




Autonomic nervous system responses to hypo- and hyperglycemia in type 2 diabetes and prediabetes

Martin H. Lundqvist,^{1,*}  Maria J. Pereira,¹ Urban Wiklund,²  Susanne Hetty,¹ 
and Jan W. Eriksson¹

¹Department of Medical Sciences, Clinical Diabetology and Metabolism, Uppsala University, 751 85 Uppsala, Sweden

²Department of Diagnostics and Intervention, Biomedical Engineering and Radiation Physics, Umeå University, 901 85 Umeå, Sweden

*Corresponding author: Department of Medical Sciences, Clinical Diabetology and Metabolism, Uppsala University, Akademiska Sjukhuset, Ingång 40, 3 tr, 75185 Uppsala, Sweden. Email: martin.lundqvist@medsci.uu.se

Abstract

Objective: Previous research points to a role of the brain in the regulation of glucose and pathogenesis of type 2 diabetes (T2D) via modulation of counter-regulatory hormone secretion and activity in the autonomic nervous system (ANS). The aim of this study was to investigate glucose-dependent responses of catecholamines and ANS activity in individuals with T2D, prediabetes (PD), and normoglycemia (NG).

Design: Cross-sectional.

Methods: Individuals with T2D ($n = 19$, 7 men, HbA1c 49 mmol/mol), PD ($n = 18$, 8 men), and NG ($n = 17$, 3 men) underwent 1 stepwise hyperinsulinemic–euglycemic–hypoglycemic and 1 hyperglycemic clamp with repeated measurements of catecholamines, symptoms, heart rate variability (HRV), and hemodynamics.

Results: The hypoglycemic response of adrenaline was augmented in T2D and PD vs NG (both $P < .05$), and there was a strong association with insulin resistance ($P < .05$ for M -value). In relation to achieved glucose levels in both clamps, noradrenaline exhibited a steeper rise during hypoglycemia in T2D vs NG and PD (both $P < .05$). There were trends toward more marked autonomic hypoglycemic symptoms in T2D vs PD and NG. By contrast, insulin resistance was associated with attenuated responses of heart rate and HRV indices P_{LF} and P_{HF} at the target glucose plateau of 2.7 mmol/L ($P < .05$), independent of BMI and HbA1c.

Conclusion: Alterations in glucose-dependent responses of counter-regulatory hormones and the ANS appear before, and probably contribute to, the onset of T2D. Together with other reported alterations in neuroendocrine pathways, the findings suggest that a maladaptation of the brain's responses to glucose fluctuations is important in T2D progression.

Keywords: type 2 diabetes, catecholamine, heart rate variability, autonomic nervous system, insulin resistance, clamp

Significance

In this study, we have shown that individuals with early-stage type 2 diabetes and prediabetes have an elevated response of the glucose-raising hormone adrenaline to low blood glucose levels. The early appearance of this phenomenon—present already before the onset of type 2 diabetes—has not been demonstrated before and suggests that it may contribute to the development of type 2 diabetes. By contrast, we found that insulin resistance was associated with attenuated autonomic nerve responses to low blood glucose levels.

Introduction

In spite of intensive research, there are still knowledge gaps regarding the pathogenic mechanisms involved in the development of type 2 diabetes (T2D). Mounting evidence supports a role of the brain in the regulation of systemic glucose levels and development of T2D.^{1,2} Glucose-sensitive neurons—particularly prevalent in the hypothalamus and brainstem—may regulate whole body glucose metabolism via modulation of activity in the autonomic nervous system (ANS) as well as hormonal pathways.^{3,4} For example, glucagon, cortisol, growth hormone, catecholamine, and the sympathetic

nervous system all raise hepatic glucose production—and thus endogenous release of glucose into the circulation—and inhibit peripheral glucose uptake, resulting in elevated systemic glucose levels. While this provides a strong and rapid physiological, so-called counter-regulatory defense against hypoglycemia, altered regulation of components of this system may in the long term also be important for the development of T2D.

In support of this concept, glucose clamp studies by our research group have previously demonstrated general elevation of glucagon levels and an augmented response of ACTH and

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Table 1. Clinical characteristics.

Variable	T2D, <i>n</i> = 19	PD, <i>n</i> = 18	NG, <i>n</i> = 17
Age, years	55 (46; 60) ^a	51 (36; 55)	37 (28; 52)
Sex, m:f	7:12	8:10	3:14
Weight, kg	97.5 (87.9; 115.6) ^a	92.8 (82.9; 116.7) ^a	78.4 (62.5; 88.0)
BMI, kg/m ²	36.7 (29.0; 40.2) ^a	31.1 (27.5; 39.1) ^a	24.4 (22.6; 29.5)
Resting heart rate, bpm	72 (65; 80) ^a	67 (58; 73)	62 (58; 70)
Systolic BP, mmHg	142 (138; 153) ^{a,b}	128 (120; 136) ^a	115 (111; 127)
Diastolic BP, mmHg	90 (85; 95) ^a	86 (78; 90)	78 (75; 83)
Fasting plasma glucose, mmol/L	7.4 (6.9; 8.3) ^{a,b}	6.0 (5.8; 6.3) ^a	5.3 (5.2; 5.5)
IFCC HbA1c, mmol/mol	49 (45; 55) ^{a,b}	36 (32; 37)	33 (32; 35)
NGSP HbA1c, %	6.6 (6.3; 7.2) ^{a,b}	5.4 (5.1; 5.5)	5.2 (5.1; 5.4)
Serum C-peptide, nmol/L	1.4 (1.2; 1.9) ^{a,b}	1.0 (0.8; 1.2) ^a	0.6 (0.5; 0.8)
HOMA-IR	6.0 (3.9; 8.4) ^{a,b}	3.2 (2.2; 4.8) ^a	1.3 (1.0; 2.2)
M-value, mg kg FFM ⁻¹ min ⁻¹	3.9 (3.0; 6.4) ^{a,b}	7.6 (4.9; 10.2) ^a	11.6 (9.3; 14.4)
Metformin, <i>n</i> (%)	14 (73.7) ^{a,b}	0 (0)	0 (0)
Antihypertensive treatment, <i>n</i> (%)	9 (47.4) ^{a,b}	1 (5.6)	0 (0)
Lipid-lowering treatment, <i>n</i> (%)	10 (52.6) ^{a,b}	1 (5.6)	0 (0)
Smoking, <i>n</i> (%)	3 (15.8)	1 (5.6)	3 (17.6)
Smoke-less tobacco, <i>n</i> (%)	3 (15.8)	1 (5.6)	1 (5.9)

