Understanding the Determinants of Sustained Beta Cell Function in Type 1 Diabetes

Doctoral Thesis

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

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ABSTRACT

Type 1 diabetes is a disease defined by the inexorable autoimmune destruction of pancreatic beta cells, leading to endogenous insulin deficiency. C-peptide, a 31 amino acid protein that joins the alpha and beta chains of insulin in the proinsulin molecule, is well established as a marker of endogenous insulin secretion. Circulating levels within people with type 1 diabetes demonstrate persistence of insulin secretion, in some cases, for many years after diagnosis. Additionally, histological analyses of donor pancreata have provided evidence for persistent immunoreactive insulin-positive beta cells. These findings have challenged the dogma that all beta cells are destroyed at, or soon after, onset of type 1 diabetes. Although it is clear there is some relationship between residual C-peptide and preserved beta cell mass, residual C-peptide alone cannot distinguish between loss of beta cell mass and reduced functionality. As such, C-peptide level remains a contested surrogate for the aetiopathological definition of type 1 diabetes, which is used across disease classification and as the end point in many intervention trials designed to preserve beta cell function.

A fundamental difference between type 1 diabetes and type 2 diabetes is that the former is characterised by rapid progression to endogenous insulin deficiency due to autoimmune beta-cell destruction. Since histological classification is impossible in living humans with type 1 diabetes, C-peptide-defined type 1 and type 2 diabetes have been used as the endpoint in the development and validation of classification models which combine clinical features and biomarkers to improve classification of disease at

diagnosis. In Chapter 2, I aimed to I validate a classification model that was previously developed on a C-peptide outcome in a clinical cohort, against a histological definition of type 1 diabetes. This classification model combined age, body mass index (BMI), autoantibody status and type 1 diabetes genetic risk score (T1D GRS), with its predictive performance tested on samples defined histologically as having type 1 diabetes and non-type 1 diabetes from the Network for Pancreatic Organ Donors with Diabetes (nPOD) biobank. Strong predictive performance of the model in this setting demonstrated that the C-peptide outcome, used in its development, is representative of histologically defined disease, confirming that C-peptide is a robust, appropriate surrogate outcome that can be used in large clinical studies where histological definition is impossible.

In the 1970s it was crystallised that type 1 diabetes is a disease mediated by the autoimmune destruction of insulin producing beta cells. Since then, the centrepiece of many disease modifying intervention trials has been to augment the survival of functional beta cells, assessed via measures of preserved C-peptide secretion. However, there are clear differences in disease progression between children and adults with recent suggestions that, even within children, differences are driven by underlying endotypes. In Chapter 3, across disease duration, I compared the trends of decline of C-peptide in a cohort of living children from the UK Genetic Resource Investigating Diabetes, to the trends of decline of pancreatic beta cells in organ donors from the combined nPOD and Exeter Archival Diabetes Biobank (EADB), through stratifying by newly described age at diagnosis associated endotypes. I demonstrated that C-peptide loss and beta cell loss, in all age at diagnoses studied, mirror one

another across duration of disease. I demonstrated that proportionally fewer children diagnosed <7 retained C-peptide after one year of diagnosis, with the levels of retained C-peptide being lower at diagnosis that those diagnosed at older ages. I showed these trends of loss are almost identical in pancreas donors, with proportionally fewer children retaining islets containing insulin positive beta cells after one year of diagnosis, with fewer insulin positive beta cells at diagnosis as compared to donors diagnosed at older ages. The results in this chapter are indicative of the differences in disease progression in children. The rapid depletion of C-peptide and beta cells in children diagnosed < 7 years is suggestive that early intervention close to or before diagnosis may be most time critical, and should additionally be considered in planning and interpretation of intervention trials.

Preserving C-peptide is unequivocally beneficial to a person diagnosed with type 1 diabetes, associating with reduced frequency and severity of self-reported hypoglycaemia and fewer long term microvascular complications, as evidenced originally from DCCT. In Chapter 4, using insights from continuous glucose monitoring (CGM) technology, I demonstrated that higher levels of endogenous insulin secretion around the time of diagnosis impact glycaemic variability, but are not associated with hypoglycaemia. The work in this chapter adds to findings from previous studies of longer duration diabetes to offer a more complete picture of the impact that variation in C-peptide levels have on glucose control in people with type 1 diabetes.

Increased use of flash and continuous glucose monitoring has enabled more detailed, daily insights into glycaemic control within type 1 diabetes, the relationships of such

with C-peptide have been explored within this thesis. This technology however offers a wealth of opportunity for exploring the lived experience type 1 diabetes, in relation to daily glucose control. In Chapter 5 I developed upon the skills I had refined in CGM data analysis, exploring the impact that free-lived high and moderate intensity exercise have on glycaemic control in type 1 diabetes, as compared to an individual's non-exercise "normal". I compare monitored glucose traces from 10 adults with type 1 diabetes that each completed three, 14-day intervention periods of: home-based high intensity interval exercise, home-based moderate intensity continuous exercise and a free-living non-exercise control period. A key part of this analysis was the careful comparison of the glucose traces in each exercise intervention period to the glucose traces within the non-exercise of control period, in order to understand how much exercise perturbed an individual from their "normal" . In this analysis I found that the exercise modes assessed increase glycaemic variability and hypoglycaemia in the 4 hours after exercise, had a modest effect on glycaemic variability overnight, but increased glycaemic variability and hypoglycaemia the day after exercise. The findings in this chapter suggest that developing focused clinical guidance around time periods post-exercise, and accounting for "everyday life", may improve the management of blood glucose in type 1 diabetes and ultimately reduce barriers to exercise.

