Insulin Increases Central Aortic Stiffness in Response To Hyperglycemia in Healthy Humans: A Randomized Four-Way Crossover Study

William B Horton (WBH2N@virginia.edu)
University of Virginia School of Medicine https://orcid.org/0000-0001-8081-1214
Linda A Jahn
University of Virginia
Lee M Hartline
University of Virginia
Kevin W Aylor
University of Virginia
Eugene J Barrett
University of Virginia School of Medicine

Research Article

Keywords: Insulin, Hyperglycemia, Octreotide, Vascular stiffness

Posted Date: October 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-72339/v2

Abstract

Increasing arterial stiffness is a feature of vascular aging that is accelerated by conditions that enhance cardiovascular risk, including diabetes mellitus. Emerging evidence demonstrates that reversal of the normal lower central to higher 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. We tested the effect of acutely increasing plasma glucose, plasma insulin, or both on hemodynamic function, central aortic stiffness (carotid-femoral pulse wave velocity), and peripheral arterial stiffness (augmentation index) in a randomized, four-way, crossover study of 19 healthy young adults. Carotid-femoral pulse wave velocity increased only during hyperglycemic-hyperinsulinemia (+0.4 m/s; p=0.02), while augmentation index did not change significantly within any intervention (all p>0.05). Heart rate significantly increased only during hyperglycemic-hyperinsulinemia (+3.62 bpm; p=0.02). There was a significant inverse correlation between the changes of central and peripheral arterial stiffness only during hyperglycemic-hyperinsulinemia. We conclude that combined hyperglycemia and hyperinsulinemia acutely increased aortic stiffness, changed the normal central-to-peripheral arterial stiffness gradient, and increased heart rate in healthy humans. (ClinicalTrials.gov number NCT03520569; registered 9 May 2018).

Introduction

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) (Zieman et al., 2005). Stiffness is not uniformly distributed throughout the arterial tree but is often patchy (Galis and Khatri, 2002, Bassiouny et al., 1994), occurring in central elastic and conduit muscular arteries while sparing smaller arteries (Zieman et al., 2005, Benetos et al., 1993, Gillessen et al., 1995). 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 (Yu and McEniery, 2020).

Increasing arterial stiffness occurs with vascular aging and is accelerated by conditions that increase cardiovascular risk, including DM (Lyle and Raaz, 2017). 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 (Bolton and Rajkumar, 2011). Notably, arterial stiffness often antedates and is itself a strong risk factor for a spectrum of cardiovascular diseases (e.g., heart failure, myocardial infarction, etc.) (Lyle and Raaz, 2017, Yu and McEniery, 2020). Data from numerous studies demonstrate that aortic stiffness (assessed by carotid-femoral pulse wave velocity (cfPWV)) is an important, independent determinant of cardiovascular event risk in multiple populations (Boutouyrie et al., 2002, Laurent et al., 2001, Ben-Shlomo et al., 2014, London et al., 2001, Cruickshank et al., 2002, Sutton-Tyrrell et al., 2005, Mitchell et al., 2010) and may explain some of the notable residual cardiovascular risk associated with even well-controlled hypertension (Niiranen et al., 2016).

In healthy young adults, the central aorta is quite elastic, while peripheral, muscular arteries are inherently stiffer (Yu and McEniery, 2020). However, studies of the general population (Mitchell et al., 2004, McEniery et al., 2005) and hypertensive persons (Benetos et al., 1993) demonstrate that age-related increases in peripheral artery stiffness are less rapid than in the central arteries (Yu and McEniery, 2020). This differential rate of stiffening results in aortic stiffness equaling or exceeding peripheral stiffness in the majority of older individuals (Yu and McEniery, 2020). 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 (Yu and McEniery, 2020, Lu et al., 2017, Mitchell, 2018, Vasan et al., 2019).

Interestingly, preferential stiffening of central over peripheral arteries occurs in type 2 DM (Cardoso et al., 2009, Kimoto et al., 2003, Kimoto et al., 2006), though mechanisms responsible for this finding are unclear. A recent editorial by Currie and Delles encouraged investigation of healthy cohorts to understand mechanisms contributing to accelerated vascular aging (Currie and Delles, 2017). 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 hemodynamic changes, central aortic stiffness with carotid-femoral pulse wave velocity (cfPWV), and peripheral arterial stiffness with augmentation index (AI) during euglycemia, hyperglycemia, euglycemic-hyperinsulinemia, and hyperglycemic-hyperinsulinemia.

Materials And Methods

Recruitment and Study Population

Recruitment for this study was achieved by public advertisement. Healthy young adults met inclusion criteria if they were ≥18 and ≤35 years old, had normal body mass index (18–25 kg/m^2), 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 University of Virginia (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 of study sequence was conducted by study personnel using a 1:1:1:1 starting allocation with a computer-generated sequence program (Urbaniak, 2013). The crossover plan was designed to assess: (1) Protocol A followed by Protocol B (or vice-versa), with subsequent crossover to Protocols C and D (in randomized order); or (2) Protocol C followed by Protocol D (or vice-versa), with subsequent crossover to Protocols A and B (in randomized order). Subjects underwent four infusion protocols (Figure 1) to test the discrete 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 to allow for a washout period between studies. Within each protocol, we measured cfPWV, AI, 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). After randomization, study personnel were blinded to subject and protocol when evaluating outcome measures. 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 (DeFronzo et al., 1979). We repeated vascular measurements over the final 30 minutes of OCT infusion.