Data for participants with T2D, PD, and NG presented as median (IQR) or *n* (%). Parts of these data have been published previously.⁷

Significant results are typed in bold.

Abbreviations: FFM, fat free mass; IFCC, International Federation of Clinical Chemistry; NG, normoglycemia; NGSP, National Glycohemoglobin Standardization Program; PD, prediabetes; T2D, type 2 diabetes.

^a*P* < .05 vs NG.

^b*P* < .05 vs PD.

cortisol upon glucose-lowering in individuals with obesity, insulin resistance, prediabetes (PD), and T2D compared with lean, insulin-sensitive, and normoglycemic individuals.⁵⁻⁷

Other researchers have observed excessive secretion of catecholamines, occurring at higher glycemic thresholds, in individuals with advanced T2D.⁸⁻¹²

The aim of this study was to examine whether ANS responses to glucose fluctuations, in similarity with glucagon and hypothalamic–pituitary–adrenal (HPA) axis responses, are unfavorably altered in early stages and pre-stages of T2D. To this purpose, we provide a comprehensive multimodal evaluation of ANS responses—including plasma catecholamine concentrations, hypoglycemic symptoms, heart rate variability (HRV), and hemodynamic measures—assessed in hypoglycemic as well as hyperglycemic clamps in the previously reported cohort of individuals with T2D, PD, and normoglycemia (NG).⁷

Methods

Participants

This cross-sectional study took place at the Uppsala University Hospital and the Department of Medical Sciences at Uppsala University between March 2018 and August 2022. The study population included 54 individuals: 19 with T2D, 18 with PD, and 17 with NG. Clinical characteristics are presented in [Table 1](#). Recruitment methods, eligibility criteria, and comprehensive baseline characteristics have been published previously.^{5,7} Participants with T2D had a median diabetes duration of 17 months (range 0-56) and a low prevalence of non-severe microvascular complications (no retinopathy or neuropathy, 4 microalbuminuria, and all estimated glomerular filtration rate > 60 mL/min/1.73 m²) and were on metformin only (*n* = 14) or no glucose-lowering drug (*n* = 5). In T2D vs PD and NG, the use of anti-hypertensive and lipid-lowering medication was more common. Smoking was rare in all groups but individuals with T2D tended to use more smokeless tobacco. The participants did not have cardiovascular diseases, other

significant comorbidities, or alcohol or drug abuse and did not use systemic corticosteroids, beta-blockers, or antipsychotic drugs. Prediabetes was defined in accordance with American Diabetes Association criteria,¹³ with fasting glucose ≥ 5.6 mmol/L (mean of 2 visits) or HbA1c ≥ 39 mmol/mol.

Experimental procedures

Each participant underwent 1 hyperinsulinemic–euglycemic–hypoglycemic clamp (henceforth denoted hypoglycemic clamp) and 1 hyperglycemic clamp experiment at ~9:00 AM after an overnight fast. The order of the clamps was randomized 1:1 with 1-5 weeks in between. The clamp experiments and other study procedures have been described in full detail previously.^{5,7} In the hypoglycemic clamp, target glucose levels were, sequentially, 5.0 mmol/L for 80 min, 3.8 mmol/L for 30 min, 3.2 mmol/L for 45 min, and 2.7 mmol/L for 30 min, followed by recovery to the normal range ([Figure 1A](#)). In the hyperglycemic clamp, thresholds were fasting levels for 30 min, +3, +6, and +9 mmol/L above fasting levels for 45 min each until recovery to the normal range ([Figure 1B](#)). Glucose levels were measured every 5 min during both clamps with a Contour Glucose Meter (Bayer Healthcare, Leverkusen, Germany). Repeatedly during both clamps (time points indicated in [Figure 2](#)), blood samples, blood pressure, and heart rate were obtained. Blood samples were drawn from a dorsal hand vein that had been arterialized with the use of a heating pad. Electrocardiogram was recorded for 5 min before clamp initiation and continuously during the clamps to assess HRV. In the hypoglycemic clamp, subjects were asked to assess hypoglycemic symptoms, using the Edinburgh Hypoglycemia Symptom Scale.¹⁴

Biochemistry

Plasma employed in the current analysis had been collected in sodium-heparin tubes, frozen at –80 °C and stored at the Uppsala Biobank (number 827). Using 3-CAT ELISA Fast Track (LDN® Immunoassays and Services, Nordhorn,

