The influence of reduced insulin sensitivity via short-term reductions in physical activity on cardiac baroreflex sensitivity during acute hyperglycemia

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Abstract

Reduced insulin sensitivity and impaired glycemic control are among the consequences of physical inactivity and have been associated with reduced cardiac baroreflex sensitivity (BRS). However, the effect of reduced insulin sensitivity and acute hyperglycemia following glucose consumption on cardiac BRS in young, healthy subjects has not been well characterized. We hypothesized that a reduction in insulin sensitivity via reductions in physical activity would reduce cardiac BRS at rest and following an oral glucose tolerance test (OGTT). Nine recreationally-active men (23±1 yrs; >10,000 steps/day) underwent 5 days of reduced daily physical activity (RA5) by refraining from planned exercise and reducing daily steps (<5,000 steps/day). Spontaneous cardiac BRS (sequence technique) was compared at rest and for 120-min following an OGTT at baseline and after RA5. A sub-study (n=8) was also performed to independently investigate the influence of elevated insulin alone on cardiac BRS using a 120-min hyperinsulinemic-euglycemic clamp. Insulin sensitivity (Matsuda index) was significantly reduced following RA5 (BL: 9.2±1.3 vs. RA5: 6.4±1.1, P<0.001). Resting cardiac BRS was unaffected by RA5 and significantly reduced during the OGTT similarly at baseline and RA5 (BL: 0 min, 28±4 vs. 120 min, 18±4; RA5: 0 min, 28±4 vs. 120 min, 21±3 ms/mmHg). Spontaneous cardiac BRS was also reduced during the hyperinsulinemic-euglycemic clamp (P<0.05). Collectively, these data demonstrate that acute elevations in plasma glucose and insulin can impair spontaneous cardiac BRS in young, healthy subjects, and that reductions in cardiac BRS following acute hyperglycemia are unaffected by reduced insulin sensitivity via short-term reductions in physical activity.
Introduction

Over half of the adults in the US do not achieve recommended levels of physical activity (57). Reduced insulin sensitivity and impaired glycemic control are among the primary consequences of sedentary behavior and physical inactivity (23, 52, 59), and contribute to the cardiovascular disease risk (25). Importantly, impairments in arterial baroreflex function, a critical negative feedback control system for beat-to-beat regulation of arterial blood pressure (BP), has been shown in conditions exhibiting reductions in insulin sensitivity and persistent hyperglycemia (21, 34, 51, 55, 73, 75). However, despite the known cardiovascular risks of chronic sedentary behavior, little is known regarding the effects of a short-term reduction in physical activity, and the influence of acute hyperglycemia following glucose consumption, on arterial baroreflex function and autonomic regulation of the cardiovascular system.

Reduced cardiac baroreflex sensitivity (BRS), which is recognized as an independent risk factor for cardiovascular morbidity and mortality (32, 68), has been documented in conditions characterized by fasting hyperglycemia, such as insulin resistance and type 2 diabetes (20, 44, 53, 55, 67). Furthermore, acute hyperglycemia following a standard oral glucose tolerance test (OGTT) has been shown to reduce cardiac BRS (63, 65); however, these studies were performed in subjects with obesity, insulin resistance, and type 2 diabetes. To our knowledge, there are currently no studies of cardiac BRS during acute hyperglycemic conditions in young, healthy individuals that would demonstrate whether these reductions are independent of comorbidities and cardiovascular complications typically present with obesity and insulin resistance. This becomes important given the growing prevalence of insulin resistance among US adults (27) as well as the increased cardiovascular risk of impaired cardiac BRS (21, 68).
Recent reports have demonstrated that short-term reductions in daily physical activity in healthy individuals reduced insulin sensitivity (43, 52). Although evidence supports impaired insulin sensitivity as a significant contributor to reduced cardiac BRS (51, 73, 75), it has not been established whether decrements in insulin sensitivity that are seen following short-term reductions in physical activity are sufficient to induce impairments in cardiac BRS at rest or in response to a glucose challenge. Thus, herein we sought to examine whether a reduction in insulin sensitivity following 5 days of reduced daily physical activity (RA5; from >10,000 to <5,000 steps/day) alters cardiac BRS at rest or during acute hyperglycemia following a glucose challenge in young, healthy subjects. We hypothesized that a decrease in cardiac BRS would parallel reductions in insulin sensitivity following RA5, and that an OGTT would further impair cardiac BRS at RA5 compared to baseline when subjects were active.