Protocol B (Isolated Hyperglycemia): Octreotide with basal insulin replacement was continuously infused for 90 minutes while euglycemia was maintained. Baseline vascular measurements were 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 (DeFronzo et al., 1979). 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 (DeFronzo et al., 1979). 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 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 whileOCT (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 then maintain BG at ~200 mg/dL. 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 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 (Townsend et al., 2015) using a Sphygmacor tonometer by the same trained operator. To minimize the effects of sympathetic activity on cfPWV measurements, participants laid were supine 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 (Horton et al., 2020).

Augmentation Index (AI): To assess peripheral arterial stiffness,we measured AI noninvasively with a Sphygmacor tonometer. AI measurements were obtained at the radial artery 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. 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. 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.

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) (Harris et al., 2019) 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 (Chai et al., 2011, Eggleston et al., 2007, Jahn et al., 2016, Wang et al., 2020). A crude sample size calculation using the Cohen's *d* effect size from a previous study using the same study design to measure changes in cfPWV during euglycemic-hyperinsulinemia (Jahn et al., 2016) indicated that a sample size of 10 subjects would have ≥90% power.

*Outcomes:*The primary outcome for each protocol was change in cfPWV and secondary outcomes for each protocol included changes in AI, 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 ± SEM or as change within protocol. All data were normally distributed, thus within-protocol changes were analyzed using either paired, two-tailed t-test or 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 AI. Linear regression was used to generate line of best fit. 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 Protocol B, 14 subjects Protocol C, and 12 subjects Protocol D. Nine subjects completed all four protocols. Three subjects voluntarily withdrew after completing only Protocols C and D, two after completing only Protocol C, and two after completing only Protocol A. One subject moved out of the area after completing Protocols A and B, one experienced scheduling difficulty after completing only Protocols A and D, and one completed Protocol D but then experienced abdominal cramping (due to octreotide) that led to study termination during their second admission. An electrode wire malfunction resulted in no post-intervention assessment of cfPWV for one subject in Protocol B.

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

Figure 3 shows the boxplots for pre- and post-intervention measures of cfPWV and AI within 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). AI trended downward within each protocol, but none of these changes reached statistical significance.

Relationship between Changes in cfPWV and 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 AI), thus we examined the relationship between change in cfPWV and change in AI within each protocol (Figure 4). A strong negative relationship ($r = -0.744$; $p = 0.011$) between AI and cfPWV was identified during hyperglycemic-hyperinsulinemia, indicating that 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 slightly increased 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 discrete effects of acute hyperglycemia and hyperinsulinemia on central and peripheral arterial stiffness in the same subjects, with several novel observations warranting discussion. First, the combination of hyperglycemia and hyperinsulinemia increased cfPWV, while isolated hyperglycemia or hyperinsulinemia alone did not. Second, hyperglycemic-hyperinsulinemia preferentially stiffened the central aorta and increased the central-to-peripheral arterial stiffness gradient, changes that are typically seen in vascular aging (Yu and McEniery, 2020). Finally, hyperglycemic-hyperinsulinemia acutely increased heart rate.

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 (Puzantian et al., 2015). 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. We note that heart rate rose significantly and mean arterial pressure trended upward during hyperglycemic-hyperinsulinemia. A positive association between heart rate and cfPWV has been demonstrated in recent studies (Logan and Kim, 2016, Tan et al., 2016). However, this effect is small and on the order of 0.02 m/s per 1 bpm change (Tan et al., 2016, Logan and Kim, 2016). Bikia et al. recently performed an in-silico evaluation of the impact of heart rate on cfPWV and confirmed that small heart rate changes only slightly affect cfPWV, but they suggested that a more clinically significant impact on cfPWV should be considered in cases wherein heart rate might vary to a greater extent (Bikia et al., 2020). The increase in heart rate and trend towards increase in blood pressure during hyperglycemic-hyperinsulinemia in our study are hypothesis-generating and suggest enhanced sympathetic nervous system activation. Norepinephrine causes vasoconstriction in most arteries and also transiently increases heart rate, resulting in increased sympathetic tone (Gordan et al., 2015). 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 (Joy et al., 2016). In contrast, insulin during euglycemia has vasodilatory effects, with insulin-mediated vasodilation and glucose uptake being functionally linked in humans (Cleland et al., 1999). 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 (Jern, 1994). As our study was not designed to establish a mechanistic basis for how glucose and insulin may work in concert to increase aortic stiffness, we did not measure sympathetic nervous system activity. Future work will focus on investigating the mechanistic basis for this observation.

We also found that hyperglycemic-hyperinsulinemia changed the normal central-to-peripheral arterial stiffness gradient. In individuals with compliant aortas, peripheral muscular artery stiffness exceeds central elastic artery stiffness (Hickson et al., 2016). With aging, central aortic stiffness increases with little change in peripheral arterial stiffness, resulting in a reversal of the normal stiffness gradient (Hickson et al., 2016, Yu and McEniery, 2020). This decreased compliance of the central vasculature subsequently alters arterial pressure and flow dynamics and impacts cardiac performance and coronary perfusion (Zieman et al., 2005). 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 (Yu and McEniery, 2020). 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 (Loehr et al., 2016). In the current study, we identified an inverse relationship for change in central (cfPWV) and peripheral (AI) stiffness during hyperglycemic-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 AI in both healthy and metabolic syndrome subjects, but increased cfPWV in metabolic syndrome subjects only (Jahn et al., 2016). 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. Here we must note that the major determinants of AI are body height, heart rate, and PWV (Smulyan et al., 1998). The inverse association between change in cfPWV and change in AI during hyperglycemic-hyperinsulinemia may reflect late return of the reflection pressure wave, which consequently decreases AI.