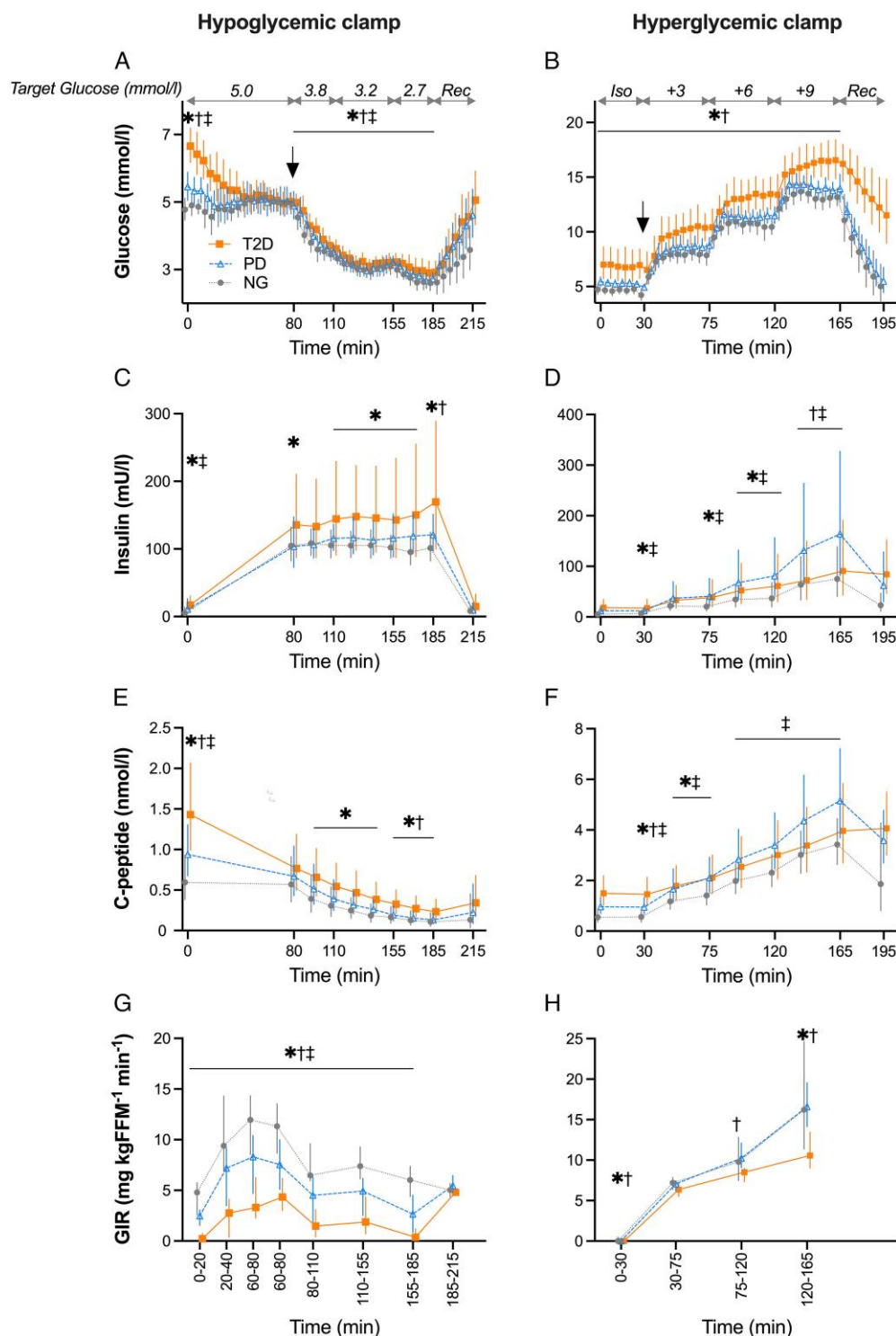


Figure 1. Glucose (A, B), insulin (C, D), and C-peptide (E, F) levels and glucose infusion rate (GIR, G, H) in hypo- (left panels) and hyperglycemic (right panels) clamps for participants with type 2 diabetes (T2D, filled squares and solid lines, $n = 18$ left panels, $n = 19$ right), prediabetes (PD, open triangles and dashed lines, $n = 18$), and normoglycemia (NG, filled circles and dashed lines, $n = 17$ left panels, $n = 16$ right). Data presented as geometric mean and SD (A-F) or median and IQR (G, H). Rec, recovery phase; Iso, isoglycemic phase; FFM, fat free mass. * $P < .05$, T2D vs NG; † $P < .05$, T2D vs PD; ‡ $P < .05$, PD vs NG; all post hoc tests. Significance at 0 min refers to mean from both clamps. Downward arrows indicate start of active phases of each clamp.

Germany) in accordance with the manufacturer's instructions, adrenaline (intra-assay coefficient of variation (CV) 11.0%-24.7% and inter-assay CV 11.1%-14.5%), noradrenaline (intra-assay CV 11.1%-14.3% and inter-assay CV 9.2%-10.9%), and dopamine (intra-assay CV 24.4%-29.8% and inter-assay CV 14.2%-28.2%) were measured. Values below the limit of detection (LOD) (10 pg/mL for adrenaline,

36 pg/mL for noradrenaline, and 49 pg/mL for dopamine) were imputed to LOD/2.

Heart rate variability

Electrocardiogram recordings were manually inspected to exclude extrasystoles and other arrhythmias from the analysis.

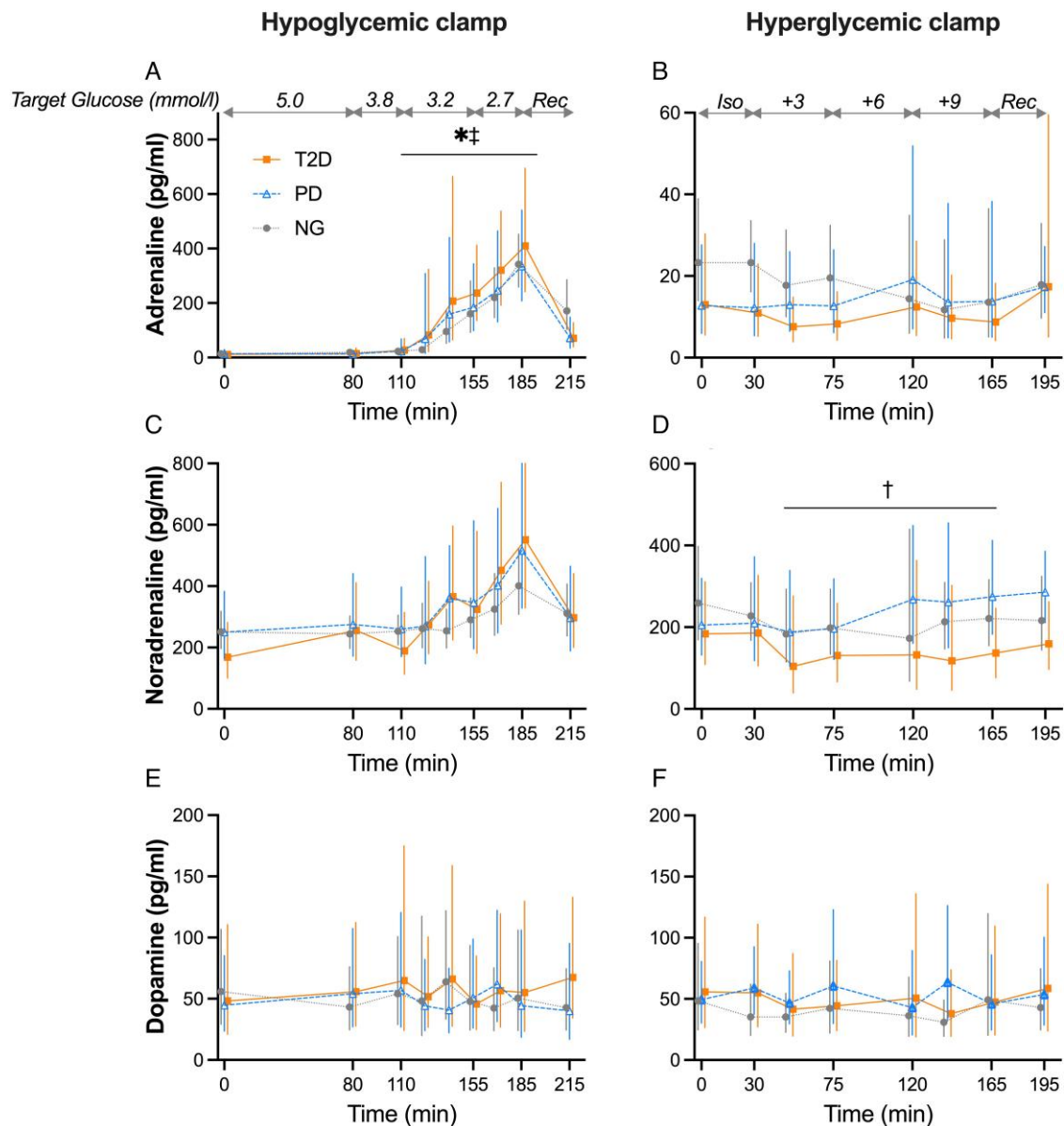


Figure 2. Levels of adrenaline (A, B), noradrenaline (C, D), and dopamine (E, F) in the hypoglycemic clamp (left panels) and hyperglycemic clamp (right panels) for participants with type 2 diabetes (T2D, filled squares and solid lines, $n = 10$), prediabetes (PD, open triangles and dashed lines, $n = 11$), and normoglycemia (NG, filled circles and dashed lines, $n = 9$). Data presented as geometric mean and SD. Rec, recovery phase; Iso, isoglycemic phase. * $P < .05$, T2D vs NG; † $P < .05$, T2D vs PD; ‡ $P < .05$, PD vs NG; all post hoc tests, adjusted for baseline.

Data were then processed automatically using Matlab Software version R2019a (MathWorks, Natick, MA, United States). Heart rate variability was analyzed by power spectrum analysis using Welch's periodogram method as described previously.⁵ The high frequency HRV component (P_{HF} 0.15-0.50 Hz) mainly reflects the parasympathetic part of cardiac autonomic modulation, while the low frequency component (P_{LF} 0.04-0.15 Hz) reflects a combination of sympathetic and parasympathetic activity. The ratio P_{LF}/P_{HF} represents the balance between sympathetic and parasympathetic activity.¹⁵

Statistical analysis

P -values $< .05$ were considered statistically significant. For group comparison of measurements at fasting (mean of both visits) and at the start of the active clamp phases, Kruskal-Wallis H tests

followed by post hoc Dunn's test were performed in SPSS for Mac (v28.0, Armonk, NY, United States: IBM corporation). Repeated measurements of catecholamines, symptoms, HRV indices, and hemodynamics during the hypoglycemic (95-185 min) and hyperglycemic (50-165 min) phases of the clamp were analyzed by linear mixed models with random intercept using the lme4 package (v.1.1.34)¹⁶ in R Statistical Software (v.4.2.2 R Core Team; 2022). Before the analysis, HRV indices during the clamps were averaged over 15-25 min in congruence with other clamp assessments. Fixed effects were time, group, the interaction group * time, and measurements at the start of the active clamp phases (indicated by downward arrows in Figure 1A and B), with appropriate transformations if needed. Fixed effects were first evaluated with type III test for fixed effects, which prompted post hoc tests of estimated marginal means using the emmeans package (v1.8.9; 2023) at each time point in case of

a significant group*time effect, or averaged across all time points in case of a significant group effect. Results were corrected for multiplicity by the false discovery rate method.

To account for possible confounding, significant group differences were further adjusted for sex, age, BMI, and *M*-value as well as their interactions with time in separate models.