In addition, because insulin is elevated along with glucose following an OGTT, we performed a second study using hyperinsulinemic euglycemic clamps in which insulin is elevated to postprandial concentrations but glucose is clamped at fasting concentrations to isolate acute effects of insulin on cardiac BRS. To our knowledge, there are no studies examining the influence of acute hyperglycemia on cardiac BRS in young, healthy humans, and the limited studies with insulin suggest either improvements (49, 50) or no change (5, 13) in cardiac BRS during acute hyperinsulinemia. As such, we hypothesized that reductions in cardiac BRS would be observed in the presence of acute hyperglycemia, but not during hyperinsulinemic euglycemic clamps when only plasma insulin concentrations are elevated in young healthy insulin sensitive subjects.
METHODS

Subjects

A total of 17 healthy young men were studied. Nine subjects (age: 23 ± 1 yrs; BMI: 23.3 ± 0.5 kg/m²) participated in study #1 (influence of 5 days of reduced daily physical activity on cardiac BRS at rest and in response to a glucose challenge) and eight subjects (age: 26 ± 2; BMI: 25.9±0.7 kg/m²) participated in study #2 (influence of acute elevations in insulin on cardiac BRS). Two subjects participated in both studies. All subjects were healthy, nonsmokers, and recreationally active but not training for competitive endurance events. Subjects had no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications. Subjects in study #1 were part of a larger published dataset examining endothelial function (11) and blood flow responses to glucose ingestion (52) following 5 days of reduced daily physical activity. Women were not studied because of the potential confounding effects of sex hormone changes across the menstrual cycle during the course of the study interventions. Protocols were approved by the University of Missouri Health Sciences Institutional Review board and written informed consent was obtained from all subjects.

Experimental Measurements

For both studies, heart rate (HR) was continuously monitored using a lead II surface ECG (Q710; Quinton, Bothell, WA, USA). Arterial blood pressure (BP) was measured on a beat-to-beat basis using servo-controlled finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Return to flow calibrations were performed and physiocal turned off before each Finometer recording. Arterial BP was also measured with an automated...
sphygmomanometer (Welch Allyn, Skaneatles Falls, NY, USA) for absolute measures of BP and to confirm Finometer measurements. Respiratory movements were monitored using a strain-gauge pneumograph placed around the abdomen (Pneumotrace, UFI, Morro Bay, CA, USA) to avoid potential confound of large respiratory excursions on cardiovascular measurements and calculate respiratory frequency.

**Study 1:** Influence of 5 days of reduced daily physical activity on cardiac BRS at rest and in response to a glucose challenge.

*Pre Intervention Procedures*

For this study, subjects were performing at least 90 min of primarily aerobic lower body exercise ≥3 days per week (self-reported) and taking greater than 10,000 steps per day (verified by pedometers). Prior to the reduced activity intervention, for 3 consecutive days, daily steps were monitored via a pedometer and average daily energy expenditure above 3 METs (metabolic equivalents, kilojoules) via a physical activity monitor (Body Media). Kilojoules were converted to kilocalories by the following equation: kilocalories = (kilojoules x 0.239). Subjects were also provided with breakfast, lunch, dinner, and snacks during the 3-day pre intervention testing to determine daily energy intake. The breakfast meal was standardized to 15% of daily energy (~450 kcal) and lunch was standardized to 24% of daily energy intake (~850 kcal), similar to habitual eating patterns of young adult Americans (69). A morning and afternoon snack (~200 kcal/snack) and an ad libitum dinner were also provided during the 3-day pre intervention testing. All meals were comprised of 57% carbohydrate, 28% fat, and 15% protein. Subjects were asked to fill out a questionnaire to assess compliance to the diet. Average daily energy intake over the 3-days was then determined.
During the 2 days prior to baseline testing and throughout the entire study intervention, the
subjects received eucaloric meals and snacks based on their respective measured intake
determined during the pre-intervention testing. A 45 minute supervised treadmill exercise session
at 60% of heart rate reserve was performed 24 hours prior to baseline testing to control for the
amount and time of day when the subjects received their last bout of structured physical activity,
and to control for exercise-induced increases in insulin sensitivity that is known to occur in
healthy subjects (23).

Five Day Reduced Daily Physical Activity Intervention (RA5)

Subjects were instructed to reduce their normal daily physical activity (>10,000 steps/day, along
with regular exercise sessions) to <5,000 steps/day for 5 days and abstain from any structured
exercise. This volume of daily steps was chosen because 5,000 steps or below is close to the
daily average step count of most sedentary individuals (6). Subjects wore pedometers to
quantitatively assess daily steps, and a physical activity monitor to estimate daily energy
expenditure. Some subjects were pushed in a wheelchair by study personnel to help facilitate
them achieving <5,000 steps/day.

Experimental Protocol

Before and after RA5 subjects underwent resting cardiovascular measurements followed by an
OGTT. Study visits were initiated between 7:00am and 9:00am after an overnight fast, and were
performed in a quiet, dimly lit, temperature controlled room (21-22°C). An intravenous catheter
was placed while resting in a supine position. After a 15 minute rest period, baseline blood
samples were obtained for measures of glucose and insulin. Arterial BP, HR and respiration were
measured for 10 min after which subjects ingested a standard glucose drink (75 grams glucose; Azer Scientific, Morgantown, PA). Subjects drank the glucose drink within 1 minute and then every 30 min up to 120 min, cardiovascular measures were made for a 5-min period followed by blood sampling for glucose and insulin.