A third point is that hyperglycemic-hyperinsulinemia acutely significantly increased heart and slightly increased mean arterial pressure. These changes mimic the increased sympathetic nervous system activity observed in insulin-resistant states like obesity and type 2 DM (Moreira et al., 2015, Esler et al., 2001). Recent work has also shown that arterial stiffness precedes any overt increase in blood pressure (Kaess et al., 2012, Weisbrod et al., 2013). Given the short duration of our study period, it is unsurprising that mean arterial pressure trended up but did not reach statistical significance.

Taken together, our findings suggest a potential mechanism for how acute metabolic alterations impact hemodynamic function, aortic stiffness, and the central-to-peripheral arterial stiffness gradient. 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. However, we must firmly caution that the acute effects observed in this study likely do not reflect the chronic impact of glucose and insulin per se on aortic stiffness. Moreover, the acute changes in cfPWV during hyperglycemic-hyperinsulinemia likely reflect alterations in arterial tone as opposed to arterial wall remodeling.

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. Third, while AI is widely used to evaluate peripheral arterial stiffness, conflicting data exist in regards to its validity (Zahner et al., 2017, Cheng et al., 2007). 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 (Beckman et al., 2001, Beckman et al., 2002, Moller et al., 1995, Joy et al., 2016) and that OCT infusion does not alter the hemodynamic effects of acute hyperglycemia (Marfella et al., 2000).

Conclusions

We conclude that the combination of hyperglycemia with hyperinsulinemia increased cfPWV, changed the normal central-to-peripheral arterial stiffness gradient, and increased 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; UVA = University of Virginia; CRU = clinical research unit; BG = blood glucose.

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.

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

Conflict of Interests: The authors declare that they have no conflicts of interest to disclose.

Funding: This work was supported in part by NIH research grants F32HL14230401, KL2TR003016, and UL1TR003015 (to WBH) and RO1DK073059 and RO1HL142250 (to EJB).

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.

References

BASSIOUNY, H. S., ZARINS, C. K., KADOWAKI, M. H. & GLAGOV, S. 1994. Hemodynamic stress and experimental aortoiliac atherosclerosis. *J Vasc Surg,* 19*,* 426–34.

BECKMAN, J. A., GOLDFINE, A. B., GORDON, M. B. & CREAGER, M. A. 2001. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation,* 103*,* 1618–23.

BECKMAN, J. A., GOLDFINE, A. B., GORDON, M. B., GARRETT, L. A. & CREAGER, M. A. 2002. Inhibition of protein kinase Cbeta prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. *Circ Res,* 90*,* 107–11.

BEN-SHLOMO, Y., SPEARS, M., BOUSTRED, C., MAY, M., ANDERSON, S. G., BENJAMIN, E. J., BOUTOUYRIE, P., CAMERON, J., CHEN, C. H., CRUICKSHANK, J. K., HWANG, S. J., LAKATTA, E. G., LAURENT, S., MALDONADO, J., MITCHELL, G. F., NAJJAR, S. S., NEWMAN, A. B., OHISHI, M., PANNIER, B., PEREIRA, T., VASAN, R. S., SHOKAWA, T., SUTTON-TYRELL, K., VERBEKE, F., WANG, K. L., WEBB, D. J., WILLUM HANSEN, T., ZOUNGAS, S., MCENIERY, C. M., COCKCROFT, J. R. & WILKINSON, I. B. 2014. 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,* 63*,* 636–646.

BENETOS, A., LAURENT, S., HOEKS, A. P., BOUTOUYRIE, P. H. & SAFAR, M. E. 1993. Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb,* 13*,* 90–7.

BIKIA, V., STERGIOPULOS, N., ROVAS, G., PAGOULATOU, S. & PAPAIOANNOU, T. G. 2020. The impact of heart rate on pulse wave velocity: an in-silico evaluation. *J Hypertens.*

BOLTON, E. & RAJKUMAR, C. 2011. The ageing cardiovascular system. *Reviews in Clinical Gerontology,* 21*,* 99–109.

BOUTOUYRIE, P., TROPEANO, A. I., ASMAR, R., GAUTIER, I., BENETOS, A., LACOLLEY, P. & LAURENT, S. 2002. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension,* 39*,* 10–5.

CARDOSO, C. R., FERREIRA, M. T., LEITE, N. C., BARROS, P. N., CONTE, P. H. & SALLES, G. F. 2009. Microvascular degenerative complications are associated with increased aortic stiffness in type 2 diabetic patients. *Atherosclerosis,* 205*,* 472–6.

CHAI, W., LIU, J., JAHN, L. A., FOWLER, D. E., BARRETT, E. J. & LIU, Z. 2011. Salsalate attenuates free fatty acid-induced microvascular and metabolic insulin resistance in humans. *Diabetes Care,* 34*,* 1634–8.

CHENG, L. T., TANG, L. J., CHENG, L., HUANG, H. Y. & WANG, T. 2007. Limitation of the augmentation index for evaluating arterial stiffness. *Hypertens Res,* 30*,* 713–22.

CLELAND, S. J., PETRIE, J. R., UEDA, S., ELLIOTT, H. L. & CONNELL, J. M. 1999. Insulin-mediated vasodilation and glucose uptake are functionally linked in humans. *Hypertension,* 33*,* 554–8.

