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Effects of insulin-induced hypoglycaemia on cardiac function in people with type 1 and type 2 diabetes and people without diabetes

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Abstract

Aims: Cardiovascular disease is the most common complication and cause of death in people with diabetes. Hypoglycaemia is independently associated with the development of cardiovascular complications, including death. The aim of this study was to assess changes in cardiac function and workload during acute hypoglycaemia in people with and without diabetes and to explore the role of diabetes type, magnitude of the adrenaline response, and other phenotypic traits.

Materials and Method: We enrolled people with type 1 diabetes ($n = 24$), people with insulin-treated type 2 diabetes ($n = 15$) and controls without diabetes ($n = 24$). All participants underwent a hyperinsulinaemic-normoglycaemic-(5.3 ± 0.3 mmol/L)-hypoglycaemic (2.8 ± 0.1 mmol/L)-glucose clamp. Cardiac function was assessed by echocardiography, with left ventricular ejection fraction (LVEF) as the primary endpoint.

Results: During hypoglycaemia, LVEF increased significantly in all groups compared to baseline (6.2 ± 5.2%, $p < 0.05$), but the increase was significantly lower in type

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1 diabetes compared to controls without diabetes (5.8 ± 3.4% vs. 9.4 ± 5.0%, $p = 0.03$, 95% CI difference: −5.0, −0.3). In people with type 1 diabetes, ΔLVEF was inversely associated with diabetes duration (β: −0.16, 95% CI: −0.24, −0.53, $p = 0.001$) and recent exposure to hypoglycaemia (β: −0.30, 95% CI: −0.53, −0.07, $p = 0.015$). Hypoglycaemia also increased global longitudinal strain (GLS) in controls without diabetes ($p < 0.05$), but this did not occur in the two diabetes subgroups ($p > 0.10$).

Conclusions: Hypoglycaemia increased LVEF in all groups, but the increase diminished with longer disease duration and prior exposure to hypoglycaemia in type 1 diabetes, suggesting adaptation to recurrent hypoglycaemia. The increment in GLS observed in controls was blunted in people with diabetes. More research is needed to determine the clinical relevance of these findings.

KEYWORDS
diabetes complications, heart failure, hypoglycaemia, type 1 diabetes, type 2 diabetes

1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in diabetes[1,2] and accounts for around half of the deaths in people with type 1 and type 2 diabetes.[3] The increased risk of morbidity and mortality caused by CVD is only partially explained by traditional risk factors, including hyperglycaemia, hypertension, and dyslipidaemia.[1,4–6] Data from previous landmark studies indicate that hypoglycaemia, especially severe hypoglycaemia, is linked to an increased risk of cardiovascular morbidity and mortality.[7–9] This association is independent of a range of known cardiovascular risk factors, suggesting that hypoglycaemia contributes causally to such events.[5] Still, the underlying mechanisms are poorly understood.

Several potentially harmful responses to hypoglycaemia may explain a direct causal relationship with CVD,[5,10] including activation of blood coagulation,[11,12] increased inflammation,[11,13] and endothelial dysfunction.[11] Furthermore, hypoglycaemia acutely stimulates the release of adrenaline, resulting in increased cardiac workload with increased heart rate, myocardial contractility, and cardiac output, which in the setting of concomitant substrate depletion may promote acute ischaemia in people with ischaemic heart disease.[14]

Few studies have investigated cardiac function during experimentally controlled hypoglycaemia. Using radionuclide ventriculography, hypoglycaemia has been found to increase ejection fraction in people without diabetes[15] and in people with type 1 diabetes.[16] Two recent papers using echocardiography reported increased left ventricular ejection fraction (LVEF) as well as global longitudinal strain (GLS) during hypoglycaemia in both type 1 and type 2 diabetes.[17,18] It remains unclear whether the cardiac effects of hypoglycaemia differ between people with type 1 or type 2 diabetes. It is also unknown whether factors associated with suppressed adrenaline responses to hypoglycaemia such as long duration of diabetes and recent exposure to hypoglycaemia also modulate the effects of hypoglycaemia on cardiac function.