In the majority of endocrine conditions, the hormone in question is measured as part of routine usual care. In diabetes this is not yet the case. In this thesis I provide evidence that C-peptide is a robust surrogate marker of functional beta cells in clinical settings and demonstrate how an estimate of a patients C-peptide reserve could

benefit clinical management. In addition to C-peptide level, I explore how exercise is another influential factor on glucose control in type 1 diabetes.

Throughout this thesis I aim to place findings within the context of the lived experience of type 1 diabetes. After all, it is the people living with type 1 diabetes that are the reason we continue our research.

ABBREVIATIONS

BMI	body mass index
CGM	continuous glucose monitoring
CSII	continuous subcutaneous insulin infusion
CV	coefficient of variation
DKA	diabetic ketoacidosis
DiViD	Diabetes Virus Detection (study)
EADB	Exeter Archival Diabetes Biobank
EXTOD	Exercise in Type 1 Diabetes (study)
GAD(A)	glutamic acid decarboxylase (antibody)
HbA_{1c}	glycated hemoglobin A_{1c}
HBGI	high blood glucose index
HIIE	high intensity interval exercise
HLA	human leukocyte antigen
HR	heart rate
IA2(A)	tyrosine phosphatase-related islet antigen 2 (antibody)
$IDAA_{1c}$	insulin dose–adjusted glycated hemoglobin A_{1c}
IQR	interquartile range
isCGM	intermittently scanned continuous glucose monitoring
JDRF	Juvenile Diabetes Research Foundation
L1H	level 1 hypoglycaemia
L2H	level 2 hypoglycaemia
LGBI	low blood glucose index

MARD	mean absolute relative difference
MDI	multiple daily injection
mIAA	micro insulin autoantibody
MICE	Moderate intensity interval exercise
MMTT	mixed-meal tolerance test
NHS	UK National Health Service
nPOD	Network for Pancreatic Organ Donors
PA	physical activity
SNP	single nucleotide polymorphisms
SEARCH	SEARCH for Diabetes in Youth (study)
T1D	type 1 diabetes
T1D-GRS	type 1 diabetes genetic risk score
TIGI	Type 1 diabetes, Immunology, Genetics and endogenous Insulin production (study)
TIR	time in range
UK GRID	UK Genetic Resource Investigating Diabetes
WHO	World Health Organisation
Znt8(A)	Zinc Transporter 8 (antibody)

NOTE: For Chapters **1, 2 and 6**, English (UK) abbreviations and spelling are used. For Chapters **3-5** English (US) abbreviations and spellings are used as these chapters are either published in, submitted to a US based journal.

CHAPTER 1: INTRODUCTION

Structure and Aims of Thesis

The overall aim of this thesis is to demonstrate that C-peptide is a robust surrogate marker of functional beta cells and examine the impact that preserving C-peptide, and other external factors, have on glucose control in type 1 diabetes across duration of disease.

In Chapter 1 I present a review of the current understanding of the clinical impacts of preserved C-peptide described across duration of diabetes, including how continuous glucose monitoring has helped provide detailed insights into the immediate impact of C-peptide on glucose control in type 1 diabetes.

In Chapter 2 I expand on the previous development of a clinical diagnostic model that combines simple clinical features and biomarkers to improve the classification of type 1 diabetes. I aim to validate this model against a histological definition of type 1 diabetes, using samples from the Network for Pancreatic Organ Donors (nPOD) biobank that have been classified as having type 1 diabetes or non-type 1 diabetes based off the current histological gold standard of disease. As the original model was developed and validated in cohorts of people with C-peptide defined type 1 diabetes and type 2 diabetes, I validate the performance of the original model and demonstrate that C-peptide is a robust appropriate surrogate outcome that can be used in large clinical studies where histological definition is impossible.

In Chapter 3 I build upon recent work describing type 1 diabetes endotypes that highly correlate with age at diagnosis. Stratifying by age at diagnosis endotypes, I describe the trends of decline in C-peptide levels in living children and young people with type 1 diabetes within the UK Genetic Resource Investigating Diabetes (UK GRID) cohort and the trends of decline in beta cells in pancreatic donors from nPOD and Exeter Archival Diabetes Biobank (EADB). I explore the similarities in the trends of decline in both settings and what this means in the context of intervention trials.

In Chapter 4 I aim to describe the impact that variation in endogenous insulin secretion close to diagnosis has on glycaemic variability and hypoglycaemia, using Continuous Glucose Monitoring (CGM) data from adults enrolled in the Exercise for Type 1 Diabetes (EXTOD) study with recent onset type 1 diabetes. I discuss how the clinical impact of C-peptide on a person with type 1 diabetes varies depending on the point from diagnosis with declining C-peptide, and how current estimates of a patient's C-peptide reserve could influence their clinical management.

In Chapter 5 I develop upon the skills I have refined in CGM data analysis and apply these to explore the impact of exercise on glycaemic control in type 1 diabetes. Utilising intermittently scanned continuous glucose monitoring, I explore the effect of two modes of home-based exercise on glycaemic variability and hypoglycaemia, as these are two of the main barriers to exercise people with type 1 diabetes express.

In Chapter 6 I present a discussion of the findings described, the implications of those findings, their limitations and future steps.