CRUICKSHANK, K., RISTE, L., ANDERSON, S. G., WRIGHT, J. S., DUNN, G. & GOSLING, R. G. 2002. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation,* 106*,* 2085–90.

CURRIE, G. & DELLES, C. 2017. Healthy Vascular Aging. *Hypertension,* 70*,* 229–231.

DEFRONZO, R. A., TOBIN, J. D. & ANDRES, R. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol,* 237*,* E214–23.

EGGLESTON, E. M., JAHN, L. A. & BARRETT, E. J. 2007. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. *Diabetes,* 56*,* 2958–63.

ESLER, M., RUMANTIR, M., WIESNER, G., KAYE, D., HASTINGS, J. & LAMBERT, G. 2001. Sympathetic nervous system and insulin resistance: from obesity to diabetes. *Am J Hypertens,* 14*,* 304S–309S.

GALIS, Z. S. & KHATRI, J. J. 2002. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res,* 90*,* 251–62.

GILLESSEN, T., GILLESSEN, F., SIEBERTH, H., HANRATH, P. & HEINTZ, B. 1995. Age-related changes in the elastic properties of the aortic tree in normotensive patients: investigation by intravascular ultrasound. *Eur J Med Res,* 1*,* 144–8.

GORDAN, R., GWATHMEY, J. K. & XIE, L. H. 2015. Autonomic and endocrine control of cardiovascular function. *World J Cardiol,* 7*,* 204–14.

HARRIS, P. A., TAYLOR, R., MINOR, B. L., ELLIOTT, V., FERNANDEZ, M., O'NEAL, L., MCLEOD, L., DELACQUA, G., DELACQUA, F., KIRBY, J., DUDA, S. N. & CONSORTIUM, R. E. 2019. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform,* 95*,* 103208.

HICKSON, S. S., NICHOLS, W. W., YASMIN, MCDONNELL, B. J., COCKCROFT, J. R., WILKINSON, I. B. & MCENIERY, C. M. 2016. Influence of the central-to-peripheral arterial stiffness gradient on the timing and amplitude of wave reflections. *Hypertens Res,* 39*,* 723–729.

HORTON, W. B., JAHN, L. A., HARTLINE, L. M., AYLOR, K. W., PATRIE, J. T. & BARRETT, E. J. 2020. Hyperglycemia does not Inhibit Insulin's Effects on Microvascular Perfusion in Healthy Humans: A Randomized Crossover Study. *Am J Physiol Endocrinol Metab.*

JAHN, L. A., HARTLINE, L., RAO, N., LOGAN, B., KIM, J. J., AYLOR, K., GAN, L. M., WESTERGREN, H. U. & BARRETT, E. J. 2016. Insulin Enhances Endothelial Function Throughout the Arterial Tree in Healthy But Not Metabolic Syndrome Subjects. *J Clin Endocrinol Metab,* 101*,* 1198–206.

JERN, S. 1994. Effects of insulin on vascular responses to mental stress and norepinephrine in human forearm. *Hypertension,* 24, 686–94.

JOY, N. G., PERKINS, J. M., MIKELADZE, M., YOUNK, L., TATE, D. B. & DAVIS, S. N. 2016. Comparative effects of acute hypoglycemia and hyperglycemia on pro-atherothrombotic biomarkers and endothelial function in non-diabetic humans. *J Diabetes Complications,* 30, 1275–81.

KAESS, B. M., RONG, J., LARSON, M. G., HAMBURG, N. M., VITA, J. A., LEVY, D., BENJAMIN, E. J., VASAN, R. S. & MITCHELL, G. F. 2012. Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA,* 308, 875–81.

KIMOTO, E., SHOJI, T., SHINOHARA, K., HATSUDA, S., MORI, K., FUKUMOTO, S., KOYAMA, H., EMOTO, M., OKUNO, Y. & NISHIZAWA, Y. 2006. Regional arterial stiffness in patients with type 2 diabetes and chronic kidney disease. *J Am Soc Nephrol,* 17, 2245–52.

KIMOTO, E., SHOJI, T., SHINOHARA, K., INABA, M., OKUNO, Y., MIKI, T., KOYAMA, H., EMOTO, M. & NISHIZAWA, Y. 2003. Preferential stiffening of central over peripheral arteries in type 2 diabetes. *Diabetes,* 52, 448–52.

LAURENT, S., BOUTOUYRIE, P., ASMAR, R., GAUTIER, I., LALOUX, B., GUIZE, L., DUCIMETIERE, P. & BENETOS, A. 2001. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension,* 37, 1236–41.

LOEHR, L. R., MEYER, M. L., POON, A. K., SELVIN, E., PALTA, P., TANAKA, H., PANKOW, J. S., WRIGHT, J. D., GRISWOLD, M. E., WAGENKNECHT, L. E. & HEISS, G. 2016. Prediabetes and Diabetes Are Associated With Arterial Stiffness in Older Adults: The ARIC Study. *Am J Hypertens,* 29, 1038–45.

LOGAN, J. G. & KIM, S. S. 2016. Resting Heart Rate and Aortic Stiffness in Normotensive Adults. *Korean Circ J,* 46, 834–840.

LONDON, G. M., BLACHER, J., PANNIER, B., GUERIN, A. P., MARCHAIS, S. J. & SAFAR, M. E. 2001. Arterial wave reflections and survival in end-stage renal failure. *Hypertension,* 38, 434–8.