Therefore, our hypothesis was that hypoglycaemia causes acute changes in cardiac function in individuals with and without diabetes. We also hypothesised that such changes would depend on the type of diabetes, disease duration, recent exposure to hypoglycaemia, and the adrenaline response to hypoglycaemia. To test these hypotheses, we assessed changes in cardiac function and workload during acute hypoglycaemia (<3.0 mmol/L) in people with and without diabetes and explore the impact of diabetes type, the adrenaline response, and other phenotypic traits.

2 | RESEARCH DESIGN AND METHOD

2.1 | Study design

This study is a two-centre experimental study performed at the Department of Endocrinology and Nephrology at Nordsjællands Hospital, Denmark, and the Department of Internal Medicine at Radboud University Medical Centre, The Netherlands, and was carried out from August 2019 until March 2021 as part of the Hypo-RESOLVE project.[19] It was approved by the local ethics committees s (H-19005936 and NL67229.091.18) and carried out according to the principles of the Declaration of Helsinki. The study was registered at clinicaltrials.gov with the number: NCT03976271.

2.2 | Study population

Participants in this study were part of a larger clamp study.[20,21] A subgroup from this study underwent echocardiography's. We recruited people with type 1 diabetes or type 2 diabetes, and two control groups without diabetes. People with diabetes were recruited from the diabetes outpatient clinics in both hospitals. Controls without diabetes were recruited using advertisements in local newspapers and

social media. All participants had to be fluent in the local language with a body mass index of 19–40 kg/m^2, age 18–80 years and blood pressure <140/90 mmHg (with or without antihypertensive treatment). People with diabetes could be included if treated with a basal-bolus insulin regimen for at least 1 year. Key exclusion criteria were: HbA_{1c} above 100 mmol/mol (11.3%), use of anti-depressive drugs, and a CVD event in the past 5 years before screening (e.g. myocardial infarction, stroke, heart failure or symptomatic peripheral arterial disease). For fertile women, pregnancy, breastfeeding, or taking no measures for birth control were exclusion criteria. A complete description of the exclusion criteria can be found in previous publications.[20]

2.3 | Study protocol

All potentially eligible study participants were invited for a medical screening, including medical history and standard physical examination. HbA_{1c} and kidney function (serum creatinine) were determined if this had not been done in the past 3 months before screening. People with diabetes were provided with an open intermittently scanned continuous glucose monitoring (CGM) device (Freestyle Libre 1®) 7 days before the experimental day to record glucose profiles and avoid hypoglycaemia (<3.0 mmol/L) 24 h before the experimental day, in which case the experiment was rescheduled. Participants on multiple dose injection were instructed to reduce their basal insulin by 25% the day before the clamp and to omit rapid-acting insulin in the morning. Participants using an insulin pump were asked to turn off the pump 1 h before arriving at the experimental site.

A thorough description of the study protocol has been published previously.[21] Briefly, participants arrived at the research unit between 07:00–08:00 a.m. after an overnight fast and having abstained from caffeine-containing substances, alcohol and tobacco 24 h and from strenuous exercise 48 h before the test. Subsequently, a catheter was placed in an antecubital vein in the dominant arm for a constant infusion of insulin (Novo Rapid®, Novo Nordisk, Bagsværd, Denmark) at a rate of 1.5 or 3.0 mU/kg/min for people with type 1 and type 2 diabetes and their matching control groups without diabetes, respectively, together with a variable infusion of 20% glucose (Baxter B.V., Deerfield, IL or Fresenius Kabi A.B). Another catheter was placed retrogradely in the contralateral hand and placed in a heated hand box (55–60°C) to arterialise venous blood. Every 5–10 min, plasma glucose levels were measured using the Biosen-C line machine (Biosen C-Line; EKF Diagnostics, Cardiff, UK). After obtaining baseline measurements, the clamp started aiming for glucose levels at 5.0–5.5 mmol/L for 30 min, after which glucose levels were allowed to decline to 2.8 mmol/L and maintained at that level for 60 min. During the clamp, blood was sampled for measurement of counterregulatory hormones and inflammatory markers,[12,19,20] conducted cognitive function tests and assessed the appearance of symptoms using questionnaire scoring. These data have been published previously.[12,19,20] After 60 min of hypoglycaemia, the insulin infusion was stopped, and the glucose level was raised to normoglycaemic levels again.