**Study 2: Influence of acute elevations in insulin on cardiac BRS**

Because insulin is elevated along with glucose following an OGTT, a second study was performed in which subjects underwent a hyperinsulinemic euglycemic clamp (n=8) to isolate acute effects of insulin on cardiac BRS. These subjects also underwent an OGTT on a separate day using equivalent experimental procedures as in study 1. In addition, in a subset of subjects (n=5), spontaneous cardiac BRS measures were made during 120-min time control experiments in which 0.9% saline was infused to match the volume administered during the hyperinsulinemic euglycemic clamp (77). All studies started between 7:00am and 9:00am after an overnight fast, and performed in a quiet, dimly lit, temperature controlled room (21-22°C). On the morning of the hyperinsulinemic euglycemic clamp study, intravenous catheters were placed in a right antecubital vein and a left hand vein for infusion of insulin/glucose and blood sampling, respectively. The left hand was placed in a heated box (50°C) for acquisition of arterialized venous blood samples (36). Insulin (Humulin, Eli Lilly, Indianapolis, IN, USA) was diluted in 0.9% saline with 5 ml of the subject’s blood and a 10 min priming insulin infusion was followed by a constant infusion at 40 mU·m⁻²·min⁻¹, for a total of 120 min (61, 77). Whole blood glucose was determined every 5 min and maintained at euglycemic concentrations matching their morning fasting glucose throughout via a variable 20% dextrose infusion. Arterial BP, HR and respiration were measured for 10 min at baseline, and for a 5-min period every 30 min for 120
min during the insulin infusion. Blood serum was collected every 30 min and stored at -80ºC for later analysis for glucose and insulin. Insulin sensitivity was estimated by the glucose infusion rate during the last 30 min of the hyperinsulinemic euglycemic clamp (18).

Data Analysis

**Spontaneous cardiac baroreflex sensitivity:** Beat-to-beat time series of systolic BP and R-R interval were analyzed using the sequence technique for estimating spontaneous cardiac BRS (Nevrokard, Izola, Slovenia). Briefly, sequences of three or more consecutive beats where systolic BP and R-R interval change in the same direction were identified as arterial baroreflex sequences. Sequences were detected only when the variation in R-R interval was greater than 0.5 ms, systolic BP changes were greater than 1 mmHg, and longer than 3 cardiac cycles. A linear regression was applied to each individual sequence and only those sequences in which R² was >0.85 were accepted. The slopes of the systolic BP and R-R interval relationships were then calculated and averaged for a measure of spontaneous cardiac BRS. Spontaneous cardiac BRS was determined for all sequences combined, and also separately for up (increase SBP: increase R-R interval) and down (decrease SBP: decrease R-R interval) sequences. Overall results were similar when HR was used as the dependent variable for these cardiac BRS measures and therefore only R-R interval measures are presented.

The beat-to-beat time series of systolic BP and R-R interval were also submitted to fast Fourier transformation (FFT) and transfer function analysis for an additional estimate of spontaneous cardiac BRS (DADiSP, DSP Development, Cambridge, MA). The transfer function gain was derived from systolic BP as an input and R-R interval as an output variable as described in detail previously (47, 48). Transfer function gain, phase and coherence were calculated in the low
frequency range (0.04 to 0.15 Hz) since this range has previously been reported to reflect primarily arterial baroreflex mechanisms (47, 48), while BP fluctuations in the high frequency range appear to be mainly induced by respiration (14, 19).

Reliability of spontaneous cardiac baroreflex sensitivity measures: To determine the duration of data collection needed during OGTT experiments to adequately and reliably estimate spontaneous cardiac BRS, we performed a study in 8 additional subjects in which spontaneous BRS measures were derived from 10 min and 5 min periods and compared. Intraclass correlation coefficients (ICC) were calculated and an ICC > 0.8 was considered acceptable. 10 min vs. 5 min of resting beat-to-beat time series of systolic BP and R-R interval yielded an ICC=0.987 for spontaneous cardiac BRS derived with the sequence technique and an ICC=0.981 for low frequency transfer function gain cardiac BRS measures. Thus, 5 min time series segments were considered a reliable duration for time points in the OGTT experiments.

Heart Rate Variability: Time domain HR variability was determined by the standard deviation of the normal-to-normal intervals (SDNN), standard deviation of differences between adjacent normal-to-normal intervals (SDSD), and the root-mean-square of successive differences in R-R interval (RMSSD). SDNN is considered an estimate of overall HR variability, and RMSSSD is an estimate of short-term components of HR variability (1). Power spectral analysis using fast Fourier transformation was also performed in the HF range (0.15-0.4). Similar to RMSSD, HF power is considered to predominantly represent parasympathetic tone (1, 38).

Blood Sample Analysis: Serum glucose measures were made using the glucose oxidase method (Fisher Diagnostics, Middletown, VA). Insulin was measured via enzyme-linked immunosorbent assays (Immulite 1000 Analyzer, Siemens, Deerfield, IL). Insulin sensitivity during the OGTT
was assessed using the Matsuda insulin sensitivity index (ISI) as previously described by our lab
and others (40, 42). Matsuda ISI was calculated as: $10,000/\text{square root of } [(G_0 \times I_0) \times (G_{\text{Ave}} \times
I_{\text{Ave}})]$, where $G_0$ and $I_0$ are baseline values for glucose and insulin, respectively, and $G_{\text{Ave}}$ and $I_{\text{Ave}}$
are average values for glucose and insulin over the course of the OGTT, respectively. The
homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as previously
described (41): $(\text{fasting glucose} \times \text{fasting insulin})/405$. Glucose and insulin measurements were
not performed in 1 subject at RA5 due to problems with the intravenous catheter.