Each chapter remains in the style of the journal it was or is planned to be published in. The published, and some unpublished, supplementary material for each chapter can be found within the corresponding sections of the Appendix. Additionally, within the Appendix I present papers I have co-authored, including a joint first author paper describing time to death and risk factors associated with mortality among COVID-19 cases in the World Health Organisation (WHO) African region. This was completed during my time in an emergency consulting role for the WHO African Regional Office as a junior data sciences expert. Independent to my PhD, I supported the epidemiological analysis of data related to COVID-19 in the African region. Additionally, I present R code and description of such that I have developed for all CGM analysis used throughout this PhD and by collaborators, which is available Open Source via my GitHub. Other work, which I have supported but that has not yet been published, are outlined within in the appendix.

C-peptide as a Marker of Beta Cell Function

Type 1 diabetes is a disease defined by the inexorable autoimmune destruction of pancreatic beta cells, leading to endogenous insulin deficiency. Connecting peptide, or C-peptide, is a well-established marker of endogenous insulin secretion, being identified in parallel with first descriptions of insulin biosynthesis in 1967 by Steiner and colleagues [1, 2]. C-peptide is a 31 amino acid protein that joins the alpha and beta chains of insulin in the proinsulin molecule, promoting efficient folding and assembly in the endoplasmic reticulum of the pancreatic beta cells during insulin biosynthesis. In the cleavage off proinsulin, C-peptide is excised where it is secreted into the portal blood stream in equimolar concentrations with insulin [3] (Figure 1).

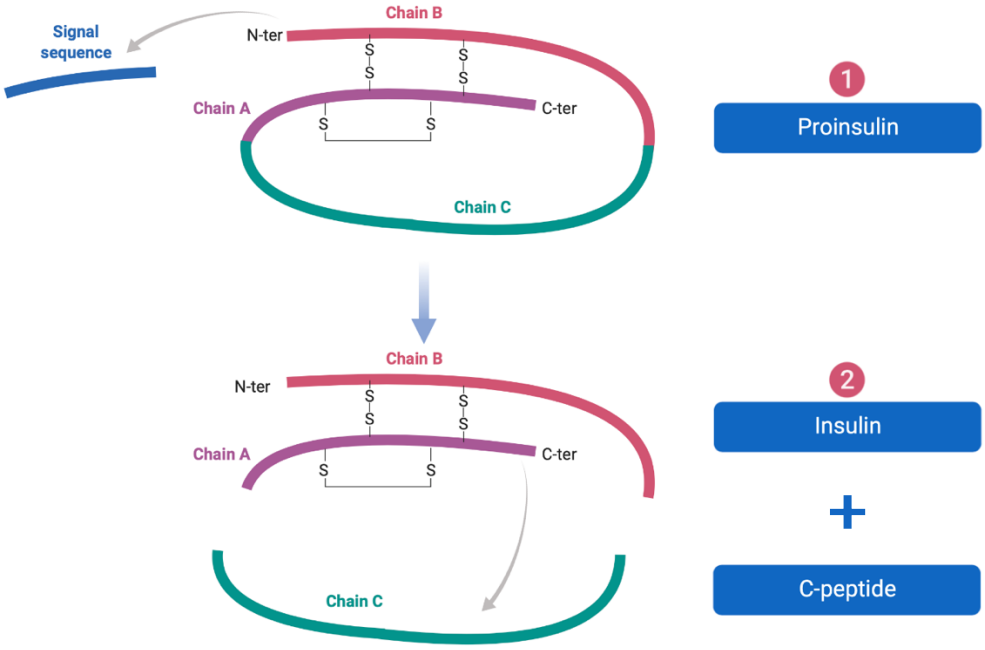

Figure 1: Schematic of insulin biosynthesis, created in BioRender.com

The differences in kinetics between C-peptide and insulin make C-peptide the better marker of endogenous insulin secretion. C-peptide experiences minimal first-pass

clearance by the liver, whereas approximately 50% of portal secreted insulin is cleared in this first-pass transit [4, 5]. C-peptide in contrast is predominantly metabolised by the kidney from the systemic circulation, with around 70% removed by glomerular filtration [6]. Therefore, unlike C-peptide peripheral insulin levels are unlikely to be far of that which was portally released. Additionally, the half-life of C-peptide is around 10 times longer than insulin (20–30 vs. 3–5 min) and it therefore circulates at concentrations approximately five times higher in the systemic circulation [7–9]. As such, from first detection in serum in the 1970s [10], C-peptide has proved clinically useful surrogate marker of endogenous insulin secretion, assisting with treatment decisions in type 1 and type 2 diabetes and monitoring the course of disease [9].

As early as 1978 [11], this measurement of serum C-peptide in individuals with type 1 diabetes led to the observation that some individuals retain measurable levels of insulin secretion for many years after diagnosis [12–19]. Additionally, evidence from histological analyses have shown the persistence of insulin-positive islets, in some cases for decades after diagnosis [18–22]. These findings have challenged the dogma that all beta cells are destroyed at, or soon after, onset of type 1 diabetes, inspiring many disease modifying intervention trials that aim to augment the survival of these residual insulin-producing beta cells.

However, due to difficulties in examination of the pancreas in living people [23], limited specimen availability [24] and the cross-sectional nature of autopsy studies, the relationship between beta cell mass and function has yet to be fully understood [25]. Although efforts are underway to improve non-invasive imaging of the pancreas for

assessments of beta cell mass in living individuals [26, 27], there are currently no methods with enough sensitivity to detect small numbers of residual beta cells [27], keeping the living pancreas largely inaccessible. As such, the majority of our histological knowledge surrounding residual beta cells, stems from studies of autopsy pancreas (Figure 2).