LU, Y., ZHU, M., BAI, B., CHI, C., YU, S., TELIEWUBAI, J., XU, H., WANG, K., XIONG, J., ZHOU, Y., JI, H., FAN, X., YU, X., LI, J., BLACHER, J., ZHANG, Y. & XU, Y. 2017. Comparison of Carotid-Femoral and Brachial-Ankle Pulse-Wave Velocity in Association With Target Organ Damage in the Community-Dwelling Elderly Chinese: The Northern Shanghai Study. *J Am Heart Assoc,* 6.

LYLE, A. N. & RAAZ, U. 2017. Killing Me Unsoftly: Causes and Mechanisms of Arterial Stiffness. *Arterioscler Thromb Vasc Biol,* 37, e1-e11.

MARFELLA, R., NAPPO, F., DE ANGELIS, L., PAOLISSO, G., TAGLIAMONTE, M. R. & GIUGLIANO, D. 2000. Hemodynamic effects of acute hyperglycemia in type 2 diabetic patients. *Diabetes Care,* 23, 658–63.

MCENIERY, C. M., YASMIN, HALL, I. R., QASEM, A., WILKINSON, I. B., COCKCROFT, J. R. & INVESTIGATORS, A. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol,* 46, 1753–60.

MITCHELL, G. F. 2018. Aortic stiffness, pressure and flow pulsatility, and target organ damage. *J Appl Physiol (1985),* 125, 1871–1880.

MITCHELL, G. F., HWANG, S. J., VASAN, R. S., LARSON, M. G., PENCINA, M. J., HAMBURG, N. M., VITA, J. A., LEVY, D. & BENJAMIN, E. J. 2010. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation,* 121, 505–11.

MITCHELL, G. F., PARISE, H., BENJAMIN, E. J., LARSON, M. G., KEYES, M. J., VITA, J. A., VASAN, R. S. & LEVY, D. 2004. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension,* 43, 1239–45.

MOLLER, N., BAGGER, J. P., SCHMITZ, O., JORGENSEN, J. O., OVESEN, P., MOLLER, J., ALBERTI, K. G. & ORSKOV, H. 1995. Somatostatin enhances insulin-stimulated glucose uptake in the perfused human forearm. *J Clin Endocrinol Metab,* 80, 1789–93.

MOREIRA, M. C., PINTO, I. S., MOURAO, A. A., FAJEMIROYE, J. O., COLOMBARI, E., REIS, A. A., FREIRIA-OLIVEIRA, A. H., FERREIRA-NETO, M. L. & PEDRINO, G. R. 2015. Does the sympathetic nervous system contribute to the pathophysiology of metabolic syndrome? *Front Physiol,* 6, 234.

NIIRANEN, T. J., KALESAN, B., HAMBURG, N. M., BENJAMIN, E. J., MITCHELL, G. F. & VASAN, R. S. 2016. Relative Contributions of Arterial Stiffness and Hypertension to Cardiovascular Disease: The Framingham Heart Study. *J Am Heart Assoc,* 5.

PUZANTIAN, H., TEFF, K. & TOWNSEND, R. R. 2015. Investigating the effect of glucose on aortic pulse wave velocity using pancreatic clamping methodology. *Biol Res Nurs,* 17, 270–5.

SMULYAN, H., MARCHAIS, S. J., PANNIER, B., GUERIN, A. P., SAFAR, M. E. & LONDON, G. M. 1998. Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol,* 31, 1103–9.

SUTTON-TYRRELL, K., NAJJAR, S. S., BOUDREAU, R. M., VENKITACHALAM, L., KUPELIAN, V., SIMONSICK, E. M., HAVLIK, R., LAKATTA, E. G., SPURGEON, H., KRITCHEVSKY, S., PAHOR, M., BAUER, D., NEWMAN, A. & HEALTH, A. B.C. S. 2005. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation,* 111, 3384–90.

TAN, I., SPRONCK, B., KIAT, H., BARIN, E., REESINK, K. D., DELHAAS, T., AVOLIO, A. P. & BUTLIN, M. 2016. Heart Rate Dependency of Large Artery Stiffness. *Hypertension,* 68, 236–42.

TOWNSEND, R. R., WILKINSON, I. B., SCHIFFRIN, E. L., AVOLIO, A. P., CHIRINOS, J. A., COCKCROFT, J. R., HEFFERNAN, K. S., LAKATTA, E. G., MCENIERY, C. M., MITCHELL, G. F., NAJJAR, S. S., NICHOLS, W. W., URBINA, E. M., WEBER, T. & AMERICAN HEART ASSOCIATION COUNCIL ON, H. 2015. Recommendations

for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension,* 66, 698–722.

URBANIAK, G. C., PLOUS, S. 2013. *Research Randomizer (Version 4.0) [*Online]. Available: http://www.randomizer.org/ [Accessed 06/04/2020].

VASAN, R. S., SHORT, M. I., NIIRANEN, T. J., XANTHAKIS, V., DECARLI, C., CHENG, S., SESHADRI, S. & MITCHELL, G. F. 2019. Interrelations Between Arterial Stiffness, Target Organ Damage, and Cardiovascular Disease Outcomes. *J Am Heart Assoc,* 8, e012141.

WANG, N., TAN, A. W. K., JAHN, L. A., HARTLINE, L., PATRIE, J. T., LIN, S., BARRETT, E. J., AYLOR, K. W. & LIU, Z. 2020. Vasodilatory Actions of Glucagon-Like Peptide 1 Are Preserved in Skeletal and Cardiac Muscle Microvasculature but Not in Conduit Artery in Obese Humans With Vascular Insulin Resistance. *Diabetes Care,* 43, 634–642.