**Statistical Analysis**

Paired t-tests were used to determine significant changes in physical activity and resting
cardiovascular and metabolic parameters following RA5. One-way repeated measures ANOVA
was used to examine changes in cardiovascular and metabolic values over the course of the 120
min OGTTs, hyperinsulinemic euglycemic clamp, and time control studies (i.e., 0, 30, 60, 90,
and 120 min time points). Two way repeated measures ANOVA was used to compare changes in
cardiovascular and metabolic values during the OGTTs before and after RA5. Student-Newman-
Keuls post hoc testing was applied where significant main effects were found. Rank order testing
was applied when necessary. Data was analyzed using SigmaPlot 13 (Systat Software Inc.) and
are presented as mean ± SE.
Results

Study 1

Reduced physical activity intervention

As expected, average steps per day (baseline: 13,058 ± 800 vs. RA5: 5,165 ± 578 steps/day, P<0.05) and kilocalories expended above 3 METs per day (baseline: 4,612 ± 420 vs. RA5: 2,151 ± 338 Kcal/day>3 METs, P<0.05) were significantly decreased with RA5 demonstrating the effectiveness of the reduced activity intervention and subject compliance. Body weight (BL: 77.0 ± 2.3 vs. RA5: 77.2 ± 2.3 Kg, P>0.05) and resting cardiovascular measures were unaffected by RA5 (Table 1). Time and frequency domain indices of heart rate variability reflective of cardiac parasympathetic tone were also not different at rest with RA5 (Table 2). Although fasting glucose was unchanged (BL: 84 ± 2 vs. RA5: 86 ± 3 mg/dl, P>0.05), fasting insulin was significantly elevated at RA5 (BL: 4.8 ± 0.5 vs. RA5: 8.3 ± 1.6 μIu/ml, P<0.05). Likewise, HOMA-IR tended to increase at RA5 indicative of a reduction in insulin sensitivity (BL: 1.4 ± 0.4 vs. RA5: 2.3 ± 0.4 units, P=0.066).

Spontaneous cardiac BRS at rest and in response to an OGTT following RA5

Resting spontaneous cardiac BRS was not different at RA5 compared to baseline (BL: 28 ± 4 vs. RA5: 28 ±4 ms/mmHg, P>0.05). Likewise, although spontaneous cardiac BRS was significantly reduced in response to an OGTT, this reduction was similar at baseline and following RA5 (P<0.001) (Figure 1A). The reduction in cardiac BRS was also apparent when sequences were separated into ups (increase SBP: increase R-R interval) and downs (decrease SBP: decrease R-R interval) with both being significantly decreased following the OGTT at baseline and RA5 (Figure 1B and 1C; both P<0.05). The SBP range and the number of spontaneous cardiac BRS
sequences were similar at rest and for all the time points during the OGTT both at baseline and after RA5 (Table 2). Similar to the sequence derived measures of spontaneous cardiac BRS, low frequency transfer function gain measures of cardiac BRS were significantly reduced in response to the OGTT and this occurred both at baseline and following RA5 (Visit: P=0.793; Time point: P=0.032; Interaction: P=0.696). For example, transfer function gain was 20 ± 3 and 14 ± 2 ms/mmHg at 0 min and 120 min, respectively, of the baseline OGTT, and 19 ± 3 and 15 ± 3 ms/mmHg at 0 min and 120 min, respectively, of the OGTT at RA5 (P<0.05).

Cardiovascular and metabolic parameters in response to OGTT

Cardiovascular parameters during the OGTT at baseline and at RA5 are summarized in Table 1. Mean, systolic and diastolic BP were unchanged during the OGTT and this was unaffected by RA5 (P>0.05). HR was significantly elevated and R-R interval significantly reduced over the course of the OGTT both at baseline and after RA5 (P<0.05). Time domain (SDNN, SDSD, and RMSSD) and high frequency heart rate variability measures were reduced during the OGTT similarly at baseline and RA5 suggesting a reduction in parasympathetic tone of the heart (Table 2). Importantly, respiratory frequency throughout the OGTT was similar at baseline and RA5 (e.g., BL: 0 min, 12 ± 1 vs. 120 min, 13 ± 1 breaths per minute; RA5: 0 min, 12 ± 1 vs. 120 min, 14 ± 1 breaths per minute, P>0.05). Elevations in serum glucose concentrations during the OGTT at RA5 were not statistically different compared to the baseline OGTT (P>0.05) (Figure 2A). Likewise, the rise in serum insulin concentrations during the OGTT was similar at baseline and RA5, although a greater insulin concentration was apparent at the 120 min time point (Figure 2B). This contributed to a greater area under the curve (AUC) serum insulin during the OGTT at RA5 compared to the baseline OGTT (BL OGTT: 9712 ± 1396 vs. RA5 OGTT: 11,546 ± 1377
a.u.) but this did not reach statistical significance (P>0.05). Nevertheless, insulin sensitivity determined from the OGTT (Matsuda ISI) was significantly reduced following RA5 compared to baseline (P<0.05) (Figure 2C).

**Study 2**

*Spontaneous cardiac BRS in response to a hyperinsulinemic euglycemic clamp*

Overall spontaneous cardiac BRS was significantly reduced during the hyperinsulinemic euglycemic clamp (Figure 3). However, this reduction in spontaneous cardiac BRS appeared to be delayed and not significant until the 120 min time point. This was in contrast to the OGTT, in which a reduction in cardiac BRS was observed at 30 min and maintained throughout (Figure 3). Only down sequences appeared to be reduced during the hyperinsulinemic euglycemic clamp (P=0.053), whereas both up and down sequences of cardiac BRS were significantly reduced during the OGTT (P<0.05), similar to observations in study #1. No changes were noted in the number of spontaneous cardiac BRS sequences detected over the course of the hyperinsulinemic euglycemic clamp (0 min, 34 ± 4 vs. 120 min, 34 ± 5; P>0.05). Although the SBP range was maintained throughout the first 90 min of the clamp (e.g. 0 min, 26 ± 1 vs. 90 min, 25 ± 1; P>0.05), there was a significant increase at the 120 min time point (31 ± 2; P<0.05 vs. 0 min). No change in spontaneous cardiac BRS was observed during 120-min time control experiments (P>0.05) (Figure 3).

*Cardiovascular and metabolic parameters in response to hyperinsulinemic euglycemic clamp*

Mean, diastolic, and systolic BP were all unchanged over the course of the hyperinsulinemic euglycemic clamp (e.g. systolic BP: 0 min, 122 ± 3 vs. 120 min, 123 ± 4; P>0.05). Likewise, HR, R-R interval, time domain (SDNN, SDSD, and RMSSD) and high frequency HR variability
did not change during the hyperinsulinemic euglycemic clamp (Table 3). By design, serum glucose concentration was maintained around fasting values during the clamp (P > 0.05), whereas serum insulin concentration was significantly elevated to postprandial values (P < 0.05) (Table 3). In this regard, the steady state insulin concentration during the hyperinsulinemic euglycemic clamp was similar to peak increase in serum insulin concentration following the OGTT. Average glucose infusion rate during the hyperinsulinemic euglycemic clamp was 8.4 ± 0.9 mg/kg/min, as expected for healthy adults with normal insulin sensitivity (7, 66).
Discussion

The primary and novel findings of the present study are that spontaneous cardiac BRS is impaired following glucose consumption in young, healthy subjects, and that a reduction in insulin sensitivity following 5 days of reduced daily physical activity is not sufficient to induce impairments in cardiac BRS at rest or contribute to further impairments in cardiac BRS following glucose consumption. In addition, we demonstrate that elevations in plasma insulin alone are also capable of reducing spontaneous cardiac BRS. Collectively, these data demonstrate for the first time that acute elevations in plasma glucose and insulin impair spontaneous cardiac BRS in young, healthy men, and that the reductions in spontaneous cardiac BRS following an OGTT appear to be unaffected by reduced insulin sensitivity via short-term reductions in physical activity.

Reduced cardiac BRS has been documented during acute hyperglycemia using a standard OGTT in conditions with known impaired cardiac BRS at rest, such as obesity, insulin resistance, and type 2 diabetes (63, 65). However, the acute effect of glucose consumption on the arterial baroreflex in young, healthy adults has not been well characterized. In the present study, we examined cardiac BRS during similar acute hyperglycemic conditions in young, healthy individuals before and after a reduction in insulin sensitivity (i.e., RA5). We rationalized that a reduction in insulin sensitivity via reductions in physical activity would contribute to impaired spontaneous cardiac BRS during acute hyperglycemia. In this regard, recent findings have shown that pharmacological insulin sensitization via pioglitazone treatment attenuates the reduction in spontaneous cardiac BRS during an OGTT in insulin resistant subjects (64). However, contrary to our hypothesis, spontaneous cardiac BRS was reduced during the OGTT at baseline and this was similar following RA5. Although the reduction in daily physical activity
significantly decreased insulin sensitivity during the OGTT, and significantly elevated fasting plasma insulin, these effects did not appear to contribute to further impairment in spontaneous cardiac BRS during the OGTT. It is possible however that the reduction in insulin sensitivity observed in the present study did not meet a threshold that would significantly impair the glycemic response to the OGTT to cause a further reduction in cardiac BRS. Indeed, no appreciable increase in serum glucose concentration was observed during the OGTT at RA5 compared to baseline (Figure 2A). However, the reduction in insulin sensitivity following the reduced physical activity intervention was comparable to previous reports of insulin sensitivity in adult subjects with or at risk of developing type 2 diabetes (2, 37). Interestingly, not only was there no impact of RA5 on cardiac BRS during the OGTT in the young, healthy men in the present study, the magnitude of reduction in cardiac BRS during the OGTT was strikingly similar to previous reports of reductions in BRS following an OGTT in subjects with impaired glucose tolerance and type 2 diabetes patients (63). Although the findings of the present study demonstrate a potent effect of hyperglycemia on spontaneous cardiac BRS, acutely reducing insulin sensitivity via a short-term reduction in physical activity does not appear to influence spontaneous cardiac BRS at rest or during acute hyperglycemia in young, healthy subjects.

While previous studies have investigated the effects of glucose on the central nervous system, the mechanism(s) responsible for the glucose-induced impairment in cardiac BRS during the OGTT remains uncertain. Glucose has been shown to exert effects on neurons in the nucleus of the solitary tract (NTS) (3, 15, 45, 72, 76), the first brainstem relay site for baroreceptor and visceral afferents. In addition, glucose injection into the hypothalamus, an important brain site for metabolic homeostasis and autonomic regulation, has been shown to decrease the firing rate of the superior vagus nerve by 39% (12) and presumably impact baroreflex control of the heart.
In the present study, significant increases in HR (13%) and reductions in HR variability (-17%) were observed following glucose consumption, in addition to a reduction in cardiac BRS, suggesting a prominent negative influence of glucose on parasympathetic control of the heart. Taken together, these findings lend support to the idea that glucose intake promotes a reduction in parasympathetic control of the heart leading to a decrease in cardiac BRS.

An elevation in circulating insulin is the main hormone response to glucose intake, thus presenting another factor for consideration when examining the cardiovascular effects following glucose ingestion. Indeed, circulating insulin can influence arterial baroreflex control via the central nervous system where insulin receptors are present in specific cardiovascular regulatory regions, including the hypothalamus and brainstem (58, 74). It is well established in animal (50) and human (77) studies that insulin increases arterial baroreflex control of sympathetic nerve activity. Acute elevations of insulin in the brain in normal healthy rats have previously been demonstrated to either enhance (49, 50) or have no effect (5, 13) on cardiac BRS. Similar to the latter studies, our group previously showed no effect of elevated systemic insulin on cardiac BRS in humans (77). However, in the present study, although delayed, a significant reduction in spontaneous cardiac BRS was seen in all subjects at 120 min of the hyperinsulinemic euglycemic clamp. Interestingly, in contrast to the OGTT, no change in HR or HR variability was observed during the hyperinsulinemic euglycemic clamp. These results are consistent with previous studies reporting a lack of change in HR and HR variability during a hyperinsulinemic euglycemic clamp (8), and bring into question whether the reduction in cardiac BRS during the hyperinsulinemic euglycemic is a direct result of an alteration in parasympathetic control of the heart as evidence suggests during the OGTT.
Although not investigated in the present study, several other peripheral hormones related to nutrient intake have been shown to influence autonomic regulation of the cardiovascular system, such as glucagon-like peptide 1 (GLP-1), leptin, and ghrelin (4, 22, 31, 33, 39). In healthy individuals, GLP-1 infusion leads to significant increases in muscle sympathetic nerve activity (10), but no detectable changes in parasympathetic control of the heart have been reported (8, 10). While studies show that leptin can also enhance central sympathetic outflow (35), investigations into the effects of leptin on cardiac BRS have yielded mixed results (4, 35). Nonetheless, elevations in circulating leptin would likely not contribute to alterations in cardiac BRS during the 2 hr OGTT because previous studies demonstrated a significant lag (≥5 hr) in the increase in plasma leptin concentration following a meal in young, lean subjects (16). Intravenous administration of ghrelin in healthy humans has been shown to enhance arterial baroreflex control of sympathetic nerve activity but not heart rate (31), suggesting that ghrelin also may not contribute importantly to the impaired spontaneous cardiac BRS that is observed during acute hyperglycemia.

Although a sedentary lifestyle has been associated with reduced cardiac BRS (29, 46, 60), to date, no studies have investigated the direct impact of a short-term reduction in daily physical activity on arterial baroreflex function. The majority of studies have focused on cross-sectional analyses or the impact of increased physical activity interventions (17, 30, 46, 62). In the present study, a reduction in resting cardiac BRS was not observed following the 5-day reduction in daily physical activity. In addition, no changes in measures of HR or HR variability were observed following the short-term reduction in physical activity, supporting a lack of change in parasympathetic control of the heart. Although it is unclear whether a longer duration of reduced physical activity would influence cardiac BRS, these results are consistent with previous findings.
in which physical activity status among young adults was not associated with cardiac BRS (46).

Monohan et al. (2000) reported in a cross-sectional study that young adults who exercised regularly did not have greater cardiac BRS compared to their sedentary counterparts. Similar results have been reported in exercise intervention studies (30). While focus in the present study was on a short-term reduction in physical activity, additional studies using prolonged decreases in daily physical activity (i.e., >5 days) in healthy, active individuals would be needed to further characterize the relationship between physical inactivity and arterial baroreflex function.

Several limitations should be considered when interpreting the findings from the present study. First, only young men were studied, and thus, care should be given in extending these findings to women. Second, subjects were inactive for 5 days and it is plausible that a longer duration of inactivity may impair resting cardiac BRS and further reduce BRS following glucose ingestion even in these young healthy subjects with normal autonomic function. Lastly, a time control experiment was not included in study #1. Although this was initially considered, the already extensive burden of the study design and duration for the subjects as well as investigators, including diet control, made it somewhat prohibitive. More importantly, in reviewing the literature, previous studies have reported high reproducibility of spontaneous baroreflex sensitivity using the sequence technique at rest and during perturbations (24, 26). Also, lack of a change in cardiac BRS at rest after 5 days of reduced physical activity further suggests time was not a factor.