2.4 | Echocardiography

At baseline and during hypoglycaemia, a cardiac ultrasound was performed. In Denmark, GE Healthcare Vivid iq and GE healthcare S70 ultrasound machines were used; in the Netherlands, Phillips CX50 and Phillips Affiniti were used. At least two consecutive heart cycles were recorded.

LVEF represents the fraction of blood from the left ventricle that is pumped out though the aortic valve, and it was determined (primarily) by Simpsons' biplane method, in all cases combined with eyeballing. Early left ventricle diastolic inflow (E), which represents the maximal velocity of the inflow to the left ventricle trough the mitral valve, was evaluated by pulse-wave Doppler interrogation on an apical four-chamber view positioning the sample volume at the mitral tip. Tissue Doppler imaging was performed on an apical four-chamber view to obtain e' values (pulsed-wave early diastolic tissue Doppler velocity). E/e' was calculated as the ratio of E and e'. GLS is a measurement of the performance of the left ventricle, and it measures the shortening of the muscle fibres. It is a more sensitive marker for detecting left ventricle function deterioration than LVEF. GLS was measured using two-dimensional speckle-tracking.[22] Due to image quality, GLS measurements could be obtained in 79% of the participants. Ten ultrasound exams were analysed by two independent cardiologists trained in evaluations of cardiac ultrasounds to determine interobserver variability, which was 7.5%.

2.5 | CGM analysis

Participants with type 1 and type 2 diabetes were equipped with a CGM 7 days prior to the clamp day. Participants with >80% of valid CGM recordings in the monitoring period were included (type 1 diabetes $n = 22$, type 2 diabetes $n = 12$, see Table 1 and Table S1 for further information). The hypoglycaemic metrics were classified according to international consensus.[23] A hypoglycaemic event was defined as two consecutive measurements (≥15 min) of glucose values <3.9 mmol/L. Events were further classified as level 1 (<3.9–3.0 mmol/L) and level 2 (<3.0 mmol/L) hypoglycaemia. See Table S1 for information on CGM metrics.

2.6 | Statistics

All normally distributed data are shown as mean ± SD, and non-normally distributed data as median [inter quartile range] ([IQR]), unless otherwise stated. According to data distribution, baseline characteristics and Δ-values between two groups were compared using independent samples t test or Mann–Whitney U test for continuous variables. In each group, paired sample t tests were used to evaluate the effect of hypoglycaemia on endpoints. The primary outcome for this analysis was change in LVEF, and secondary outcomes were changes in GLS and E/e'. We performed univariate analyses to determine potential predictors for each dependent variable. Predictors

TABLE 1 Participant's characteristics.

	Type 1 diabetes	Type 2 diabetes	Type 1 controls without diabetes	Type 2 controls without diabetes
Participants, *n*	24	15	8	16
Gender (M/F)	11/11	9/6	4/4	8/8
Age, year	48.5 [28.8–62.3]**	62.0 [55.0–68.0]	39.5 [24.5–62.3]	57.0 [52.3–61.8]
Duration of diabetes, year	24.3 ± 13.3**	15.4 ± 7.6	–	–
HbA_1c mmol/mol (%)	62 ± 11 (7.8 ± 1.0)	64 ± 11 (8.0 ± 1.0)	33 ± 3 (5.2 ± 0.3)*	36 ± 2 (5.4 ± 0.2)**
Body mass index (kg/m^2)	26.4 ± 3.5**	29.0 ± 4.3	23.2 ± 3.6*	28.0 ± 4.4
Smoking status (yes/no)	3/21	0/16	0/8	0/16
Adrenaline (AUC) (nmol/L)	1.7 [1.1–2.8]	3.3 [1.2–6.4]	2.5 [1.3–3.7]	2.7 [1.1–3.7]
CGM prior to clamp, *n*	24	15	–	–
CGM (>80% active sensor time)	22	12	–	–
Hypoglycaemia, episodes/week	5.6 [3.1–7.7]	1.1 [0.0–4.8]*	–	–
Diabetes complications				
Retinopathy, *n* (%)	6 (25.0)	2 (13.3)	–	–
Neuropathy, *n* (%)	3 (16.7)	2 (13.3)	–	–
Nephropathy, *n* (%)	1 (4.2)	1 (6.7)	–	–
Glucose lowering medication				
CSII, *n* (%)	7 (29.2)	1 (6.7)	–	–
MDI, *n* (%)	17 (70.8)	14 (93.3)	–	–
Metformin, *n* (%)	–	9 (60)	–	–
SGLT-2i, *n* (%)	–	1 (7)	–	–
GLP-1 agonist, *n* (%)	–	2 (13)	–	–
Sulfonylurea, *n* (%)	–	3 (20)	–	–
Other medication				
BP lowering, *n* (%)	13 (54)	12 (80)	–	1 (6)
Lipid lowering, *n* (%)	6 (25)	7 (47)	–	–
Other, *n* (%)	14 (58)	13 (87)	–	2 (13)