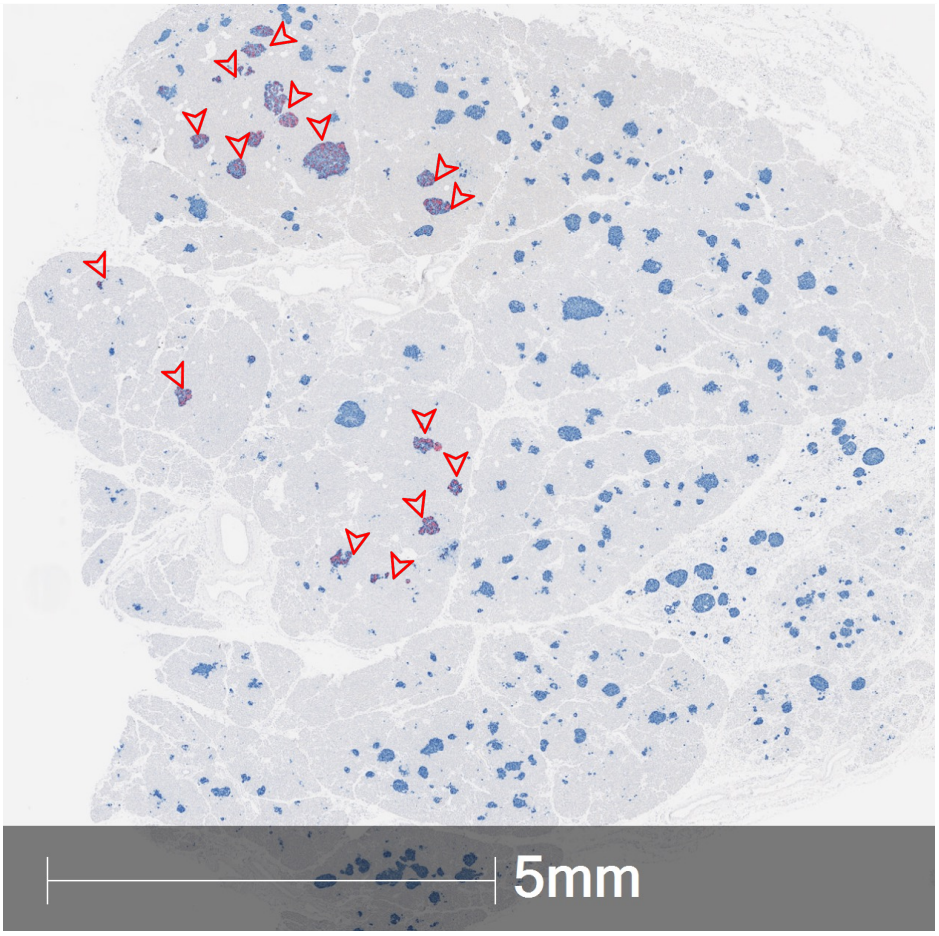

Figure 2: Micrograph of autopsy pancreas from nPOD donor 6484 (location: pancreas other) with type 1 diabetes, demonstrating insulin positive islets (red arrow) stained for insulin (red) denoting beta cells and glucagon (blue) denoting alpha cells and insulin negative islets showing only glucagon staining.

The largest collections of such are held in the Exeter Archival Diabetes Biobank (EADB) [28] and the Network for Pancreatic Organ Donors with Diabetes (nPOD) [29]. EADB is a 50-year-old archival biobank mainly comprising of non-systematically collected autopsy samples from young people (<20 years old) with recent-onset disease (<2 years), a feature unique to this biobank placing it as the world's largest collection of pancreas tissue from individuals diagnosed with diabetes under the age of 10 years [24]. These rare archival samples, although able give valuable insight into the pathogenesis of type 1 diabetes in young children, are of varying quality which preclude some applications in addition to lacking linked measurements of C-peptide due to the age of the biobank. In contrast, nPOD curated circa 2007 closely works with organ procurement organisations for the systematic and standardised processing of pancreatic tissue and other samples, enabling a wider range of applications. Additionally, at donation C-peptide levels are stored within the biobank, but must be interpreted with caution due to the end-of-life circumstances of collection and possible degradation due to glucotoxicity [30]. However, many of the donors in nPOD have a much older onset of disease with the majority often longer duration. Therefore, a combination of these two biobanks is our largest and most encompassing source for understanding the pathophysiology of type 1 diabetes in the pancreas. This still is far however from the extensive, within donor, analysis required to fully elucidate the relationship between beta cell mass and function, but at present there are no large systematic studies of C-peptide in clinical type 1 diabetes cohorts in whom post-death pancreas samples are available. A recent study however has shown that within children and young adults (<18) there are similarities in the trends of decline of beta

cell loss in pancreas donors (from the EADB and nPOD biobanks) and the trends of decline in C-peptide in the living across disease duration [31].

Importantly, highlighted within this work is that both beta cell mass and function is markedly heterogeneous in people with type 1 diabetes even varying from onset, which although is a well-established finding, is worth emphasising in the context of intervention and prevention trials. As the youngest children at diagnosis have lower levels of C-peptide and lower beta cell mass, it is this group in which intervention is most time-critical. Additionally, across all age of diagnosis, a proportion of individuals had presence of insulin containing islets and measurable C-peptide > 10 years from diagnosis, although markedly reduced. This echoes findings from previous studies [11–20, 32] and calls to the line of discussion surrounding the source of these persistent beta cells in long duration type 1 diabetes: if all are in fact functional or just "sleeping", which has been reviewed elsewhere [25]. It is however unequivocal that endogenous insulin, as measured by C-peptide and paralleled by histological observations, does persist in long duration type 1 diabetes.

