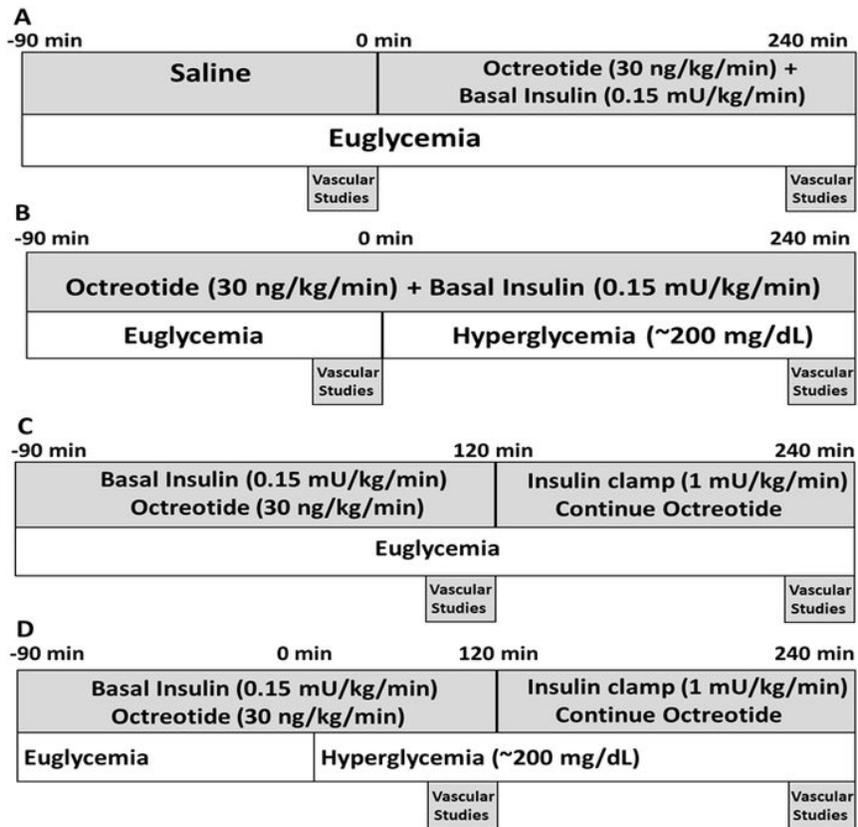


Figure 1

Experimental protocols. (A= euglycemia; B= hyperglycemia; C= euglycemic-hyperinsulinemia; D= hyperglycemic-hyperinsulinemia).