Note: Data are *n* (%), mean ± SD or median [IQR].
Abbreviations: CSII, continuous subcutaneous insulin infusion; GLP-1, Glucagon-like peptide-1; MDI, multiple daily injections; SGLT-2i, sodium glucose co-transporter 2 inhibitor.
*$p < 0.05$ versus type 1 diabetes.**$p < 0.05$ versus type 2 diabetes.

included were: age, baseline glucose, diabetes duration, HbA1c (mmol/L), total number of CGM-recorded hypoglycaemia (<3.9 mmol/L) and level 1 and 2 events and area under the curve for adrenaline concentration during the hypoglycaemic clamp procedure. After that, multivariate regression analysis was done to investigate the potential independent effect of each predictor. The following comparisons were made: Type 1 versus type 2 diabetes, type 1 diabetes versus (matched) controls without diabetes and type 2 diabetes versus (matched) controls without diabetes. The level of statistical significance was set at 5% (two-sided). IBM SPSS Statistical Software, version 25.0 (IBM, Armonk, NY), was used for analysis.

3 | RESULTS

A total of 63 people were included. People with type 2 diabetes were older and had a higher body mass index and a shorter duration of diabetes than the group of included people with type 1 diabetes. People with type 1 diabetes and their respective controls without diabetes were overall well matched, albeit that body mass index was somewhat higher in the type 1 diabetes group. The group with type 2 diabetes and their controls without diabetes were well matched for age, sex, and body mass index (Table 1).

3.1 | Plasma glucose during clamp procedure

Baseline plasma glucose values did not differ significantly between the type 1 diabetes and type 2 diabetes subgroups (12.4 ± 4.1 vs. 9.6 ± 4.7 mmol/L, $p = 0.075$), but were clearly higher than in controls without diabetes (5.7 ± 0.5 and 5.9 ± 0.5 mmol/L, both $p < 0.01$ vs. diabetes subgroups). All groups reached a mean normoglycaemic plateau at 5.3 ± 0.3 mmol/L and a hypoglycaemia plateau at 2.8 ± 0.1 mmol/L, with a coefficient of variation at 5.8% and 4.3%, respectively (Figure 1).