It is well established, with evidence originally from the landmark Diabetes Control and Complications Trial, that persistent detectable C-peptide in long duration disease is associated with numerous positive clinical outcomes for people with type 1 diabetes [33–35]. It is for this reason that C-peptide is accepted as a primary outcome measure in intervention trials designed to preserve or improve beta cell function [36]. Although the benefits of preserved C-peptide in longer duration disease it is well documented, in more recent years evidence has emerged to suggest that C-peptide retention has a

measurable impact from the point of diagnosis for a person with type 1 diabetes [37, 38]. These recent studies show what has long been demonstrated in islet transplant recipients; high or low beta cell function impacts glyceamic control differently [39]. With advances in assay sensitivity and glucose monitoring technology, it is increasingly possible to gain an insight into the impact of preserved C-peptide across an individual's timeline of disease.

In this review I discuss the clinical use and impact of preserved C-peptide in people with type 1 diabetes from diagnosis through to long-duration disease.

Utility of C-peptide in Clinical Practice

As reviewed elsewhere [9], C-peptide is used in clinical practice in preference to insulin measurement when assessing beta cell function, due to negligible first pass clearance by the liver and because levels are not affected by exogenous insulin administration [7, 8]. Approximately half of peripherally secreted C-peptide is removed by the kidneys and therefore C-peptide levels must be interpreted with caution in renal failure in which blood levels of C-peptide can be falsely elevated [40, 41]. C-peptide can be measured in a fasting or non-fasting (random) sample or by formal stimulation test. The most commonly used stimulation test for C-peptide is Mixed Meal Tolerance Test (MMTT), which involves a liquid mixed meal containing standard proportions of protein, fat and carbohydrate [42]. During the test, blood is drawn for the measurement of C-peptide at 30 minutes pre-liquid mixed meal ingestion, at liquid mixed meal ingestion and at 30 minutes intervals post meal, until 120 minutes. The C-peptide level at 90 minutes is most commonly used as a measure of stimulated C-peptide, as this is approximately

the point post-liquid mixed meal where C-peptide is at a peak [43]. The choice of testing condition depends on both the clinical or research question and on practical considerations. There have been several consensus statements that suggest stimulated C-peptide from the MMTT is a gold standard measure for endogenous insulin secretion leading to its adoptions as an outcome measure in clinical studies [36, 42, 44]. However, while formal stimulation tests are most accurate and reproducible for research purposes, a fasting or non-fasting ('random') sample is usually suitable in clinical practice [9]. As sampling and detection methods have evolved, measuring beta cell function by C-peptide has become a cheap and easily accessible test that can even be employed in home-settings for clinical use. C-peptide is reported in several different units, commonly either in nmol/L or pmol/L, or occasionally ng/L. 1 nmol/L = 1000 pmol/L = 3 ng/L. Throughout this thesis the units of pmol/L are used.

C-peptide has been highlighted as a useful clinical tool for classification of diabetes. As type 1 diabetes is characterized by rapid progression to endogenous insulin deficiency due to autoimmune beta cell destruction, C-peptide levels taken within the first few years of diagnosis may be useful in confirming type 1 diabetes if results are low (<200pmol/L). However, utility is greatest 3-5 years post diagnosis as there may be a substantial overlap of C-peptide levels between type 1 and type 2/monogenic diabetes at the time of diagnosis [9]. In more recent years, classification models which combine clinical features and biomarkers, such as autoantibodies and T1D-GRS, have been shown to be more discriminative of diabetes type at diagnosis than any one feature alone [45–47]. C-peptide-defined type 1 and type 2 diabetes have been used

as the endpoint in the development and validation of these models, since histological classification, a widely accepted gold-standard in many other diseases, is impossible within type 1 diabetes [23]. Recent histological validation [48] of such models has strengthened evidence for the classification uses of C-peptide within these settings.

As assays have become more cost effective, reliable and widely available, there is grounds for C-peptide to become an established tool in the management of diabetes. In the majority of endocrine conditions, the hormone in question is measured as part of usual routine care, in diabetes this is not yet the case. There is accumulating evidence that suggests that the benefit of C-peptide preservation is much more complex, and clinical impact to the patient could depend on their point from diagnosis [38].

The Diabetes Control and Complications Trial and Beyond

The landmark Diabetes Control and Complications Trial has been the cornerstone in the descriptions of rates of progression to microvascular complications and severe, life-threatening hypoglycaemia in relation to beta cell function. Initial analyses have highlighted that within the intensive insulin therapy arm of the trial the loss of beta cell function was markedly slowed [35]. Although this was a success, those that were intensively treated were at much higher risk of severe hypoglycaemia (self-reported), a barrier to intensive insulin therapy that still remains today despite improvements to insulin formulas. However, those that retained the ability to secrete higher levels of C-peptide in response to a stimulus, had a significant risk reduction in severe hypoglycaemia in addition to retinopathy progression [33–35]. Within those intensively

treated, retaining a C-peptide response, the risk of progression to other microvascular complications was also lower, however this is presumably mediated by the lower HbA_{1c} within the intensively treated arm observed across the years of follow up [34]. Importantly, these low HbA_{1c} values were achieved with substantially less exogenous insulin, which unequivocally plays a key role in the reduction of hypoglycaemia observed [33–35].