Glucose and insulin levels during clamps could differ between the groups, by design during hyperglycemia and also to a lesser extent during hypoglycemia. In cases where such differences were suspected to enhance observed group differences in specific glucose-dependent responses, auxiliary sensitivity analyses were undertaken with adjustment for glucose and/or insulin levels. Group differences in glucose responsiveness of key outcome variables were investigated by linear mixed models with glucose levels from both clamps, group, and the interaction group*glucose. Associations of *M*-value (glucose infusion rate in mg/kg fat-free mass/min from 40 to 80 min of the hypoglycemic clamp), BMI, and HbA1c with ANS responses were also evaluated in separate models. In case of significant main or interaction effects, estimated marginal means or trends, as appropriate, were analyzed post hoc. More details regarding statistical analysis are provided in [Supplement S1](#). Figures were constructed in GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, CA, United States).

Ethics

All study procedures were performed in accordance with the Declaration of Helsinki. The study was approved by the local Research Ethics Committee of Uppsala (DNR 2017/550) with amendments approved by the Swedish Ethical Review Authority (DNR 2019-04166, 2021-05714-02). Participants were informed verbally and in writing about the study and signed an Informed Consent Form.

Results

One participant with T2D did not complete the hypoglycemic clamp for personal reasons, and 1 subject with NG did not complete the hyperglycemic clamp due to difficulties in achieving venous access. Heart rate variability was not possible to analyze for 7 participants in the hypoglycemic clamp (4 T2D, 2 PD, and 1 NG) and 8 participants in the hyperglycemic clamp (4 T2D, 2 PD, and 2 NG) because of arrhythmias ($n=2$) or technical issues (all others). Catecholamines were analyzed for a subset of 30 participants with T2D ($n=10$, 7 females, median age 49 years, BMI 37.4 kg/m²), PD ($n=11$, 6 females, median age 52 years, BMI 34.4 kg/m²), and NG ($n=9$, 8 females, median age 49 years, BMI 23.1 kg/m²).

Achieved clamp glucose, insulin, and C-peptide levels are displayed in [Figure 1](#) along with glucose infusion rates. In accordance with previous work,^{5,7} glucose levels were generally slightly higher and glucose infusion rates were lower in T2D followed by PD and NG. In T2D vs NG and PD, achieved insulin levels were higher and C-peptide levels were less suppressed during the hypoglycemic clamp, while the increase during the hyperglycemic clamp was attenuated in T2D and NG vs PD. Summary of models with significant group effects is provided in [Supplement S2](#).

Catecholamines

Catecholamine levels did not differ between the groups in fasting conditions or at the start of the active clamp phases.

During the hypoglycemic phase, baseline-adjusted adrenaline levels were higher in T2D and PD vs NG ([Figure 2A](#)). The group difference was attenuated below the significance level after adjustment for BMI and *M*-value ([Supplement S2 and S3](#)). Noradrenaline levels appeared to rise to higher levels in T2D and PD vs NG, but the difference was not significant ($P=.076$ for interaction with time; [Figure 2C](#)). Dopamine levels did not differ between the groups ([Figure 2E](#)). In the hyperglycemic clamp, noradrenaline levels were lower in T2D vs PD ([Figure 2D](#)), whereas adrenaline and dopamine levels did not differ between the groups ([Figure 2B and F](#)).

As glucose levels declined toward hypoglycemia, noradrenaline rose more steeply in T2D vs PD and NG, whereas for adrenaline, this was significant only for T2D vs NG ([Figure 3A and B](#); all $P < .05$ for interaction between glucose and group, model details in [Supplement S4](#)).

Hypoglycemic symptoms

Hypoglycemic symptom scores did not differ between the groups at the start of the clamps ([Table 2](#)). There were trends toward higher autonomic symptom scores in T2D vs PD ($P=.068$) at target glucose 2.7 mmol/L, but otherwise, total and subdomain symptom scores did not differ between the groups.

Heart rate variability and hemodynamic measures

P_{LF} and P_{HF} were lower in T2D vs NG, whereas P_{LF}/P_{HF} and heart rate did not differ between the groups in fasting conditions and at the start of the active clamp phases ([Figure 4 A-H](#)). As displayed in [Figure 3C](#), P_{HF} was generally lower in T2D vs NG, indicative of lower parasympathetic activity, throughout the glycemic range. At the end of the hypoglycemic phase, the relative increase in heart rate was higher in NG vs PD and T2D but adjustment for age, sex, BMI, *M*-value, and glucose levels generally attenuated the group differences below the significance level ([Figure 4G; Supplement S2 and S3](#)). Systolic and diastolic blood pressure was highest in T2D followed by PD, and diastolic blood pressure decreased less in PD vs T2D and NG during the hypoglycemic phase and in T2D vs NG during the hyperglycemic phase ([Supplement S5](#)).

Association between ANS responses and metabolic parameters in the full cohort

In pooled data from all groups, associations between ANS responses and *M*-value, BMI, and HbA1c were investigated in separate linear mixed models ([Supplement S6-S8](#)). Thus, low *M*-value (ie, insulin resistance) was associated with enhanced adrenaline response to hypoglycemia ([Supplement S6](#)). Furthermore, low *M*-value and high BMI were associated with lower decrease of P_{LF} and P_{HF} and lower increase of heart rate at the end of the hypoglycemic phase ([Supplement S6 and S7](#)). There was also an association between high HbA1c and lower increase of heart rate at the end of the hypoglycemic phase ([Supplement S8](#)). Adjustment for glucose and insulin levels attenuated some HRV and heart rate associations ([Supplement S6-S8](#)). *M*-value displayed the strongest independent associations with these responses in multivariate models, also including HbA1c and/or BMI ([Supplement S9](#)). *M*-value was also independently and positively associated with overall levels of P_{HF} during the hypoglycemic clamp, suggesting general suppression of parasympathetic activity in insulin-resistant individuals

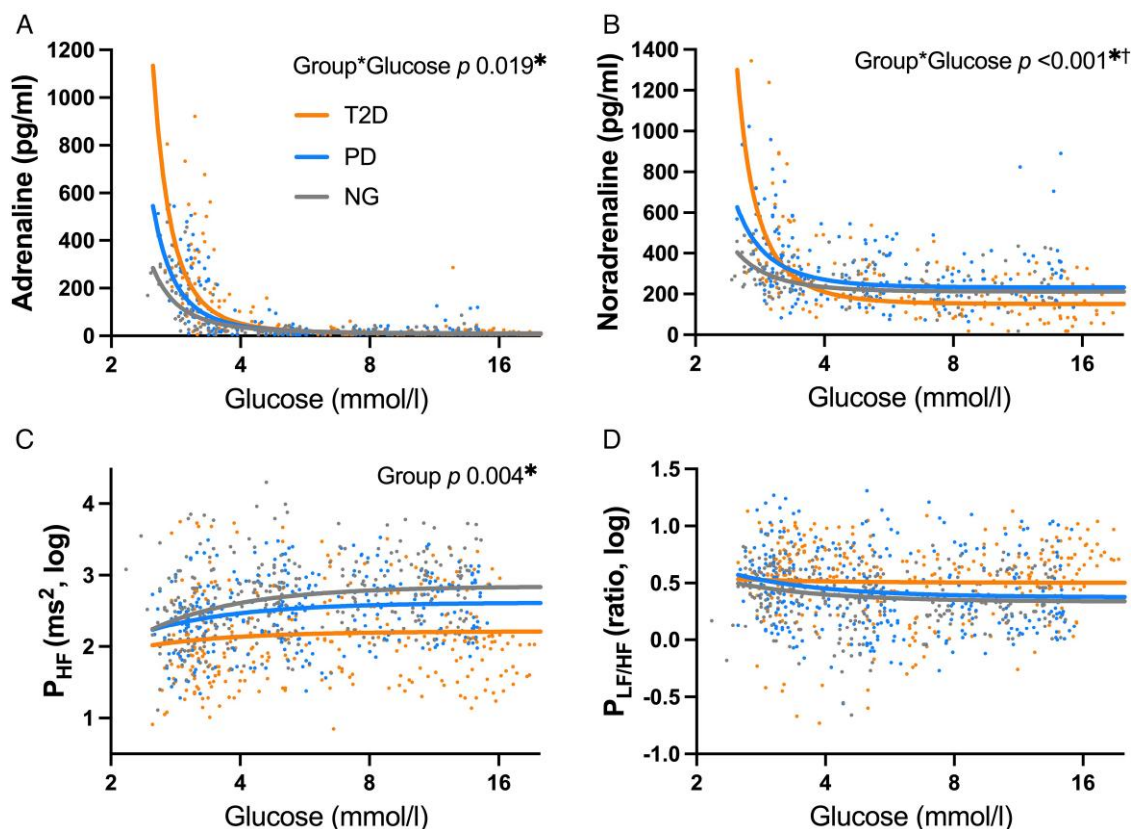


Figure 3. Association between glucose levels and catecholamines or heart rate variability (HRV) measures. Adrenaline (A), noradrenaline (B), power of the high frequency (P_{HF} , C), and the ratio between low frequency and high frequency power (P_{LF}/P_{HF} , D) across hypo- normo- and hyperglycemic clamp conditions for participants with type 2 diabetes (T2D, orange, $n = 10$ A, B; $n = 15$ C, D), prediabetes (PD, blue, $n = 11$ A, B; $n = 16$ C, D), and normoglycemia (NG, gray, $n = 9$ A, B; $n = 16$ C, D). Dots represent real data, and lines represent predicted values from linear mixed models as described in [Supplement S1](#). x-axes are on the log 2 scale. * $P < .05$, T2D vs NG; [†] $P < .05$, T2D vs PD; both post hoc tests.

Table 2. Hypoglycemic symptoms.

Time point		0 min	95 min	125 min	170 min	215 min
Target glucose		NA	3.8 mmol/L	3.2 mmol/L	2.7 mmol/L	NA
Total symptoms	T2D	13 (11; 14)	14 (12; 15)	15 (13; 16)	21 (18; 27)	18 (14; 19)
	PD	12 (11; 15)	14 (13; 15)	17 (15; 18)	21 (18; 22)	16 (15; 18)
	NG	13 (12; 17)	14 (13; 17)	15 (14; 18)	20 (16; 25)	19 (15; 25)
Autonomic symptoms	T2D	5 (4; 6)	5 (4; 7)	6 (5; 7)	11 (9; 14) ^a	9 (6; 10)
	PD	5 (4; 6)	6 (5; 7)	7 (6; 8)	9 (7; 11)	8 (7; 9)
	NG	5 (5; 8)	6 (5; 7)	6 (6; 8)	9 (7; 14)	10 (7; 13)
Neuroglycopenic symptoms	T2D	5 (5; 6)	5 (5; 7)	6 (5; 7)	7.5 (6; 9)	7 (5; 8)
	PD	5 (5; 6)	6 (5; 6)	8 (6; 9)	9 (7; 9)	6 (5; 7)
	NG	7 (5; 10)	6 (5; 7)	7 (5; 7)	8 (7; 10)	7 (5; 10)
Malaise symptoms	T2D	2 (2; 2)	2 (2; 2)	2 (2; 3)	2 (2; 3)	2 (2; 3)
	PD	2 (2; 2)	2 (2; 3)	3 (2; 3)	3 (2; 3)	2 (2; 3)
	NG	2 (2; 2)	2 (2; 2)	2 (2; 3)	2 (2; 3)	2 (2; 2)

Total and subdomain Edinburgh Hypoglycemic Symptom Scale scores in participants with type 2 diabetes (T2D), prediabetes (PD) and normoglycemia (NG) at different stages of the hypoglycemic clamp. Data presented as median (IQR).

Abbreviation: NA, not applicable; NG, normoglycemia; PD, prediabetes; T2D, type 2 diabetes.

^a $P = .068$ for T2D vs PD; $P = .131$ for T2D vs NG.