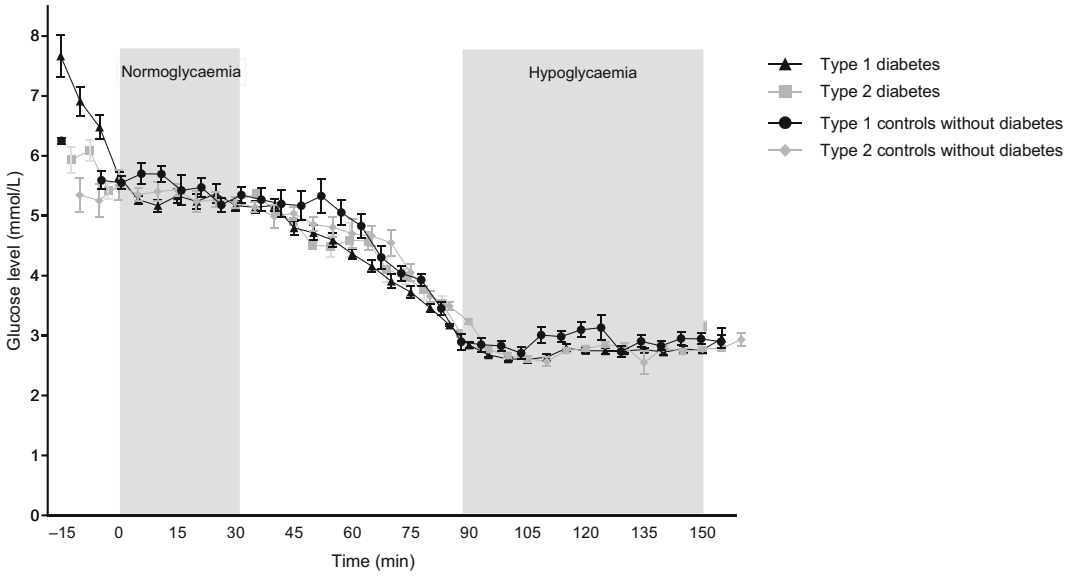

FIGURE 1 Glucose levels during the glucose clamp.

3.2 | Left ventricular ejection fraction

At baseline, LVEF was comparable between people with type 1 and their matched control group (57.7 ± 3.5% vs. 56.9 ± 4.6% $p = 0.582$, 95% CI difference: −2.29, 4.01), whereas LVEF was lower in participants with type 2 diabetes as compared to their matched control group (55.8 ± 4.1% vs. 59.8 ± 6.3%, $p = 0.012$, 95% CI difference: −7.09, −0.94). There were no differences in baseline LVEF between the two diabetes groups ($p = 0.129$, 95% CI difference: −0.59, 4.48). LVEF increased significantly from baseline to hypoglycaemia in all groups (by 6.2 ± 5.2%, $p < 0.05$) (Figure 2A), but the increase was significantly lower in the group of type 1 diabetes compared to controls without diabetes (5.8 ± 3.4% vs. 9.4 ± 5.0%, $p = 0.03$, 95% CI difference: −5.0, −0.3). In type 1 diabetes, higher age ($p = 0.005$), longer diabetes duration ($p = 0.001$), and CGM-recorded hypoglycaemia in the week before the clamp ($p = 0.049$) were all inversely associated with ΔLVEF in univariate analysis, but not area under the curve for adrenaline ($p = 0.5$). In multivariate analysis, diabetes duration (β: −0.16, 95% CI: −0.24, −0.53, $p = 0.001$) and CGM-recorded hypoglycaemia level 1 (β: −0.30, 95% CI: −0.53, −0.07, $p = 0.015$) remained negatively associated with ΔLVEF.

3.3 | Global longitudinal strain

GLS was measured in 50 out of 63 participants. Baseline GLS averaged −19.5 ± 2.4% and did not differ between the four subgroups. In response to hypoglycaemia, GLS increased significantly in both the control group to type 1 diabetes (−2.5 ± 2.3%, $p = 0.029$, 95% CI difference: 0.35, 4.66) and the control group to type 2 diabetes (−3.6 ± 3.9%, $p = 0.014$, 95% CI difference: 0.86, 6.04), but did not change in the two diabetes subgroups ($p > 0.10$) (Figure 2B). In type 2 diabetes, HbA_{1c} was associated with ΔGLS in univariate analysis ($p = 0.029$), but not in multivariate analysis.

3.4 | *E*/*e*′-mean

Baseline *E*/*e*′-mean was higher in participants with type 1 diabetes than their control group (6.5 ± 1.5 vs. 5.0 ± 2.2 m/s, $p = 0.037$, 95% CI difference: 0.10, 2.92), as well as in participants with type 2 diabetes compared to their control group (8.5 ± 3.7 vs. 6.1 ± 1.6 m/s, $p = 0.04$, 95% CI difference: 0.12, 4.60). Baseline *E*/*e*′-mean did not differ between the two diabetes groups ($p = 0.094$, 95% CI difference: −4.42, 0.39). Hypoglycaemia did not affect *E*/*e*′-mean in any of the groups ($p > 0.10$), (Figure 2C).

4 | DISCUSSION

In this study assessing cardiac function during experimental hypoglycaemia by echocardiography, hypoglycaemia increased LVEF in people with type 1 diabetes, type 2 diabetes, and without diabetes. Apart from a slightly lower response in the group with type 1 diabetes compared to controls, there were no differences between the groups. GLS did not change in response to hypoglycaemia in the two diabetes groups, but it increased in the two control groups, whereas *E*/*e*′-mean was not affected by hypoglycaemia in any of the four groups. Together, these findings suggest an acute effect of hypoglycaemia on cardiac function in all people, which is modified by the presence of diabetes, type 1 diabetes in particular.