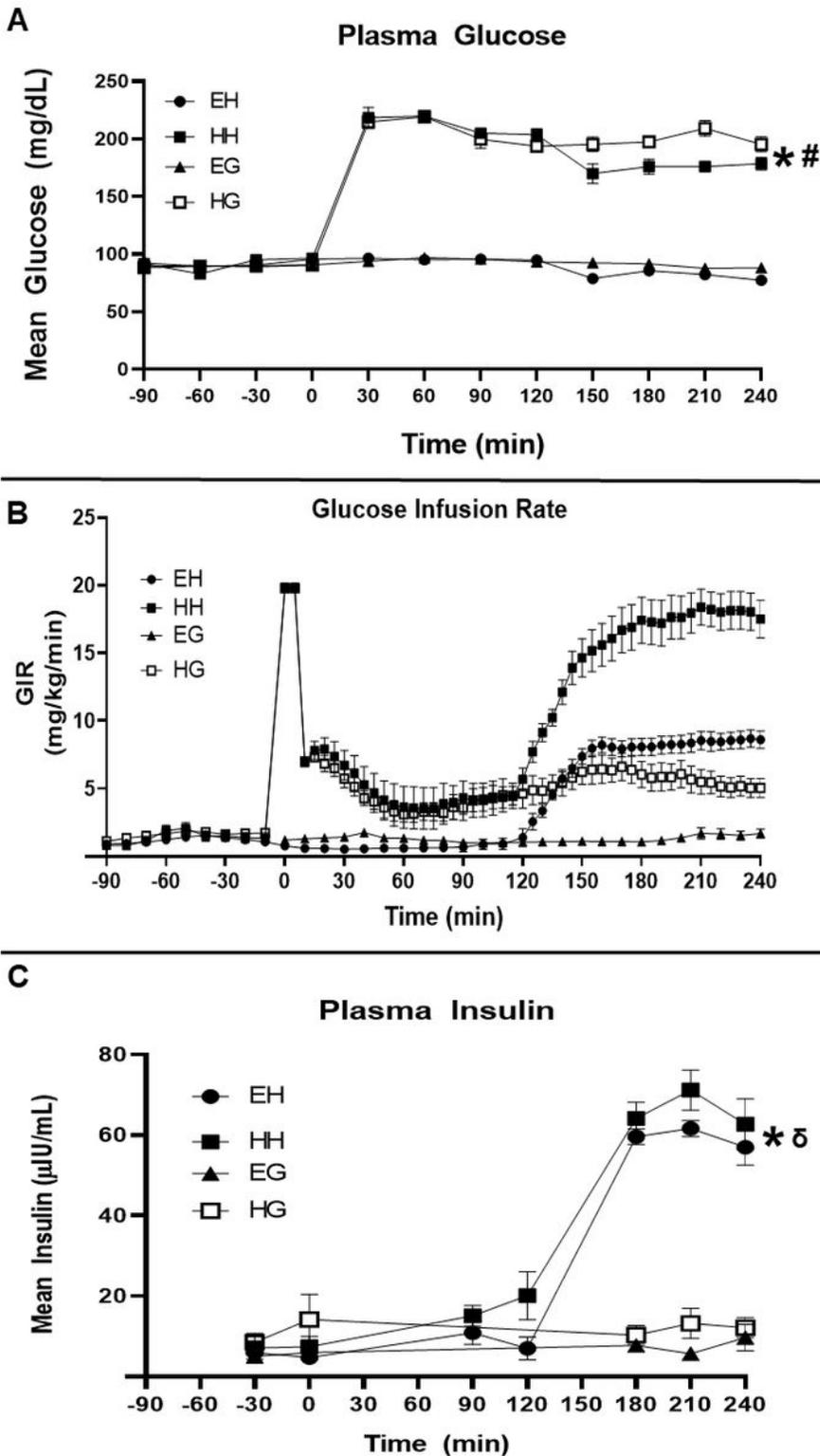


Figure 2

Time course for mean plasma glucose (Panel A), mean glucose infusion rate (Panel B), and mean plasma insulin (Panel C) throughout each infusion protocol. (Min= minutes; GIR= glucose infusion rate; EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia). * $p < 0.001$ when compared to baseline. # $p < 0.01$ when compared to EG or EH. $\delta p < 0.001$ when compared to EG or HG.

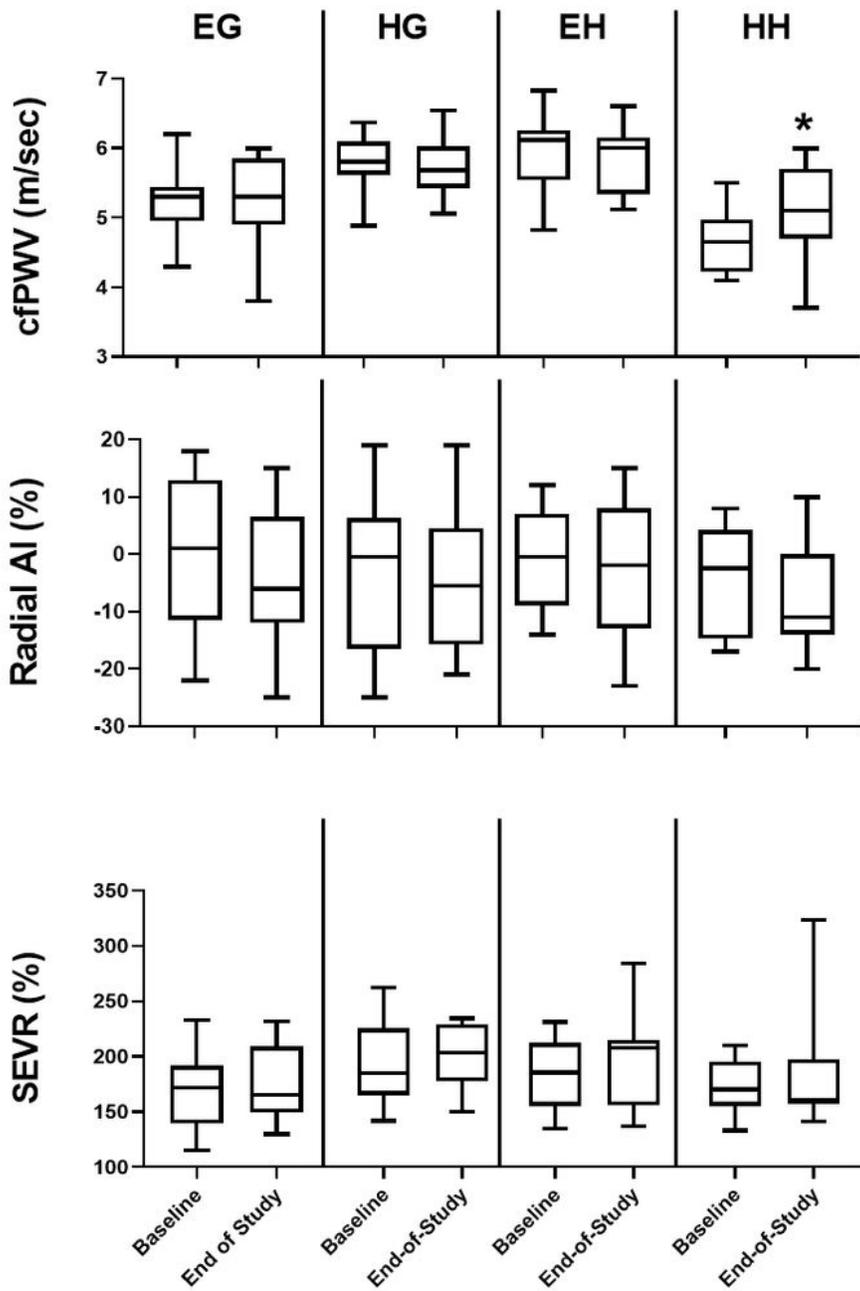


Figure 3

Boxplots detailing pre-intervention (baseline) and post-intervention (end of study) changes in cfPWV (Panel A), radial AI (Panel B), and SEVR (Panel C) within each protocol. Boxplots present five-point data summary (i.e., minimum, first quartile, median, third quartile, and maximum values). (cfPWV= carotid-femoral pulse wave velocity; AI= augmentation index; SEVR= subendocardial viability ratio; EG=

euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia). * $p < 0.03$ when compared to baseline.

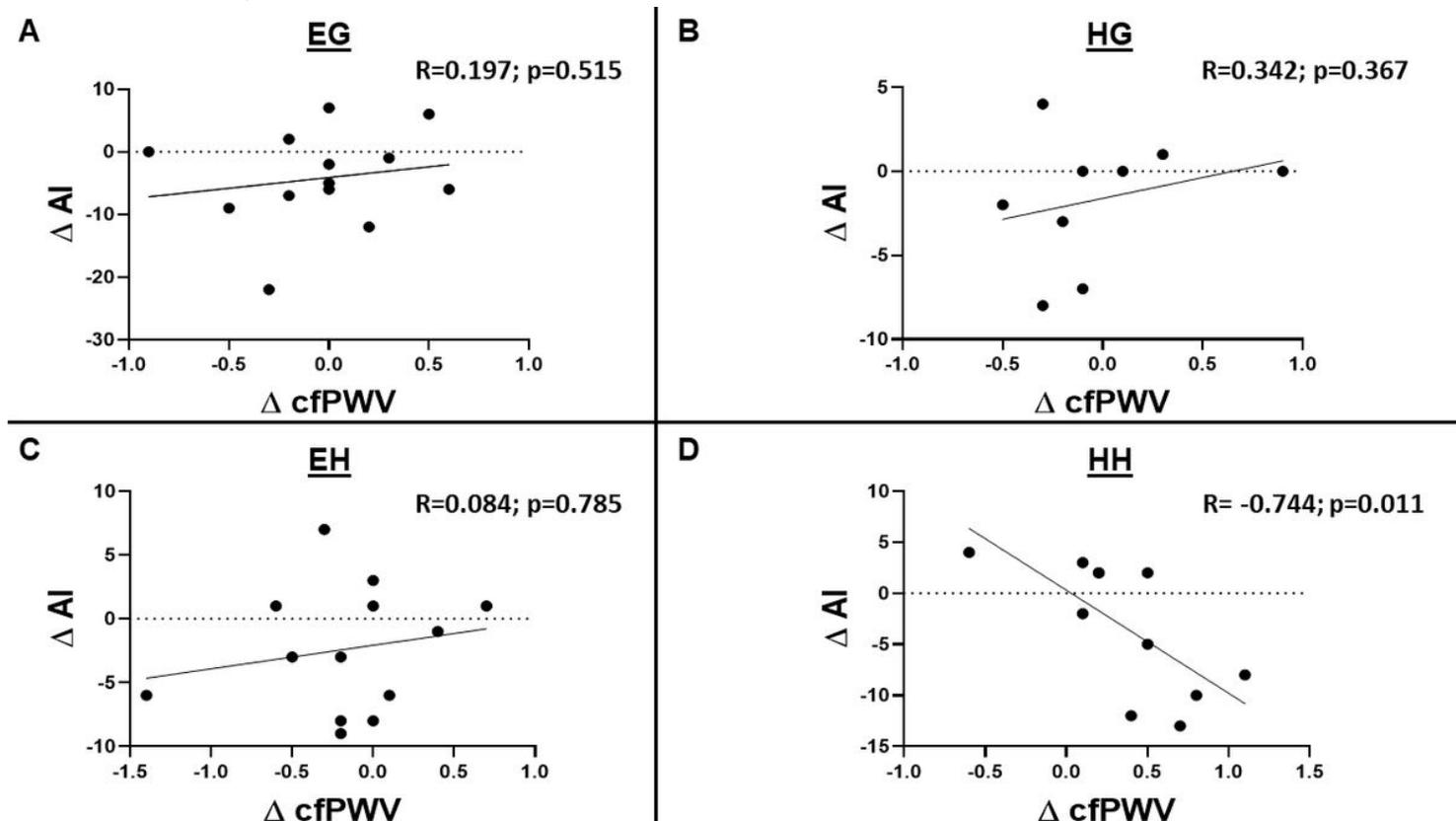


Figure 4

Relationship between change in cfPWV and change in AI during euglycemia (Panel A), hyperglycemia (Panel B), euglycemic-hyperinsulinemia (Panel C), and hyperglycemic-hyperinsulinemia (Panel D). Spearman's correlation was used to evaluate the relationship between variables. Linear regression was used to generate line of best fit. cfPWV is expressed in m/sec while radial AI is expressed as percentage. (EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia).