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Heart rate variability and HbA1c predict plasma interleukin-6 response to psychosocial stress challenge in trauma-exposed women with type 2 diabetes

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a major public health problem in the United States. Although cardiovascular autonomic functioning, blood glucose control, and inflammation are known to play a role in T2DM, the interaction between these variables remains largely unexplored, particularly in the context of stress. To address this gap, we examined the relationship between these variables in a sample that is uniquely vulnerable to the health consequences of T2DM.

Methods: Participants were 37 trauma-exposed Black women with a diagnosis of T2DM. High frequency heart rate variability (HF-HRV), blood glucose control (HbA1c), and a stressor-evoked biomarker of inflammation (interleukin 6; IL-6) were obtained as part of a larger study of the genetic risk factors for and consequences of trauma exposure.

Results: The interaction of HbA1c and HF-HRV was significantly associated with IL-6 response calculated as area under the curve with respect to ground. Post-hoc simple slopes analyses revealed HbA1c, rather than HF-HRV, as the moderator in this association such that higher HF-HRV conferred higher circulating levels of IL-6 only in the presence of lower HbA1c, ($\beta = 0.60$, $t = 3.51$, $p = .001$).

Conclusions: Cardiovascular autonomic functioning and blood glucose control were significantly associated with stressor-evoked IL-6 responses when controlling for BMI and age. Moreover, the association between cardiovascular autonomic functioning and inflammation varied at different levels of HbA1c. This highlights the possibility that individuals with trauma exposure and T2DM may benefit from stratification by HbA1c levels for research analysis and treatment decision making.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has risen rapidly over the course of the last several decades and has become a major public health concern in the United States (Geiss et al., 2014). T2DM, sometimes referred to as 'adult onset' or 'non-insulin-dependent' diabetes, is a metabolic disorder characterized by chronic hyperglycemia, or elevated blood sugar (Smushkin and Vella, 2010). A diagnosis of T2DM, which is

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typically made in the presence of elevated HbA1c or fasting blood glucose levels, confers elevated risk of cardiovascular disease, autonomic neuropathy, renal disease, and various other health complications and mortality (Smushkin and Vella, 2010). Approximately 8.6% of American adults, or 21 million individuals, are currently diagnosed with T2DM (Bullard et al., 2018).

Among the complications associated with T2DM, increased risk for cardiovascular disease is particularly well documented and accounts for more than 50% of all diabetes-related deaths (Einarson et al., 2018). Diabetic cardiovascular autonomic neuropathy (CAN), which is a frequent and serious complication of diabetes marked by damage to the autonomic nerve fibers that innervate the heart and blood vessels (Vinik et al., 2003; Vinik and Ziegler, 2007), confers a nearly five-fold increase in risk of cardiovascular mortality (Serhiyenko and Serhiyenko, 2018). CAN is indicative of cardiovascular autonomic dysfunction and is therefore most commonly assessed using measures of heart rate variability (HRV; Vinik et al., 2003). HRV represents the variability in the time interval between heartbeats and is typically categorized into one of two frequency bands – low (0.04–0.15) or high (0.15–0.40; Vinik et al., 2003). High frequency HRV (HF-HRV) is unique in that it is regarded as an index of parasympathetic, or vagal, control (Benichou et al., 2018). Higher HF-HRV generally confers a greater ability for one's autonomic nervous system to adapt to novel circumstances (Benichou et al., 2018), and it is for this reason that HF-HRV is considered a measure of autonomic flexibility and is typically higher in 'healthier' individuals (McCraty and Shaffer, 2015). On average, individuals with T2DM present with significantly lower HF-HRV than healthy controls (Benichou et al., 2018). In a study of 3000 men and women with T2DM, those with *lower* HRV were at significantly increased risk of cardiovascular mortality (Kataoka et al., 2004). In other words, even within a clinical sample of individuals with categorically low HRV, more severe autonomic dysfunction conferred worse health outcomes.

Much like autonomic dysfunction, chronic inflammation is a frequent and well-documented feature of T2DM (Calle and Fernandez, 2012). In fact, the findings of a 2014 study indicated that circulating inflammation levels – specifically, interleukin-6 (IL-6) – marked the difference between obese women with and without T2DM (van Beek et al., 2014). Given the well-established link between T2DM and cardiovascular disease (Tsalamandris et al., 2019), it is unsurprising that chronically elevated inflammation is similarly present among individuals with cardiovascular disease (Ruparelia et al., 2017). Taken together, these findings suggest that inflammation may serve as the link between these conditions (Ziegler, 2005). Studies also show that higher levels of inflammation – such as those observed in T2DM and cardiovascular disease – are consistently associated with lower HF-HRV, or decreased autonomic function (Williams et al., 2019). It is important to note, however, that baseline inflammation is biologically distinct from stressor-evoked inflammation. While baseline inflammation reflects the body's immune activity while at rest, stressor-evoked inflammation reactivity represents the body's immune response to a stressful stimulus. At present, there is a paucity of research exploring inflammatory responses to stress in relation to T2DM and autonomic function. However, researchers exploring inflammation within other contexts have found that greater IL-6 reactivity to psychosocial stress was associated with decreased cardiometabolic functioning in perimenopausal women (Zannas et al., 2020), as well as with trauma exposure in adult women and men (Carpenter et al., 2010; Tell et al., 2018).

There is considerable evidence to suggest that trauma exposure increases the risk for T2DM and its related conditions (Huffhines et al., 2016; Roberts et al., 2015; Vaccarino et al., 2014). In particular, individuals living in low-income communities are more likely to experience chronic, repeated exposure to violence and trauma, and individuals living in those communities are disproportionately likely to be Black as a result of historic disenfranchisement in the United States (Gluck et al., 2021). Likewise, prevalence rates of T2DM are higher among Black people (Golden et al., 2012) and Black individuals have worse T2DM severity (indicated by higher HbA1c levels; Herman, 2016) compared to white individuals. Given that trauma exposure and subsequent psychopathology are also linked with autonomic dysfunction and chronic inflammation (Brudey et al., 2015), it is critical to study T2DM and these physiological phenomena among trauma-exposed Black populations.

1.1. Experiment overview

It is clear that cardiovascular autonomic functioning, blood glucose control, and inflammation play critical roles in the onset and maintenance of T2DM, yet the interaction between these measures remains largely unexplored. To address this gap, we examined their associations in trauma-exposed Black women with T2DM. Cardiovascular autonomic functioning was assessed using HF-HRV, blood glucose control was assessed using HbA1c levels, and stressor-evoked inflammation was represented by IL-6 levels in response to the Trier Social Stress Test (TSST). We hypothesized that 1) worse blood glucose control (higher HbA1c levels) at baseline would predict higher IL-6 levels during the TSST, and 2) the relationship between HbA1c and stressor-evoked IL-6 reactivity would be moderated by baseline cardiovascular autonomic functioning (HF-HRV).

2. Materials and methods

2.1. Participants

Participants were 37 trauma-exposed Black women with a diagnosis of T2DM. A diagnosis of T2DM was determined either from the participant's medical record or at the time of enrollment by one of the following criteria: 1) fasting blood glucose $\geq$126 mg/dl, 2) 2-h glucose tolerance test blood glucose >200 mg/dl, or 3) hemoglobin HbA1C > 6.5%. See Table 1 for participant demographics. Trauma exposure was defined for this sample as exposure to any traumatic event in childhood and/or adulthood (e.g., sexual assault, childhood abuse or neglect, serious accident or injury, natural disaster, etc.). In this sample, 78.4% of individuals reported experiences of childhood maltreatment (i.e., childhood physical, sexual, or emotional abuse, or physical or emotional neglect), with childhood maltreatment scores ranging from 25 to 100 (M = 39.14) on a scale of 25–125. Additionally, 91% of individuals have experienced 2 or more trauma types according to the Traumatic

Table 1
Participant demographics.

Age Range	28–65
Sex, N (%)	
Female	37 (100%)
Race, N (%)	
Black/African American	37 (100%)
Ethnicity, N (%)	
Non-Hispanic/Non-Latinx	37 (100%)
Highest Level of Education, N (%)	
Less than 12th Grade	1 (4.8%)
12th or High School Graduate	5 (23.8%)
GED	1 (4.8%)
Some College or Technical School	3 (14.3%)
Technical School Graduate	2 (9.5%)
College Graduate	7 (33.3%)
Missing	2 (9.5%)
Employment Status, N (%)	
Currently Employed	28 (75.7%)
Not Currently Employed	9 (24.3%)
Household Monthly Income, N (%)	
$0 - $249	5 (13.5%)
$250 - $499	5 (13.5%)
$500 - $999	6 (16.2%)
$1000 - $1999	14 (37.8%)
$2000 or more	6 (16.2%)
Missing	1 (2.7%)

Note. $N = 37$.

Experiences Inventory. Of note, 31.4% met criteria for current PTSD at the time of the study and 31.4% met for lifetime but not current PTSD, for a total of 62.9% of individuals with lifetime PTSD.

2.2. Procedure

Participants were recruited as part of a larger study on the genetic risk factors for and consequences of trauma exposure (The Grady Trauma Project; Gillespie et al., 2009; Gluck et al., 2021). Specifically, they were recruited from primary care and OB/GYN clinics at the Grady Memorial Hospital in Atlanta, GA, which serves a majority Black, low-income, trauma-exposed community. From this broad sample, women with T2DM and a history of trauma exposure were invited to participate in the current sub-study assessing metabolic and neuroendocrine dysfunction. All procedures were in accordance with the ethical standards of Emory Institutional Review Board and the Research Oversight Committee of Grady Memorial Hospital, Atlanta, GA, as well as the United States Federal Policy for the Protection of Human Subjects. Written informed consent was obtained from all participants following an explanation of the study's procedures.

2.3. Cardiovascular autonomic function

Resting high-frequency heart rate variability (HF-HRV) was measured through electrocardiogram (ECG) sensors using the Biopac MP150 for Windows (Biopac Systems, Inc, Goleta, CA) with the BioNomadix wireless ECG amplifier. Three Ag/AgCl electrodes filled with electrolyte gel were attached to the torso in the lead II position. ECG were sampled at 1000 Hz and analyzed using MindWare HRV module (MindWare Technologies, Ltd, Gahanna, OH). The ECG signal was amplified by a gain of 2000, filtered with a Hamming windowing function, and with a 60-Hz notch filter. HRV was quantified during 1-min intervals by spectral analysis of the time-sampled inter-beat interval series, according to the methods recommended by the Society for Psychophysiological Research Committee on HRV (Berntson et al., 1997). HF-HRV was sampled from 0.12 to 0.40 Hz and was transformed by natural log. HF-HRV was measured from the 1-min intervals during two baseline pre-TSST time points, once 15 min pre-TSST and once immediately pre-TSST. Resting HF-HRV for the present study was calculated as the mean of these two baseline values.

2.4. Blood glucose control

Blood glucose control was assessed using the glycated hemoglobin (HbA1c) test, which indicates an individual's average blood glucose levels over the last 90 days (Eyth and Naik, 2020). Specifically, it measures what percentage of hemoglobin is glycated, or covered with glucose. Should this percentage exceed 6.5, a diagnosis of T2DM may be made (Smushkin and Vella, 2010). This glycated hemoglobin test is a widely used measure of blood glucose control in individuals with diabetes (Saudek and Brick, 2009), and is highly predictive of diabetes-related complications and mortality (Sherwani et al., 2016).

2.5. Stressor-evoked inflammation response

Stressor-evoked inflammation was assessed as the IL-6 response to a psychosocial laboratory stressor, the Trier Social Stress Test (TSST). This test is known to induce changes in autonomic, endocrinological, and immunological activity (Marsland et al., 2017; Rodríguez-Medina et al., 2019). The test consists of a 10-min anticipation and preparatory phase and a subsequent 10-min public speaking and mental arithmetic task in front of three trained staff members. TSSTs were conducted at the same time of day across the entire study (i.e., procedures always began at 1 PM following a 2-h rest period after arrival at the clinical research center. Plasma samples were obtained from blood drawn from an IV catheter at multiple points across the TSST: once 15 min pre-TSST, again 15 min after the start of the TSST, and then at 15-min intervals until 90 min post-TSST. Following this, blood was drawn hourly while subjects rested in bed for an additional 3 h (for a total of 5 h after the onset of the stressor). On average, initial pre-TSST blood draws occurred at approximately 12:50pm EST, and final post-TSST blood draws occurred at approximately 5:00pm EST. Plasma was obtained by centrifugation immediately after a blood sample was drawn and then frozen at −80 °C until batch analyses to determine concentrations of IL-6 using commercially available ELISA kits from R&D Systems (Minneapolis, MN) according to manufacturer instructions. Not all plasma samples described above were analyzed for concentrations of IL-6. Instead, based on our prior work as well as work by others (Marsland et al., 2017; Pace et al., 2009, 2010; Quinn et al., 2018), we only analyzed IL-6 concentrations in the baseline sample (i.e, 15 min pre-TSST), as well as samples collected 30, 90, 150, 210, and 270 min after the start of the TSST. This was done to examine the relevant aspect of the TSST IL-6 response "curve" that increases from baseline by 60 min, and then continues to increase thereafter. Intra- and inter-assay coefficients of variation were 9% and 9.2%, respectively. The lower limit of detection of the assay was 0.067 pg/ml. To quantify inflammation levels across the TSST, area under the curve measurements with respect to ground (AUCg) and increase (AUCi) were calculated for IL-6 (Pruessner et al., 2003).