WEISBROD, R. M., SHIANG, T., AL SAYAH, L., FRY, J. L., BAJPAI, S., REINHART-KING, C. A., LOB, H. E., SANTHANAM, L., MITCHELL, G., COHEN, R. A. & SETA, F. 2013. Arterial stiffening precedes systolic hypertension in diet-induced obesity. *Hypertension,* 62, 1105–10.

YU, S. & MCENIERY, C. M. 2020. Central Versus Peripheral Artery Stiffening and Cardiovascular Risk. *Arterioscler Thromb Vasc Biol,* 40, 1028–1033.

ZAHNER, G. J., GRUENDL, M. A., SPAULDING, K. A., SCHALLER, M. S., HILLS, N. K., GASPER, W. J. & GRENON, S. M. 2017. Association between arterial stiffness and peripheral artery disease as measured by radial artery tonometry. *J Vasc Surg,* 66, 1518–1526.

ZIEMAN, S. J., MELENOVSKY, V. & KASS, D. A. 2005. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol,* 25, 932–43.

Tables

Table 1. Baseline Subject Characteristics and Demographics.

Variable	Mean ± SEM
Sex	12 Female; 7 Male
Age (years)	24.4 ± 1.0
dy Mass Index (kg/m^2)	22.4 ± 0.4
ic Blood Pressure (mmHg)	114.9 ± 2.6
lic Blood Pressure (mmHg)	66.5 ± 1.9
ıg Blood Glucose (mg/dL)	87.8 ± 1.4
:al Cholesterol (mg/dL)	162.4 ± 5.4
)L Cholesterol (mg/dL)	92.1 ± 5.1
)L Cholesterol (mg/dL)	59.9 ± 2.9
'riglycerides (mg/dL)	63.6 ± 5.0

SEM = standard error of mean

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

Parameter	Protocol	Assessment	n	Mean ± SEM	P-value
PWV	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	
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	