Our findings on LVEF are in line with previous reports. A recent study assessing cardiac function with echocardiography showed an increment in LVEF in people with type 1 diabetes in response to hypoglycaemia that was comparable to our findings.[17] Furthermore, our LVEF findings are in line with a study examining the LVEF response to hypoglycaemia immediately preceded by hyperglycaemia in people with type 2 diabetes.[18] Other studies using radionuclide ventriculography showed comparable LVEF responses to other stimuli (i.e. cold-pressor stimulation and exercise) in healthy

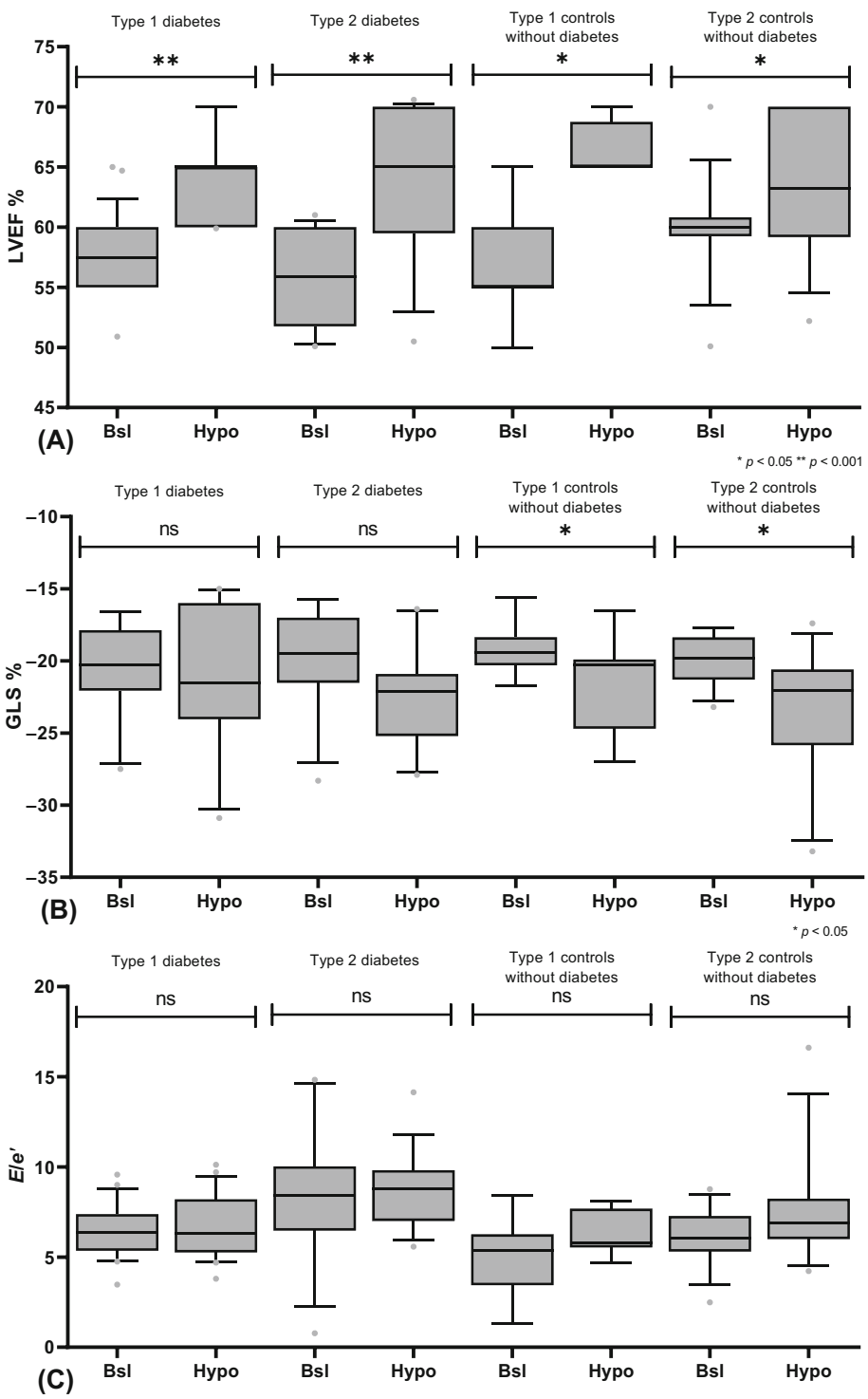

FIGURE 2 Cardiac function results of baseline and hypoglycaemia per subgroup for left ventricular ejection fraction (A), global longitudinal strain (B), and *E*/*e*′ (C). Data are shown in a box, representing 25th to 75th percentiles and whiskers with 10th and 90th percentiles. $^{*}p < 0.05$ and $^{**}p < 0.001$.

people and in people with type 1 diabetes and counterregulatory failure.[24]

Our study expands previous knowledge by showing that the LVEF increment was smaller in type 1 diabetes than in controls and that among people with type 1 diabetes, long duration of diabetes and prior hypoglycaemia exposure was associated with attenuated LVEF responses to hypoglycaemia. This is interesting as it follows the pattern of many other responses to hypoglycaemia, including hormonal counter regulation, sympathoadrenal activation, and symptom responses.[25–27] Thus, particularly in the early phase of type 1 diabetes, these responses are reversibly blunted following exposure to recurrent hypoglycaemia[28] and along the course of the disease, they may result in more profound counterregulatory failure and impaired awareness of hypoglycaemia.[29] Several potential mechanisms may explain the reduced LVEF response in people with hypoglycaemia prior to the experiment. Firstly, it could be due to blunting of the

counterregulatory catecholamine response. However, the fact that there was no association between the magnitude of the adrenaline response and the LVEF change during hypoglycaemia is an argument against that. The lacking adrenaline effect combined with the impact of exposure to recurrent hypoglycaemia prior to the experiment could hypothetically suggest reduced beta-adrenergic sensitivity as a potential explanation. This is supported by some, but not all, experimental studies that have reported reduced beta-adrenergic sensitivity because of repetitive exposure to hypoglycaemia.