2.6. Data analysis

Outliers were defined as individuals with scores ≥3 standard deviations above the mean for each variable. One outlier was identified for each of our four primary variables – HF-HRV, HbA1c, IL-6 AUCg, and IL-6 AUCi; these 4 cases were removed from analyses. Upon removal of these cases, in addition to the removal of 13 cases with missing data, our final sample was $N = 37$. Missing data occurred due to problems with blood sampling (e.g., catheter dysfunction) and scheduling difficulties. Two linear stepwise regressions were conducted: one with IL-6 AUCg as the dependent variable and one with IL-6 AUCi as the dependent variable. For both models, predictor variables included HbA1c (Step 1), HF-HRV (Step 2), and their interaction (Step 3). The HbA1c by HF-HRV interaction term was calculated by mean-centering both variables and then multiplying these mean-centered values. Age and body mass index (BMI) were included as covariates in Step 4 of both models. All calculations were computed using SPSS version 24 with a significance level of $p < .05$.

3. Results

The current sample is characterized by an average HbA1c level of 7.75 (SD = 1.77), IL-6 AUCi level of 420.45 (SD = 431.04), IL-6 AUCg level of 1174.34 (SD = 691.87), and BMI of 35.22 (SD = 6.80). Significant correlations were observed between IL-6 AUCg and HF-HRV ($r = 0.35$, $p = .034$), IL-6 AUCi and HF-HRV ($r = 0.37$, $p = .023$), and IL-6 AUCg and IL-6 AUCi ($r = 0.69$, $p < .001$).

In terms of the regression predicting IL-6 AUCi, the overall model was not significant, $F(5,36) = 1.59$, $p = .192$. HF-HRV was significant when entered in Step 2 ($\beta = 0.36$, $t = 2.20$, $p = .035$), but it was not significant in subsequent steps after the addition of other predictors. The regression model predicting IL-6 AUCg was significant and accounted for 37% of the variance in IL-6 AUCg, $F(5,36) = 3.64$, $p = .010$. HF-HRV was significant when entered in Step 2 ($\beta = 0.38$ $t = 2.33$, $p = .026$), and the HbA1c by HF-HRV interaction was also significant, ($\beta = -.37$, $t = -2.27$, $p = .030$). As indicated by the results from both regression models, HbA1c did not significantly predict IL-6 levels during the TSST, which was in contrast to our first hypothesis. However, the significant HbA1c by HF-HRV interaction suggested that this null finding could be due to a moderation effect (i.e., perhaps HbA1C only predicts IL-6 at certain levels of HF-HRV).

Given that the HbA1c by HF-HRV interaction was only significant in the model predicting IL-6 AUCg, simple slopes analyses were conducted only for this model. In order to test our hypothesis that HF-HRV was a moderator of the association between HbA1c and IL-6, simple slopes

analyses were conducted at high and low HF-HRV levels (calculated by subtracting and adding one standard deviation, respectively). Unexpectedly, HbA1c levels were not significantly associated with IL-6 AUCg at either high or low levels of HF-HRV. When an interaction is significant but neither simple slopes model is significant, this suggests that the incorrect moderator was tested. In other words, our finding indicated that HbA1c, rather than HF-HRV, may be the moderator. We, therefore, conducted post-hoc simple slopes analyses and tested the effects of HF-HRV on IL-6 AUCg at high and low levels of HbA1c. The model at high levels of HbA1c was not significant, but the model at low levels of HbA1c was significant and suggested that in this condition, higher levels of HF-HRV predicted higher IL-6 AUCg, ($\beta = 0.60$, $t = 3.51$, $p = .001$). This indicates that HbA1c moderated the association between HF-HRV and IL-6 AUCg, such that greater autonomic function was associated with increased inflammatory responses to acute stress induced by the TSST, and this association was strongest when HbA1c levels were low. See Table 2 for a summary of regression results.

4. Discussion

T2DM is an increasingly prevalent metabolic disorder associated with elevated blood glucose levels, inflammation, and autonomic dysfunction. Despite this, the ways in which these variables interact remain largely unexplored. The current study explored the interaction of baseline HbA1c levels, stressor-evoked IL-6 reactivity to the TSST, and baseline HF-HRV among trauma-exposed Black women with T2DM. Our results suggest that HbA1c moderates the relationship between baseline HF-HRV and stressor-evoked IL-6 such that higher HF-HRV is associated with higher stress-evoked IL-6 levels only in the presence of low HbA1c.