**Perspectives**

The arterial baroreflex plays an important role in the short-term maintenance of BP. Nonetheless, a decrease in cardiac BRS with glucose ingestion does not appear to be specific to insulin resistance and type 2 diabetes as we now demonstrate it occurs in healthy recreationally
active young men (i.e., at baseline prior to RA5). Such reductions in cardiac BRS have implications for the maintenance of BP following nutrient intake (e.g., postprandial hypotension), but this is likely offset by concomitant insulin-mediated increase in central sympathetic outflow and improved sympathetic baroreflex sensitivity (9, 50, 70, 77), at least in young healthy subjects. This would induce compensatory peripheral vasoconstriction for maintenance of BP and likely explain the low prevalence of postprandial hypotension in young, healthy adults. However, in addition to impairment in cardiac BRS following acute hyperglycemia (63-65), subjects with insulin resistance also demonstrate a blunting of insulin-mediated elevations in central sympathetic outflow (65, 71) that can further impair BP regulation. Indeed, significant reductions in BP (>20 mmHg) have been reported in patients with type 2 diabetes during acute hyperglycemia (28, 54, 56). Further examination of arterial baroreflex function and neural cardiovascular responses following nutrient intake are needed to better understand its contributions to impaired BP regulation, particularly in obese, insulin resistant subjects and patients with type 2 diabetes.

In summary, we demonstrate that spontaneous cardiac BRS is reduced following an OGTT in young, healthy subjects. Likewise, cardiac BRS is also reduced when insulin is elevated but glucose is clamped at fasting concentrations with a hyperinsulinemic-euglycemic clamp. We also show that a reduction in insulin sensitivity via a short-term reduction in physical activity does not impair cardiac BRS at rest or contribute to further impairment in cardiac BRS following glucose consumption. Collectively, these data demonstrate for the first time that cardiac BRS is impaired following glucose consumption in young, healthy individuals, and that these reductions are likely due to combined elevations in plasma glucose and insulin, but appear unaffected by reduced insulin sensitivity following a short-term reduction in physical activity.
Grants

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions


Acknowledgements

We thank Charla Jay, R.N., for assistance and the participants for time and cooperation. This research was submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy for Seth W. Holwerda.
References


Figure 1. Mean summary data showing reduction in overall (Panel A), up (increase SBP: increase R-R interval; Panel B) and down (decrease SBP: decrease R-R interval; Panel C) spontaneous cardiac BRS during the oral glucose tolerance test (OGTT) at baseline and following 5 days of reduced physical activity (RA5). Data presented as mean ± SE.

Figure 2. Mean summary data showing serum glucose (Panel A) and insulin (Panel B) responses to the oral glucose tolerance test (OGTT) at baseline and following 5 days of reduced physical activity (RA5). Insulin sensitivity (Matsuda insulin sensitivity index; ISI) was determined from serum glucose and insulin responses to the OGTT at baseline and following RA5 (Panel C). *P<0.05 vs. Baseline. Data presented as mean ± SE.

Figure 3. Mean summary data showing changes in overall spontaneous cardiac BRS during the hyperinsulinemic euglycemic clamp (Panel A), the OGTT (Panel B), and time control experiments (Panel C) in study #2. *P<0.05 vs. 0 min time point. Data presented as mean ± SE.
Table 1. Cardiovascular parameters during the OGTT at baseline and at RA5

<table>
<thead>
<tr>
<th></th>
<th>Visit</th>
<th>Time point</th>
<th>P-value (2-way RM ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>113 ± 3</td>
<td>117 ± 3</td>
<td>117 ± 3</td>
</tr>
<tr>
<td>RA5</td>
<td>118 ± 2</td>
<td>120 ± 4</td>
<td>120 ± 5</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
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<tr>
<td>RA5</td>
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<td>64 ± 4</td>
</tr>
<tr>
<td><strong>Mean BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>81 ± 3</td>
<td>81 ± 3</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>RA5</td>
<td>85 ± 2</td>
<td>82 ± 3</td>
<td>82 ± 4</td>
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<tr>
<td><strong>R-R Interval (ms)</strong></td>
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<td></td>
</tr>
<tr>
<td>BL</td>
<td>1155 ± 49</td>
<td>1063 ± 57</td>
<td>1077 ± 54</td>
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<tr>
<td>RA5</td>
<td>1200 ± 69</td>
<td>1130 ± 62</td>
<td>1090 ± 66</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>53 ± 2</td>
<td>58 ± 3</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>RA5</td>
<td>52 ± 3</td>
<td>55 ± 3</td>
<td>57 ± 3</td>
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Data presented as mean ± SE.
<table>
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<tr>
<th>HRV parameters</th>
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<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>P-value (2-way RM ANOVA)</th>
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</thead>
<tbody>
<tr>
<td>SDNN (ms)</td>
<td>BL</td>
<td>85 ± 12</td>
<td>85 ± 13</td>
<td>74 ± 11</td>
<td>90 ± 12</td>
<td>67 ± 10</td>
<td>0.739 0.030 0.153</td>
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<tr>
<td></td>
<td>RAS</td>
<td>91 ± 10</td>
<td>93 ± 14</td>
<td>84 ± 12</td>
<td>75 ± 11</td>
<td>67 ± 7</td>
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<tr>
<td>SDSD (ms)</td>
<td>BL</td>
<td>73 ± 12</td>
<td>74 ± 12</td>
<td>62 ± 11</td>
<td>66 ± 12</td>
<td>53 ± 10</td>
<td>0.207 0.014 0.212</td>
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<tr>
<td></td>
<td>RAS</td>
<td>80 ± 8</td>
<td>87 ± 15</td>
<td>78 ± 11</td>
<td>60 ± 8</td>
<td>61 ± 7</td>
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<tr>
<td>RMSSD (ms)</td>
<td>BL</td>
<td>73 ± 11</td>
<td>74 ± 12</td>
<td>62 ± 11</td>
<td>66 ± 12</td>
<td>53 ± 10</td>
<td>0.208 0.014 0.212</td>
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<tr>
<td></td>
<td>RAS</td>
<td>80 ± 8</td>
<td>87 ± 15</td>
<td>78 ± 11</td>
<td>60 ± 8</td>
<td>61 ± 7</td>
<td></td>
</tr>
<tr>
<td>HF power (ms²)</td>
<td>BL</td>
<td>1410 ± 487</td>
<td>1011 ± 330</td>
<td>959 ± 375</td>
<td>1137 ± 417</td>
<td>835 ± 423</td>
<td>0.686 0.008 0.392</td>
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<tr>
<td></td>
<td>RAS</td>
<td>1178 ± 246</td>
<td>1118 ± 454</td>
<td>1153 ± 491</td>
<td>758 ± 183</td>
<td>880 ± 308</td>
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</table>