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

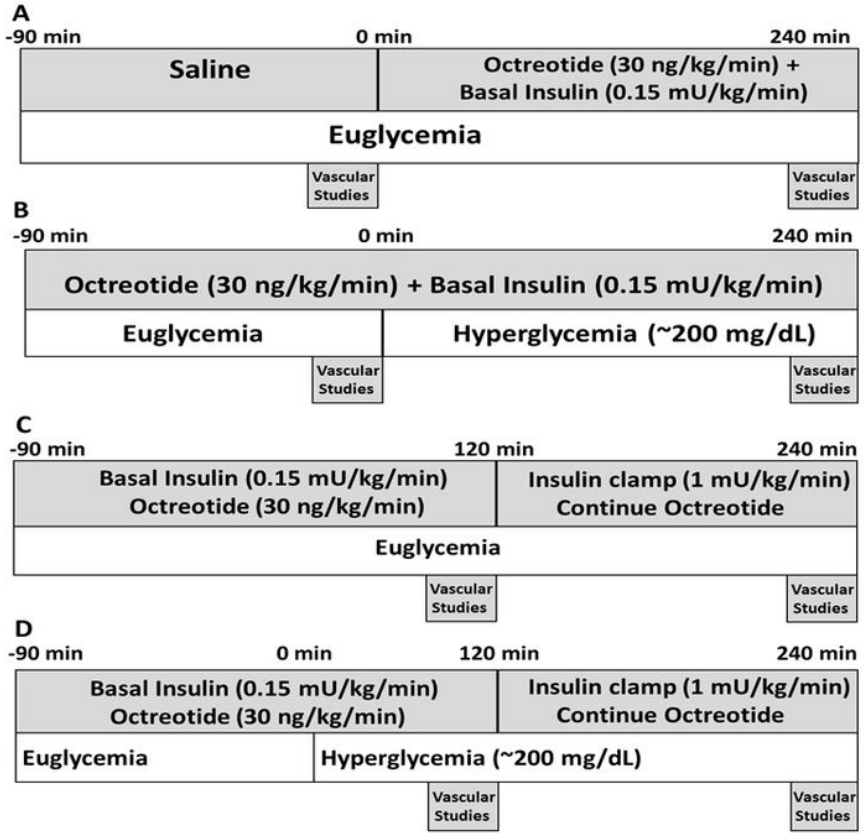

Figure 1

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

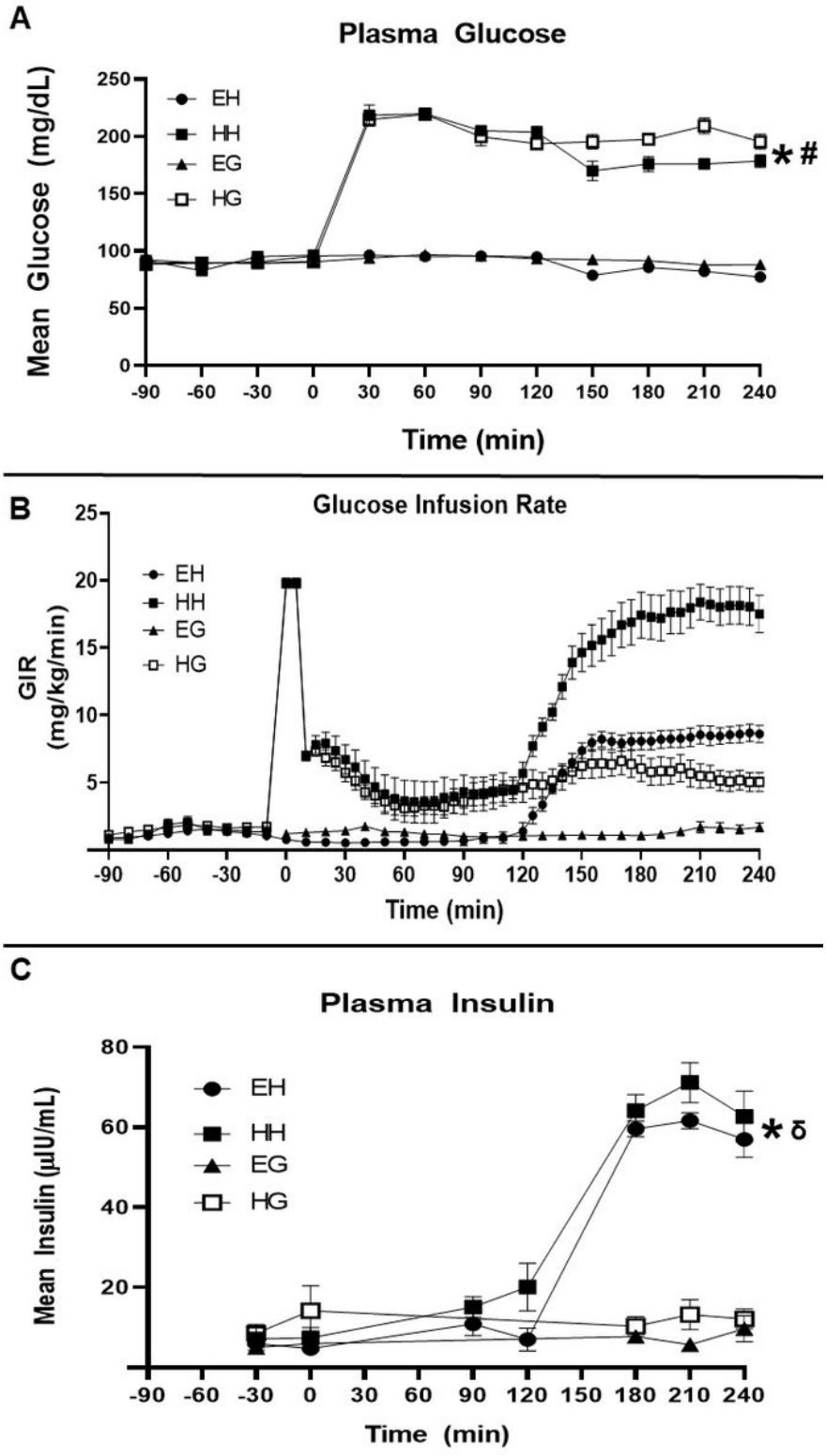

Figure 2

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. δp<0.001 when compared to EG or HG.