[30,31] Alternatively, blunting of the sympathetic response to recurrent hypoglycaemia could explain the association between hypoglycaemia exposure and reduced LVEF response. A potential reason for the association between long disease duration and reduced LVEF could be an effect of subclinical cardiovascular autonomic neuropathy, which develops in many patients along the course of diabetes.[32,33] Thus, it is well-known that autonomic dysfunction may impair the heart's ability to respond adequately to physiological stressors, including hypoglycaemia, leading to an attenuated LVEF response.[32,33] Finally, structural changes in the myocardium such as remodelling and fibrosis, which occur frequently in diabetes,[34,35] are also potential cause of the reduced LVEF response with longer diabetes duration.[36] Thus, the blunted LVEF response in type 1 diabetes could either be of reversible adaptive nature or due to structural alterations. Future research should address this issue. The fact that the LVEF response to hypoglycaemia was preserved in type 2 diabetes could be explained by much less hypoglycaemic exposure prior to the clamp and/or a shorter disease duration (~24 vs. 15 years).

Compared to LVEF,[37] GLS is a more sensitive method to detect early systolic dysfunction.[38,39] The majority of longitudinal myocardial fibres (as measured in GLS) are located in the subendocardium, resulting in these fibres being more vulnerable to increased haemodynamic load and ischaemia.[38] In our study, we found hypoglycaemia to affect GLS only in people without diabetes, but not in those with diabetes. A previous study among people with type 2 diabetes also found no effect of hypoglycaemia on GLS,[18] although another study reported a significant change in GLS in people with type 1 diabetes.[17] These findings may be explained by early signs of discrete diabetic myocardial changes not yet measurable by LVEF. This is supported by our findings that baseline E/e'-mean was significantly higher in people with type 1 and type 2 diabetes compared to controls without diabetes, suggesting an early sign of diastolic dysfunction in those with diabetes.