There has since been further study to assess the minimum level of C-peptide that translates a clinical benefit to a patient with type 1 diabetes. Through modelling C-peptide continuously, Lachin et al demonstrated that, within intensively treated DCCT participants with 1-5 year disease duration, there was an inverse linear relationship with retinopathy progression to the detection of the assay (30 pmol/L). However, hypoglycaemia, HbA_{1c} and insulin dose only held this association above levels of 80 pmol/L [34]. In those of much longer disease, having any detectable levels (30-190pmol/L) conferred significantly lower risk of severe hypoglycaemia than those with undetectable levels [34].

Since the end of the DCCT, there have been improvements in the analytical sensitivity of C-peptide assays, allowing detection of 10-fold lower concentrations of C-peptide. Persistent levels of C-peptide, is now recognised to be common in type 1 diabetes, with many long-duration patients retaining low, but detectable levels [49, 50]. A recent large, representative study by Jeyam et al within the Scottish Diabetes Research Network Type 1 Bioresource Study [51] has since demonstrated a continuous inverse association of serious self-reported hypoglycaemia and incident retinopathy with

random C-peptide down to a limit of detection of 3 pmol/L, with striking risk reductions for hypoglycaemia seen at very minimal levels of C-peptide (5-20pmol/L). Much weaker associations were demonstrated between C-peptide and HbA_{1c} and insulin dose, with benefits only observed with C-peptide levels of at least 200 pmol/L, similar to that described in the DCCT cohort [34, 52] and supporting findings from smaller cross-sectional studies [53]. In studies of islet transplant recipients, striking reductions in severe hypoglycaemia have been demonstrated within the follow up period, even at minimal beta cell function, with much more modest effects on HbA_{1c} and insulin dose [39, 54–56].

In terms of disease progression, it is undeniable that C-peptide level has its most profound impact on hypoglycaemia and progression of retinopathy. The strong associations of C-peptide with hypoglycaemia and retinopathy, independent of other factors, indicate the two may be related. It is well established that acute decreases in blood glucose have been associated with reduction of retinal responses [57, 58] and that worsening may occur early within disease [59]. It is however only postulation that frequent hypoglycaemia may worsen retinopathy overtime as there are currently no studies in humans addressing this question.

The influence C-peptide has on HbA_{1c} and insulin dose is minimal, perhaps because many people with type 1 diabetes within these studies already have levels of C-peptide too low to demonstrate substantial benefit. As such, an intervening therapeutic agent may not preserve C-peptide at a high enough level to substantially reduce insulin requirement or improve long term glucose control in those with active disease

progression, but could arguably still improve quality of life for people with type 1 diabetes by reducing hypoglycaemia and retinopathy risk by preserving measurable levels of C-peptide for as long as possible in a patient.

As low levels of C-peptide have a marked impact on hypoglycaemia, it would suggest that even minimal beta cell functionality is enough to preserve the paracrine effect between the beta and alpha cell. It is possible that whilst some beta cell functionality persists, alpha cells can continue to respond in times of hypoglycaemia by secreting glucose raising hormones, such as glucagon, and mediate protection. There have been few recent studies addressing the intrinsic relationship between glucagon levels, stimulated C-peptide and hypoglycaemia in type 1 diabetes with many past studies using non-specific assays which may have led to erroneous measurements [60]. With new assays [61] one recent small study has demonstrated that within induced hypoglycaemia, C-peptide positive patients had higher glucagon concentrations than C-peptide negative patients, in addition to more pronounced endogenous glucose production [62].

The Immediate Clinical Impact of C-peptide

Over the past 5 decades, the development of technologies such as flash and continuous glucose monitoring (CGM) have afforded more detailed, daily insights in to glucose control over that of the 3-4 month estimate HbA_{1c} provides. CGM derived measures such as glycaemic variability and time spent above (hyperglycaemia), below (hypoglycaemia) and within clinically defined glucose ranges, have made the lived experience of people with type 1 diabetes, in relation to daily glucose control, more

accessible to monitor and potentially improve. Recent studies, utilising CGM, have highlighted that persistent C-peptide has an impact on these CGM derived measures of glucose control which does in fact vary depending on the individual's point from diagnosis.

As discussed in the previous section, studies exploring these benefits of preserved C-peptide in long-duration type 1 diabetes, or post-islet transplantation have shown that persistence of C-peptide is associated with decreased variation, more time spent in range (3.9-10 mmol/L) and protection from hypoglycaemia [53, 54, 56, 63–65], in addition to lower HbA_{1c} [52, 64] and lower insulin doses [53].

In contrast, C-peptide level very close to diagnosis does not seem to confer differential protection from hypoglycaemia, where stimulated levels can reach >1500pmol/l. However, higher levels within these ranges are associated with lower glycaemic variability, more time in range, and less hyperglycaemia [37, 38]. In post-islet transplantation settings, where the levels of C-peptide can resemble that close to diagnosis, it has been shown that higher beta cell functionality is necessary to prevent hyperglycaemia whereas minimal function is necessary to reduce hypoglycaemia [39]. It is possible that the C-peptide levels at diagnosis, in addition to other factors related to a short duration of type 1 diabetes, may offer more protection from hypoglycaemia and, as such, hypoglycaemia is uncommon compared to long-duration type 1 diabetes, when endogenous insulin secretion is much lower or absent.