([Supplement S6 and S9](#)). In the hyperglycemic clamp, BMI was negatively associated with P_{LF} at 120 min, and there was a trend between BMI and total symptoms at the end of the hypoglycemic phase ($P = .066$).

Discussion

In this study, we aimed to characterize autonomic neurohormonal responses to short-term hypo- and hyperglycemia in

individuals with T2D, PD, and NG. We present evidence of an enhanced hypoglycemia-induced secretory response of adrenaline in individuals with early-stage T2D and PD that is associated with insulin resistance. In keeping with these findings, we also found a tendency toward higher burden of perceived autonomic symptoms during profound hypoglycemia in individuals with T2D. Contrarily, autonomic nerve and hemodynamic responses during hypoglycemia appeared to be more reactive in normoglycemic, lean, and

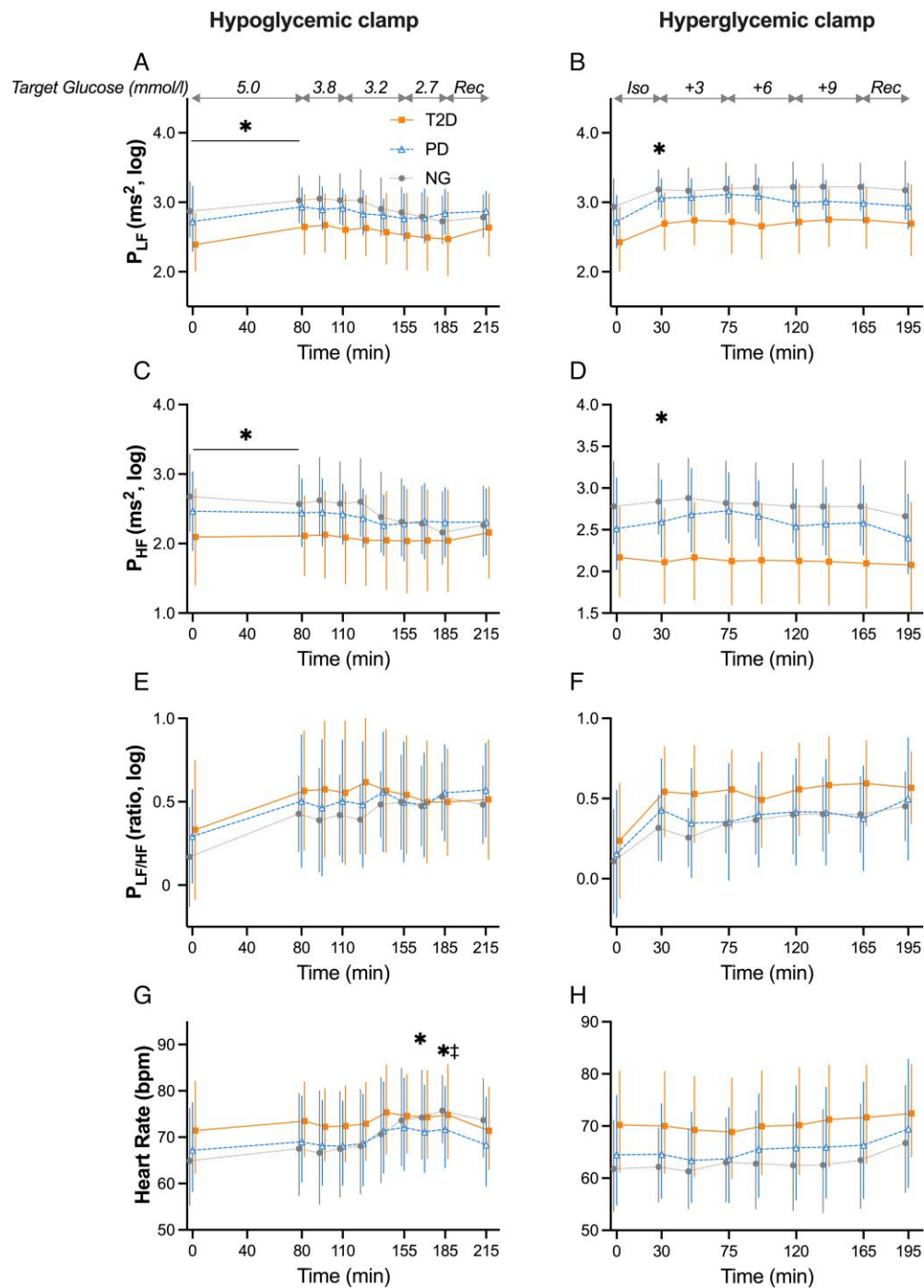


Figure 4. Heart rate variability within the low frequency (P_{LF} , A, B), high frequency (P_{HF} , C, D), the ratio between low frequency and high frequency power (P_{LF}/P_{HF} , E, F), and heart rate (G, H) for participants with type 2 diabetes (T2D, filled squares and solid lines, $n = 15$), prediabetes (PD, open triangles and dashed lines, $n = 16$), and normoglycemia (NG, filled circles and dashed lines; $n = 16$ left panels, $n = 14$ right panels). Data presented as mean and SD (E, F) or geometric mean and SD (all others). Rec, recovery phase; Iso, isoglycemic phase. * $P < .05$, T2D vs NG; † $P < .05$, PD vs NG; ‡ both post hoc tests, adjusted for baseline. Significance at 0 min refers to mean from both clamps.

insulin-sensitive individuals. The current results support and expand our previous findings of altered glucose-dependent neurohormonal regulation as an early feature in the development of T2D^{5,7} that may contribute to the progression and maintenance of the disease.