**BRS parameters**

<table>
<thead>
<tr>
<th>SBP range (mmHg)</th>
<th>Visit</th>
<th>23 ± 4</th>
<th>26 ± 4</th>
<th>23 ± 2</th>
<th>28 ± 5</th>
<th>24 ± 2</th>
<th>P-value (2-way RM ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>23 ± 4</td>
<td>26 ± 4</td>
<td>23 ± 2</td>
<td>28 ± 5</td>
<td>24 ± 2</td>
<td>0.839 0.519 0.495</td>
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<tr>
<td>RAS</td>
<td>26 ± 2</td>
<td>28 ± 3</td>
<td>23 ± 1</td>
<td>24 ± 2</td>
<td>21 ± 1</td>
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</table>

<table>
<thead>
<tr>
<th># Overall BRS seq</th>
<th>Visit</th>
<th>28 ± 5</th>
<th>29 ± 5</th>
<th>31 ± 5</th>
<th>33 ± 4</th>
<th>33 ± 5</th>
<th>P-value (2-way RM ANOVA)</th>
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<tbody>
<tr>
<td>BL</td>
<td>28 ± 5</td>
<td>29 ± 5</td>
<td>31 ± 5</td>
<td>33 ± 4</td>
<td>33 ± 5</td>
<td>0.446 0.181 0.922</td>
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<tr>
<td>RAS</td>
<td>28 ± 4</td>
<td>27 ± 5</td>
<td>28 ± 4</td>
<td>30 ± 6</td>
<td>33 ± 5</td>
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</table>

Data presented as mean ± SE.
Table 3. HRV and metabolic parameters during the hyperinsulinemic euglycemic clamp

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<tr>
<th>HRV parameters</th>
<th>Time point</th>
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<th></th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
<td>120 min</td>
<td></td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>70 ± 6</td>
<td>65 ± 8</td>
<td>69 ± 6</td>
<td>74 ± 5</td>
<td>77 ± 12</td>
<td>0.561</td>
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<tr>
<td>SDSD (ms)</td>
<td>64 ± 10</td>
<td>56 ± 11</td>
<td>64 ± 12</td>
<td>60 ± 11</td>
<td>69 ± 14</td>
<td>0.639</td>
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<tr>
<td>RMSSD (ms)</td>
<td>64 ± 10</td>
<td>56 ± 10</td>
<td>64 ± 12</td>
<td>60 ± 11</td>
<td>69 ± 14</td>
<td>0.639</td>
</tr>
<tr>
<td>HF power (ms²)</td>
<td>1049 ± 295</td>
<td>963 ± 349</td>
<td>1031 ± 317</td>
<td>933 ± 296</td>
<td>1297 ± 495</td>
<td>0.789</td>
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<table>
<thead>
<tr>
<th>Metabolic parameters</th>
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<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>87 ± 4</td>
<td>77 ± 6</td>
<td>79 ± 5</td>
<td>86 ± 4</td>
<td>82 ± 4</td>
<td>0.239</td>
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<tr>
<td>Insulin (μIU/mL)</td>
<td>6 ± 1</td>
<td>50 ± 7*</td>
<td>49 ± 6*</td>
<td>52 ± 6*</td>
<td>52 ± 7*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE. *P<0.05 vs. 0 min.
Figure 1

A) Overall cardiac BRS

B) Up cardiac BRS

C) Down cardiac BRS
Figure 3

A) Hyperinsulinemic euglycemic clamp

B) OGGT

C) Time control

RM ANOVA: P=0.034

RM ANOVA: P<0.001

RM ANOVA: P=0.453