Although there are few studies assessing the benefits of C-peptide close to diagnosis, it is possible that metabolic or physiological differences between individuals at diagnosis may have more of an impact on glycaemic variability than previously thought, with the level C-peptide present at this point possibly defining the manifestation of the individuals type 1 diabetes. As the clinical impact of C-peptide varies across diagnosis (Figure 3), there is grounds to suggest that measuring C-peptide level frequently from the point of diagnosis could be an important step towards precision medicine in type 1 diabetes, enabling development of the best approach in managing the newly diagnosed patient and pathway throughout their disease. Additionally, within the context of clinical trials, testing disease-modifying interventions that promote preservation of beta cell function would not only have long term impacts but could have substantial shorter term clinical benefits.

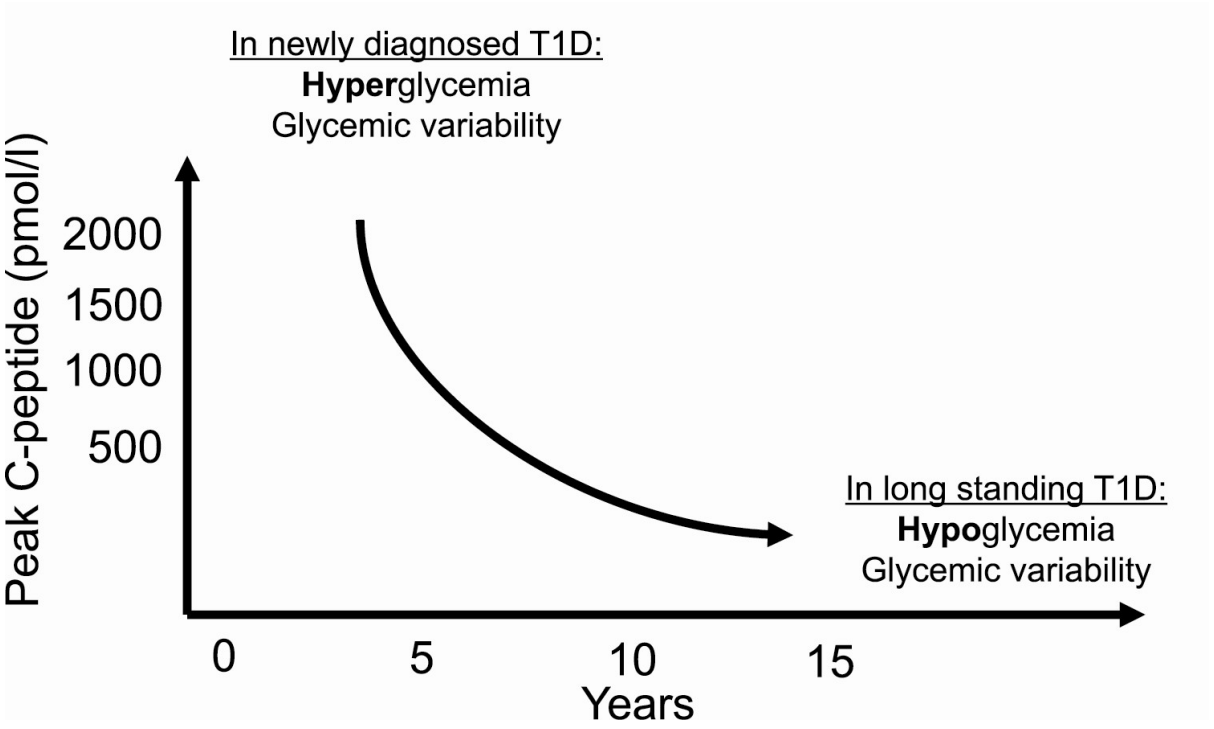

Figure 3 Summary of the impact variation in C-peptide level has on glucose control in people with type 1 diabetes across diabetes duration. In newly diagnosed type 1 diabetes (T1D), C-peptide variation impacts do not affect hypoglycemia, as demonstrated in longer-duration type 1 diabetes. C-peptide variation affects glycemic variability near to diagnosis of type 1 diabetes and at long-duration disease. Adapted from [38].

Preserving C-peptide

Many of the studies discussed in the previous sections contribute to the fact that C-peptide is now accepted by regulatory authorities as a primary end point in intervention trials aiming to preserve or restore β-cell function [66]. Within each of these studies, the C-peptide level at a certain point in time has been suggested to mitigate future risk of progression of certain complications. However, as type 1 diabetes is a disease of beta cell destruction, these C-peptide levels will decline over time. It's reasonable to suggest that if a therapeutic agent was able to continue to preserve C-peptide above

a given level, then many of the benefits described in these studies will only be enhanced. However, there is not yet a consensus on what level of C-peptide secretion constitutes a clinically useful therapeutic effect, with guidance stating only that an "Intervention will likely need to be initiated as soon as possible after manifestation of the disease to have a chance of showing a meaningful benefit" [66]. Guidance further states that clinical intervention trials must have co-primary endpoints of C-peptide (change from baseline), HbA_{1c}, severe hypoglycaemia incidence and reduction in insulin requirements [66]. With the current evidence highlighting that the most substantial effects of preserved C-peptide on HbA_{1c} and insulin dose only occur at high levels, it would indeed suggest that for therapeutics to succeed they would need to be trialled very close to, if not before clinical diagnosis. Adding further complexity, it is frequently observed, that those diagnosed with type 1 diabetes at the youngest ages (<7 years) have lower levels of C-peptide at diagnosis [43, 67–69]. It is known that there are clear differences in disease progression between children and adults [68, 69] with recent suggestions that even within children there are differences driven by underlying endotypes [17, 21] (Figure 4). This only emphasises the necessity to improve prediction of type 1 diabetes [70] and highlights that many trials could be deemed unsuccessful depending on the age of the participants and how close they are to diagnosis. It is worth noting that in recent immunotherapy trials of Teplizumab in people at and pre diagnosis of type 1 diabetes [71, 72], all participants were >7 years of age. With the suggestion that the youngest children perhaps experience a quicker progression to type 1 diabetes, driven possibly by a different mechanism to those diagnosed at older ages [21], one could speculate if the effects demonstrated in these trials would be replicated in children of younger ages. The importance of these