The question arises whether the observed cardiac responses to hypoglycaemia are of clinical significance. In this context, it is notable that the effects of hypoglycaemia on LVEF and GLS in our control groups were of the same magnitude as those of challenging myocardial function with a dobutamine stress test in healthy people and people with hypertension.[38] This suggests that clinically significant level 2 hypoglycaemia, which many people with insulin-treated diabetes may experience on weekly or even daily basis,[40] may impact cardiac workload in a manner like a dobutamine stress test. There is a well-established association between occurrence of severe hypoglycaemia and increased risk of CVD and mortality in diabetes, which is most pronounced in type 2 diabetes.[5] Recently, an analysis of the Hypo-RESOLVE pooled clinical database has expanded this by also showing associations between mild hypoglycaemia and cardiovascular events irrespective of background CVD, mainly in type 2 diabetes.[41] Compared to type 1 diabetes, type 2 diabetes is characterised by much higher cardiovascular risk, much less hypoglycaemic exposure, but according to our study, greater LVEF response to hypoglycaemia. Thus, hypoglycaemic effects on cardiac work could hypothetically contribute to increased risk of development of cardiac disease, or in particular, worsening in those with established heart failure or ischaemic heart disease.

A strength of this study is that we included a broad population of people with diabetes and controls without diabetes and that we used a standardised protocol for induction of hypoglycaemia. The present study also has limitations. Due to image quality, GLS was obtained in 79% of the participants with missing examinations in all groups. This may be the reason why we did not see a response in the participants with diabetes. The use of myocardial perfusion markers could potentially show subclinical heart disease, but since it was not the aim of the study, and this sub-study was part of a larger study, this was not performed. Secondly, the number of participants included in this study was rather small, resulting in a risk of insufficient power to identify smaller differences. Thirdly, the first echocardiography was performed at baseline, i.e. when glucose levels were considerably higher in the diabetes subgroups than in the control groups. However, this probably reflects the real-world situation, particularly in the participants with high blood glucose levels. Fourthly, the study was performed at two study sites, thus requiring two researchers to perform the measurements. However, the interobserver variability in the echocardiographic readings was 7.5%, which is considered acceptable, thus ensuring sufficient quality and reliability of the data presented in this article. Finally, we acknowledge that it would have been relevant to study how people with diabetes and established CVD respond to hypoglycaemia, as the association between hypoglycaemia and cardiovascular events is most evident in this group. However, inducing experimental hypoglycaemia in people with CVD may raise ethical concerns due to concerns about triggering arrhythmias and other cardiovascular complications. Cardiac ultrasound, while useful in many scenarios, is a less accurate method for analysing cardiac function compared to magnetic resonance imaging. Subtle differences in the heart's structure and performance can be missed due to suboptimal quality of pictures and interobserver variability. However, applying (hypoglycaemic) clamps in the MR facility is extremely challenging, as well as costly, particularly for a large data set.

In conclusion, hypoglycaemia resulted in clinically significant LVEF increments in people with type 1 and type 2 diabetes and people without diabetes. GLS only increased in people without diabetes and was not affected by hypoglycaemia in people with diabetes. In participants with type 1 diabetes, the cardiac response to hypoglycaemia was modulated by recent hypoglycaemic exposure and diabetes duration in accordance with many other responses to hypoglycaemia, suggesting existence of adaptive processes or structural alterations, the nature of which currently remains unknown. Increased cardiac

workload during hypoglycaemia may contribute to explain the association between hypoglycaemia and major adverse cardiovascular events in diabetes. This association deserves further attention.

AUTHOR CONTRIBUTIONS

TWF, CV, UP-B, and BG designed the study. TWF and CV performed the experiments and collected the data. MW and ALD performed the analyses of the cardiac ultrasound examinations. CHS analysed the CGM data. TWF analysed the data and wrote the first version of the manuscript. All authors discussed the results and implications and provided feedback on the manuscript at all stages.

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CONFLICT OF INTEREST STATEMENT

TWF has been employed by Novo Nordisk after the completion of the study. CV, MW, ALD and CHS: none. PLK has received lecture fees from AstraZeneca, Sanofi, and Novo Nordisk. BG has received research support from Novo Nordisk. UP-B has served on advisory boards for Novo Nordisk, Sanofi-Aventis, and Vertex and has received lecture fees from Novo Nordisk and Sanofi-Aventis.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.16283.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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