Our initial hypothesis that blood glucose control (HbA1c) would predict stressor-evoked IL-6 reactivity to the TSST was unsupported by this analysis. While there is evidence to suggest that HbA1c predicts baseline inflammation in nondiabetic adults (Gustavsson and Agardh, 2009; Liu et al., 2015), this has not been confirmed among individuals with T2DM, or for situations in which inflammation levels were measured dynamically in response to a stressor. Although blood glucose control did not predict IL-6 levels in this sample, we found that IL-6, which served in this analysis as a biomarker of inflammation, was significantly predicted by the interaction between blood glucose control and cardiovascular autonomic functioning. Of note, we found significant associations only when IL-6 was calculated as the area under the curve with respect to ground (AUCg); when IL-6 was calculated as the area under the curve with respect to increase (AUCi), the interaction was not significant. This can in large part be explained by the distinct quantitative differences between AUCg and AUCi. While IL-6 AUCi represents only the time-dependent (i.e., phasic) change in inflammation across the TSST, IL-6 AUCg represents the magnitude of the IL-6 response to the TSST in combination with baseline concentrations of IL-6 present before the TSST. In other words, the present study found that HF-HRV only associated with TSST-induced IL-6 reactivity when preexisting baseline IL-6 concentrations were included in area under the curve (i.e., AUCg) calculations. When IL-6 reactivity to the TSST was not inclusive of pre-existing baseline IL-6 (i.e., AUCi), HF-HRV did not associate with IL-6. These two calculations paint two meaningfully distinct pictures of the same dataset, such that their relation to each other and to variables of interest will differ from sample to sample (Pruessner et al., 2003). Further research exploring IL-6 reactivity calculated as AUCg versus AUCi is required to understand the clinical implications of these differences.

Our moderation analyses indicated that HbA1c, rather than HF-HRV, was the moderating variable, such that higher HF-HRV conferred heightened stressor-evoked IL-6 reactivity only in the presence of lower HbA1c. This was unexpected for two reasons: 1) cardiovascular autonomic function is frequently utilized as a moderator variable due to consistent findings that lower HF-HRV is associated with worse outcomes across a number of health conditions (Kataoka et al., 2004; Masi et al., 2007; Tsuji et al., 1996), and 2) blood glucose control has rarely been identified as a moderator in the relevant literature. While we would typically expect high HF-HRV to be associated with lower levels of inflammation, it is plausible that we observed an 'optimal' level of inflammatory response to stress, as inflammation is the body's natural response to protect itself from harm and may only be damaging at extreme levels or when activated chronically. In other words, it is possible that 'healthier' individuals (i.e., those with lower HbA1c) can mount a healthy stress response that includes the transient release of IL-6 more flexibly than those with higher HbA1c. We believe that this finding may be due to the unique intersection of trauma-exposure, race, and diabetes status (lower v. higher HbA1c) observed in our sample. For example, a paper by Steptoe et al. (2014) found that while individuals with T2DM have higher baseline IL-6 levels than healthy controls, they showed blunted IL-6 responsivity post-stress. Although this finding does not specifically address our HF-HRV finding, it may help explain why we observed a greater IL-6 response among 'healthier' individuals with T2DM. There is a paucity of literature at the intersection of T2DM, race, and trauma, and we believe that our findings can serve as a starting point for future research. Additionally, while plasma IL-6 levels are frequently utilized as a biomarker of inflammation reactivity, several studies have shown IL-6 to act not only as a pro-inflammatory cytokine, but also as an anti-inflammatory myokine (Pedersen and Febbraio, 2008; Petersen and Pedersen, 2005; Wedell-Neergaard et al., 2019). Thus, our finding that high HF-HRV was associated with higher levels of IL-6 in the presence of lower HbA1c (indicating a 'healthier' state) may be in part be explained by IL-6's anti-inflammatory properties. Of note, the clinical studies that have identified IL-6 as a myokine have been conducted exclusively in the context of IL-6 release from skeletal muscle tissue in response to exercise. Given the specificity of these findings, it is unclear whether they can be generalized to help explain the findings of the present study.

When evaluating the implications of this analysis, it is important to

Table 2
Stepwise regression for HF-HRV and HbA1c Moderation.

	R^2	F	β	t	SE	p
Step 1	.003	0.10				
HbA1c			.053	.31	65.92	.756
Step 2	.140	2.77				
HbA1c			.139	.85	63.75	.401
Baseline HF-HRV			.381	2.33	80.33	.026*
Step 3	.235	3.37*				
HbA1c			.010	.06	65.92	.952
Baseline HF-HRV			.289	1.77	80.13	.086
HbA1c X Baseline HF-HRV			−.338	−2.02	37.31	.052
Step 4	.370	3.64*				
HbA1c			.003	.02	62.44	.983
Baseline HF-HRV			.302	1.92	77.42	.065
HbA1c X Baseline HF-HRV			−.365	−2.27	35.80	.030*
BMI			.352	2.44	14.72	.021*
Age			−.092	−.60	11.73	.555
Dependent variable: IL-6 AUCg						
HbA1c (simple slopes)						
At low HF-HRV			.297	1.76		.089
At high HF-HRV			−.290	−1.23		.230
Dependent variable: IL-6 AUCg						
HF-HRV (post-hoc simple slopes)						
At low HbA1c			.595	3.51		.001*
At high HbA1c			.008	.036		.971
Dependent variable: IL-6 AUCg						