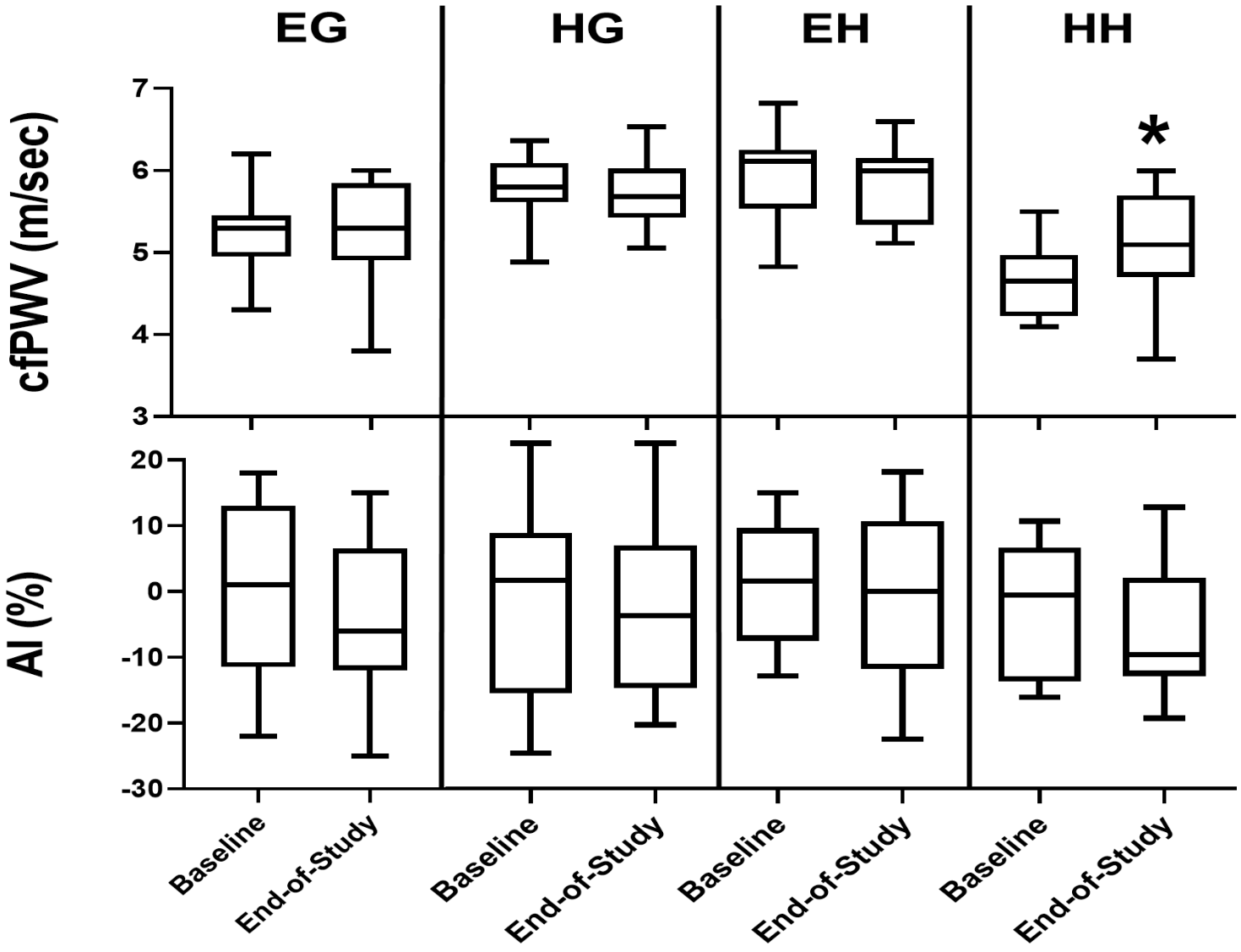

Figure 3

Boxplots detailing pre-intervention (baseline) and post-intervention (end of study) changes in cfPWV (Panel A) and AI (Panel B) 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; EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia). *$p<0.03$ when compared to baseline.

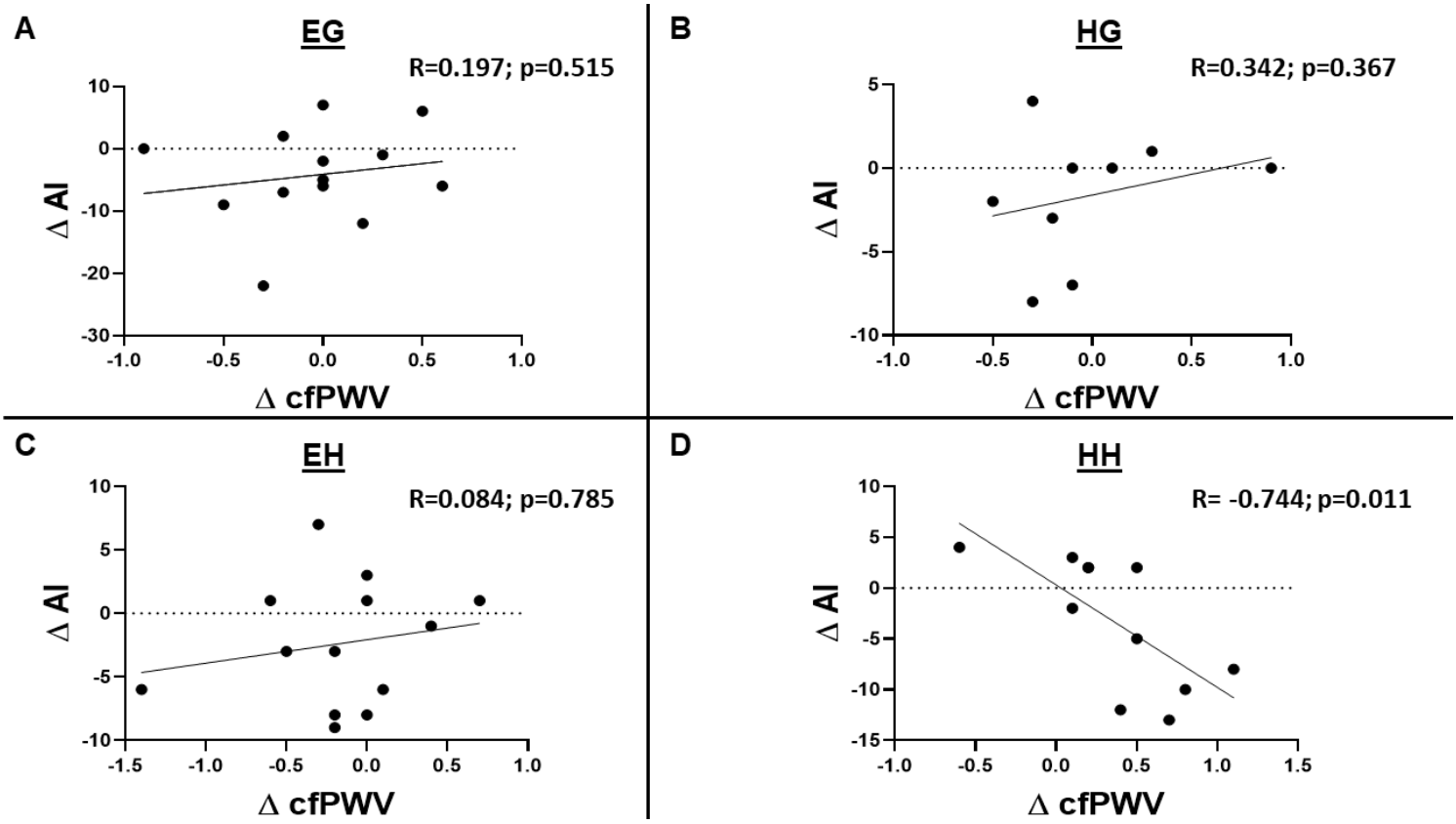

Figure 4

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). cfPWV is expressed in m/sec while AI is expressed as percentage. (EG= euglycemia; HG= hyperglycemia; EH= euglycemic-hyperinsulinemia; HH= hyperglycemic-hyperinsulinemia).