The main finding in this study was an enhanced response of adrenaline to hypoglycemia in individuals with T2D and PD compared with NG. While our findings are in line with previous investigations of individuals with more advanced T2D⁸⁻¹²

and the reported reduction of catecholamine levels following weight loss,^{17,18} the existence of adrenaline hyperresponsiveness to hypoglycemia already at the stage of PD and its association with insulin resistance have not been previously elucidated to the best of our knowledge. Since increased adrenaline responses were found in T2D and PD already in the low normal glycemic range (illustrated in Figure 3) and postprandial hypoglycemic episodes are quite common in PD¹⁹ as well as in diabetes-prone conditions such as PCOS²⁰ and metabolic dysfunction-

associated liver disease,²¹ we suggest that adrenaline hypersecretion to glucose fluctuations may contribute to the development of T2D at early stages by promoting insulin resistance on an everyday basis. In contrast to the hypoglycemic domain, catecholamine concentrations in the normo-hyperglycemic range were, if anything, lower in individuals with T2D and did not display any discernable dynamic trend. This is in keeping with previous studies of circulatory levels of catecholamines in T2D,²² while elevated diurnal urinary levels have been linked to insulin resistance.²³

Neither hypo- nor hyperglycemia had any considerable impact on the circulatory levels of dopamine, which to our knowledge have not been demonstrated before. Other stressful stimuli, such as exercise and cold provocation, have been shown to lead to small but noticeable increases in dopamine in the circulation,^{24,25} suggesting other pathways and mechanisms to be at play in these responses. Moreover, although pharmacological dopamine treatment indisputably has systemic effects,²⁶ the endocrine effect of endogenous dopamine may be of subordinate importance in comparison with the regional effects of dopamine as a neurotransmitter.

Individuals with T2D tended to experience more profound symptoms of hypoglycemia specifically within the autonomic domain (ie, sweating and heart palpitations). Previously, elevated thresholds for hypoglycemic symptoms have been observed in T2D,^{8,11} and we have also reported a tendency toward more marked hypoglycemic symptoms in overweight compared with lean individuals.⁵ Interestingly and for unknown reasons, the enhanced catecholamine response for individuals with PD did not similarly translate into an augmented symptomatic response.

Notwithstanding the clearly elevated catecholamine responses in individuals T2D and PD, the hemodynamic responses to hypoglycemia seem to be less adaptive than in individuals with NG. In line with our previous work,⁵ we also found obesity and, more clearly, insulin resistance to be associated with an attenuated increase in heart rate and lesser decrease in HRV during profound hypoglycemia. However, these findings may be partially explained by between-subject variation in achieved glucose and insulin levels, as demonstrated when exploratively adjusting for these variables. Heart rate variability indices, in particular P_{HF} , were generally reduced in T2D and insulin-resistant individuals, indicative of suppressed parasympathetic activity. This is fully compatible with some previous evidence.²⁷⁻²⁹ As for the dynamic trajectory during hypoglycemia, the current results are in keeping with 1 previous study, where individuals with T2D exhibited attenuated responses in several HRV indices.³⁰ Diurnal variation in HRV has also been reported to be lower in T2D.²² Although the participants with T2D in this study had a short disease duration and a low prevalence of other complications, these findings may partly reflect subclinical cardiac autonomic neuropathy, which may be present at diagnosis of T2D and even in PD.³¹ Besides the potential role of maladaptive ANS responses to promote insulin resistance and T2D development, there may be implications for future cardiovascular morbidity, eg, via blood pressure elevation, endothelial dysfunction, and risk of arrhythmias.³²

The strengths of this study are the relatively large sample size, the well-characterized study population, and the implementation of multimodal investigations through the entire physiologic glycemic range. There are however some important limitations. First, the groups were not well balanced

according to clinical characteristics, but this was adjusted for in the best reachable manner in the statistical analyses. Second, concomitant glucose-lowering and antihypertensive medication, all more prevalent in participants with T2D, may have influenced some results.^{33,34} Third, we did not gather information regarding self-monitoring of glucose or hypoglycemic events. Fourth, although multiplicity adjustment was carried out for post hoc testing in the models, we did not correct for the multitude of models that were performed. This may have increased the risk of type I errors. Fifth, the cross-sectional nature of this study does not permit conclusions regarding causality.

In conclusion, enhanced glucose-dependent responses, not only of glucagon and the HPA axis as previously reported,⁷ but also of adrenaline are found in T2D and PD. We hypothesize that these elevated responses to falling glucose levels maintain glucose levels at higher levels via aggravation of insulin resistance and ultimately contribute to the development of T2D. The reduction of parasympathetic activity observed in T2D may further contribute to the maintenance of insulin resistance, and the attenuated autonomic nerve responses to hypoglycemia in these individuals may indicate that autonomic nerve dysfunction is an early feature in the course of T2D. Taken together, the current and other recent findings point to a role of the brain and its neuroendocrine effector pathways in the development of insulin resistance and T2D. Future studies should aim to further elucidate the causal role of the observed autonomic nerve and hormonal alterations for the development of T2D, ideally by studies involving long-term follow-up and targeted interventions.

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Supplementary material

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Authors' contributions

Martin Lundqvist (Conceptualization [supporting], Data curation [lead], Formal analysis [lead], Investigation [equal], Methodology [supporting], Project administration [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Maria Pereira (Data curation [supporting], Formal analysis [supporting], Project administration [supporting], Resources [supporting], Supervision [supporting],

Writing—review & editing [supporting]), Urban Wiklund (Formal analysis [supporting], Software [lead], Writing—review & editing [supporting]), Susanne Hetty (Data curation [supporting], Formal analysis [supporting], Project administration [supporting], Writing—review & editing [supporting]), and Jan W. Eriksson (Conceptualization [lead], Funding acquisition [lead], Investigation [lead], Methodology [lead], Project administration [lead], Resources [lead], Supervision [lead], Writing—review & editing [equal])

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Data availability

Data will be made available upon reasonable request to the corresponding author.

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