Note. *$p < .05$; HF-HRV = high frequency heart rate variability; IL-6 = interleukin-6.
AUCg = area under the curve with respect to ground.

consider the limitations presented by our study sample and design. Perhaps our greatest limitation is that of a small sample size and low power; however, we find this limitation to be eclipsed by the well-characterized nature of our sample. A review of related literature reveals that studies with likewise small and well-characterized samples are similarly under powered (e.g., Parish et al., 2016). Additionally, given that this analysis was conducted among trauma-exposed Black women with T2DM, our findings may not be generalizable beyond this clinical sample. That said, this clinical sample is uniquely vulnerable to the health consequences of T2DM and is therefore an exceptionally relevant focus of study. While this may have limited the generalizability of our results, it also presented us with the unique opportunity to explore biomarkers of cardiovascular and metabolic disorders among individuals for whom T2DM presents a disproportionately elevated mortality risk. While men are more likely to be diagnosed with T2DM, women typically have more severe complications and a greater risk of both all-cause and cardiovascular mortality (Arnetz et al., 2014). That said, future studies should aim to explore the interrelatedness of metabolic, inflammatory, and cardiovascular measures among both men and women with T2DM. In addition, future studies would also benefit from inclusion of additional metabolic biomarkers such as leptin and assessment of blood glucose prior to, during, and after the TSST or other experimental psychosocial stress task. The interpretability of our results and characterization of our sample was limited by the absence of these variables in our analysis.

It is clear from the literature and the present study's findings that blood glucose control, cardiovascular autonomic function, and inflammation are intricately linked, and a deeper understanding of the nature of these associations (with regard to strength, directionality, etc.) across differing populations can inform how we understand and treat T2DM and its related health consequences. For instance, our identification of blood glucose control as a moderator in the relationship between cardiovascular autonomic function and inflammation highlights the possibility that individuals with trauma exposure and T2DM may benefit from stratification by HbA1c levels for research analysis and treatment decision making (see Rusu et al., 2012 for an example of this). While our highly characterized sample of trauma-exposed Black women with T2DM allowed us to investigate these variables in a uniquely vulnerable population, future studies should seek to investigate this phenomenon across multiple phenotypes.

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Declaration of competing interest

KJR has received consulting income from Alkermes, research support from NIH, Genomind and Brainsway, and he is on scientific advisory boards for Janssen and Verily, all of which is unrelated to the present work. CFG has received consulting income from Cohen Veterans Bioscience which is unrelated to the present work.

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References

Arnetz, L., Ekberg, N.R., Alvarsson, M., 2014. Sex differences in type 2 diabetes: focus on disease course and outcomes. Diabetes Metab. Syndr. Obes. 7, 409–420.
Benichou, T., Pereira, B., Mermillod, M., Tauveron, I., Pfabigan, D., Maqdasy, S., Dutheil, F., 2018. Heart rate variability in type 2 diabetes mellitus: a systematic review and meta-analysis. PLoS One 13, e0195166.
Berntson, G.G., Bigger Jr., J.T., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., Nagaraja, H.N., Porges, S.W., Saul, J.P., Stone, P.H., van der Molen, M.W., 1997. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 34, 623–648.
Brudey, C., Park, J., Wiaderkiewicz, J., Kobayashi, I., Mellman, T.A., Marvar, P.J., 2015. Autonomic and inflammatory consequences of posttraumatic stress disorder and the link to cardiovascular disease. Am. J. Physiol. Regul. Integr. Comp. Physiol. 309, R315–R321.
Bullard, K.M., Cowie, C.C., Lessem, S.E., Saydah, S.H., Menke, A., Geiss, L.S., Orchard, T.J., Rolka, D.B., Imperatore, G., 2018. Prevalence of diagnosed diabetes in adults by diabetes type - United States, 2016. MMWR Morb. Mortal. Wkly. Rep. 67, 359–361.
Calle, M.C., Fernandez, M.L., 2012. Inflammation and type 2 diabetes. Diabetes Metab. 38, 183–191.
Carpenter, L.L., Gawuga, C.E., Tyrka, A.R., Lee, J.K., Anderson, G.M., Price, L.H., 2010. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. Neuropsychopharmacology 35, 2617–2623.
Einarson, T.R., Acs, A., Ludwig, C., Panton, U.H., 2018. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovasc. Diabetol. 17, 83.
Eyth, E., Naik, R., 2020. Hemoglobin A1C. In: StatPearls. StatPearls Publishing, Treasure Island (FL).
Geiss, L.S., Wang, J., Cheng, Y.J., Thompson, T.J., Barker, L., Li, Y., Albright, A.L., Gregg, E.W., 2014. Prevalence and incidence trends for diagnosed diabetes among adults aged 20 to 79 years, United States, 1980-2012. JAMA 312, 1218–1226.
Gillespie, C.F., Bradley, B., Mercer, K., Smith, A.K., Conneely, K., Gapen, M., Weiss, T., Schwartz, A.C., Cubells, J.F., Ressler, K.J., 2009. Trauma exposure and stress-related disorders in inner city primary care patients. Gen. Hosp. Psychiatr. 31, 505–514.
Gluck, R.L., Hartzell, G.E., Dixon, H.D., Michopoulos, V., Powers, A., Stevens, J.S., Fani, N., Carter, S., Schwartz, A.C., Jovanovic, T., Ressler, K.J., Bradley, B., Gillespie, C.F., 2021. Trauma exposure and stress-related disorders in a large, urban, predominantly African-American, female sample. Arch. Womens. Ment. Health. https://doi.org/10.1007/s00737-021-01141-4.
Golden, S.H., Brown, A., Cauley, J.A., Chin, M.H., Gary-Webb, T.L., Kim, C., Sosa, J.A., Sumner, A.E., Anton, B., 2012. Health disparities in endocrine disorders: biological, clinical, and nonclinical factors–an Endocrine Society scientific statement. J. Clin. Endocrinol. Metab. 97, E1579–E1639.
Gustavsson, C.G., Agardh, C.-D., 2009. Inflammatory activity increases with haemoglobin A1c in patients with acute coronary syndrome. Scand. Cardiovasc. J. 43, 380–385.
Herman, W.H., 2016. Are there clinical implications of racial differences in HbA1c? Yes, to not consider can do great harm. Diabetes Care.
Huffhines, L., Noser, A., Patton, S.R., 2016. The link between adverse childhood experiences and diabetes. Curr. Diabetes Rep. 16, 54.
Kataoka, M., Ito, C., Sasaki, H., Yamane, K., Kohno, N., 2004. Low heart rate variability is a risk factor for sudden cardiac death in type 2 diabetes. Diabetes Res. Clin. Pract. 64, 51–58.
Liu, S., Hempe, J.M., McCarter, R.J., Li, S., Fonseca, V.A., 2015. Association between inflammation and biological variation in hemoglobin A1c in U.S. Nondiabetic adults. J. Clin. Endocrinol. Metab. 100, 2364–2371.
Marsland, A.L., Walsh, C., Lockwood, K., John-Henderson, N.A., 2017. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav. Immun. 64, 208–219.
Masi, C.M., Hawkley, L.C., Rickett, E.M., Cacioppo, J.T., 2007. Respiratory sinus arrhythmia and diseases of aging: obesity, diabetes mellitus, and hypertension. Biol. Psychol. 74, 212–223.
McCraty, R., Shaffer, F., 2015. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. Glob. Adv. Health Med. 4, 46–61.
Pace, T.W.W., Negi, L.T., Adame, D.D., Cole, S.P., Sivilli, T.I., Brown, T.D., Issa, M.J., Raison, C.L., 2009. Effect of compassion meditation on neuroendocrine, innate immune and behavioral responses to psychosocial stress. Psychoneuroendocrinology 34, 87–98.
Pace, T.W.W., Negi, L.T., Sivilli, T.I., Issa, M.J., Cole, S.P., Adame, D.D., Raison, C.L., 2010. Innate immune, neuroendocrine and behavioral responses to psychosocial stress do not predict subsequent compassion meditation practice time. Psychoneuroendocrinology 35, 310–315.
Parish, R.C., Todman, S., Jain, S.K., 2016. Resting heart rate variability, inflammation, and insulin resistance in overweight and obese adolescents. Metab. Syndr. Relat. Disord. 14, 291–297.
Pedersen, B.K., Febbraio, M.A., 2008. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol. Rev. 88, 1379–1406.
Petersen, A.M.W., Pedersen, B.K., 2005. The anti-inflammatory effect of exercise. J. Appl. Physiol. 98, 1154–1162.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone

concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Quinn, A.M., Williams, A.R., Sivilli, T.I., Raison, C.L., Pace, T.W.W., 2018. The plasma interleukin-6 response to acute psychosocial stress in humans is detected by a magnetic multiplex assay: comparison to high-sensitivity ELISA. Stress 21, 376–381.
Roberts, A.L., Agnew-Blais, J.C., Spiegelman, D., Kubzansky, L.D., Mason, S.M., Galea, S., Hu, F.B., Rich-Edwards, J.W., Koenen, K.C., 2015. Posttraumatic stress disorder and incidence of type 2 diabetes mellitus in a sample of women: a 22-year longitudinal study. JAMA Psychiatry 72, 203–210.
Rodríguez-Medina, D.A., Leija-Alva, G., Domínguez-Trejo, B., Hernández-Pozo, M.D.R., Cruz Albarrán, I.A., Morales-Hernández, L.A., Marmolejo-Ramos, F., 2019. Effects of the trier social stress test on the distributions of IL-6 and MAP levels. Heliyon 5, e01580.
Ruparelia, N., Chai, J.T., Fisher, E.A., Choudhury, R.P., 2017. Inflammatory processes in cardiovascular disease: a route to targeted therapies. Nat. Rev. Cardiol. 14, 133–144.
Rusu, I., Wong, S.H., Barbazetto, I., 2012. The effect of hemoglobin A1c on bevacizumab response in patients with diabetic macular edema. Invest. Ophthalmol. Vis. Sci. 53, 415.
Saudek, C.D., Brick, J.C., 2009. The clinical use of hemoglobin A1c. J. Diabetes Sci. Technol. 3, 629–634.
Serhiyenko, V.A., Serhiyenko, A.A., 2018. Cardiac autonomic neuropathy: risk factors, diagnosis and treatment. World J. Diabetes 9, 1–24.
Sherwani, S.I., Khan, H.A., Ekhzaimy, A., Masood, A., Sakharkar, M.K., 2016. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomark. Insights 11, 95–104.
Smushkin, G., Vella, A., 2010. What is type 2 diabetes? Medicine 38, 597–601.
Steptoe, A., Hackett, R.A., Lazzarino, A.I., Bostock, S., La Marca, R., Carvalho, L.A., Hamer, M., 2014. Disruption of multisystem responses to stress in type 2 diabetes: investigating the dynamics of allostatic load. Proc. Natl. Acad. Sci. U. S. A. 111, 15693–15698.
Tell, D., Mathews, H.L., Burr, R.L., Witek Janusek, L., 2018. During stress, heart rate variability moderates the impact of childhood adversity in women with breast cancer. Stress 21, 179–187.
Tsalamandris, S., Antonopoulos, A.S., Oikonomou, E., Papamikroulis, G.-A., Vogiatzi, G., Papaioannou, S., Deftereos, S., Tousoulis, D., 2019. The role of inflammation in diabetes: current concepts and future perspectives. Eur. Cardiol. 14, 50–59.
Tsuji, H., Larson, M.G., Venditti Jr., F.J., Manders, E.S., Evans, J.C., Feldman, C.L., Levy, D., 1996. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 94, 2850–2855.
Vaccarino, V., Goldberg, J., Magruder, K.M., Forsberg, C.W., Friedman, M.J., Litz, B.T., Heagerty, P.J., Huang, G.D., Gleason, T.C., Smith, N.L., 2014. Posttraumatic stress disorder and incidence of type-2 diabetes: a prospective twin study. J. Psychiatr. Res. 56, 158–164.
van Beek, L., Lips, M.A., Visser, A., Pijl, H., Ioan-Facsinay, A., Toes, R., Berends, F.J., Willems van Dijk, K., Koning, F., van Harmelen, V., 2014. Increased systemic and adipose tissue inflammation differentiates obese women with T2DM from obese women with normal glucose tolerance. Metabolism 63, 492–501.
Vinik, A.I., Maser, R.E., Mitchell, B.D., Freeman, R., 2003. Diabetic autonomic neuropathy. Diabetes Care 26, 1553–1579.
Vinik, A.I., Ziegler, D., 2007. Diabetic cardiovascular autonomic neuropathy. Circulation 115, 387–397.
Wedell-Neergaard, A.-S., Lang Lehrskov, L., Christensen, R.H., Legaard, G.E., Dorph, E., Larsen, M.K., Launbo, N., Fagerlind, S.R., Seide, S.K., Nymand, S., Ball, M., Vinum, N., Dahl, C.N., Henneberg, M., Ried-Larsen, M., Nybing, J.D., Christensen, R., Rosenmeier, J.B., Karstoft, K., Pedersen, B.K., Ellingsgaard, H., Krogh-Madsen, R., 2019. Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: a randomized controlled trial. Cell Metabol. 29, 844–855 e3.
Williams, D.P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M.N., Sternberg, E.M., Thayer, J.F., 2019. Heart rate variability and inflammation: a meta-analysis of human studies. Brain Behav. Immun. 80, 219–226.
Zannas, A.S., Gordon, J.L., Hinderliter, A.L., Girdler, S.S., Rubinow, D.R., 2020. IL-6 response to psychosocial stress predicts 12-month changes in cardiometabolic biomarkers in perimenopausal women. J. Clin. Endocrinol. Metab. 105. https://doi.org/10.1210/clinem/dgaa476.
Ziegler, D., 2005. Type 2 diabetes as an inflammatory cardiovascular disorder. Curr. Mol. Med. 5, 309–322.