endotypes is something yet to be fully elucidated, however should be considered in the planning and interpretation of intervention trials designed to prevent disease progression or promote beta cell retention and function.

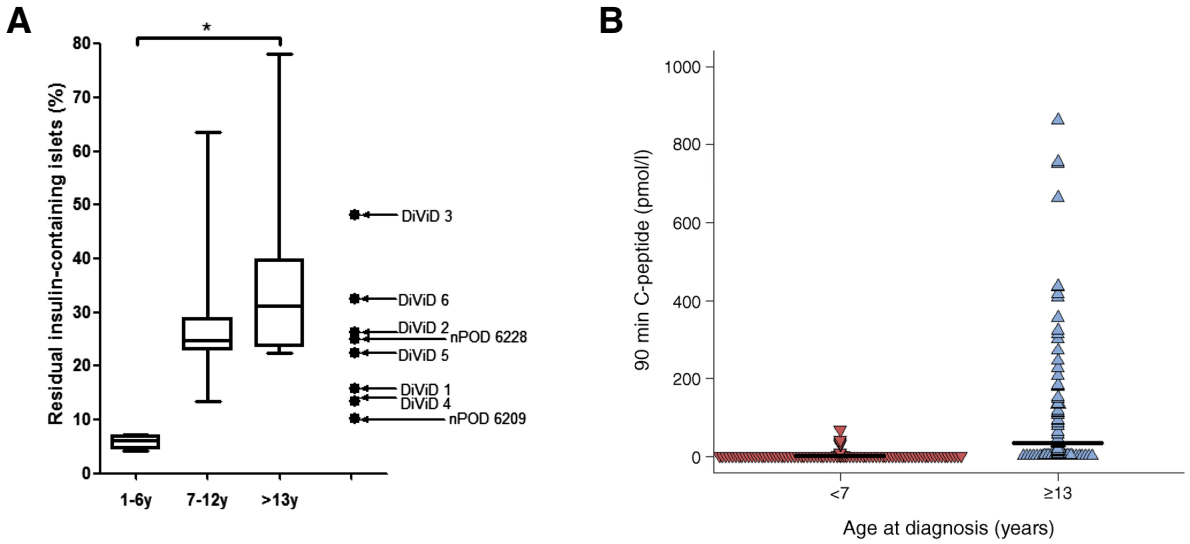

Figure 4 (A) Box plot of 20 donors from the EADB collection with recent-onset type 1 diabetes (< 3 months), displaying the proportion of residual insulin containing islets found in pancreas sections, stratified by age at diagnosis (proposed age at diagnosis associated endotypes). Arrows compare these proportions with those recently diagnosed from other cohorts: biopsy samples from 6 living donors from the Diabetes Virus Detection (DiViD) study (diagnosed >20 years) and 2 nPOD donors (nPOD donor 6228, diagnosed at 13 years of age; nPOD donor 6209, diagnosed at <5 years of age). *P < 0.001 relative to those who received a diagnosis at 1–6 years of age. Adapted from [21]. (B) Dot plot showing 90-minute stimulated C-peptide values in individuals from the Type 1 diabetes, Immunology, Genetics and endogenous Insulin production (TIGI) study cohort diagnosed at <7 years (n=87; red) or ≥13 years (n=84; blue). Black bars represent median values for each group. Adapted from [17].

Additionally, it would go amiss not to scrutinise the guidance calling for a therapeutic to show “reductions in severe hypoglycaemia”. Echoing the lessons from islet transplantation and studies close to diagnosis of type 1 diabetes, high C-peptide levels

seemingly offer a protection from hypoglycaemia, such that hypoglycaemia is in fact uncommon close to diagnosis. There is, of course, other factors in play that may contribute to the low levels of hypoglycaemia observed in this “honeymoon period”, like reduced insulin dose requirements, but it is crucial that intervention trials take this into account in planning and interpretation.

A noteworthy observation is that many individuals in the “honeymoon period” newly diagnosed type 1 diabetes, demonstrate some improvement in glycaemic control and reduced insulin dose requirements within the first year just with usual care, which can last for months [73–75]. This partial clinical remission is assumed to be due to beta cell recovery through reduced glucotoxicity. Insulin secretion is triggered through the opening of voltage-dependent calcium channels, however in chronic hyperglycaemia, a hyperstimulated metabolism, coupled to hyperexcitability of the beta cell membrane, leads to chronically elevated [Ca^{2+}], a pathway known to play a role in loss of beta cell function in diabetes [76, 77]. The DCCT was first to demonstrate the clinical phenomenon of beta cell recovery, showing that improved metabolic control by intensive insulin therapy associated with improved residual stimulated C-peptide [35]. More recently the smaller Diabetes Virus Detection (DiViD) study demonstrated a similar result in vitro, with isolated pancreatic islets from adults with newly onset type 1 diabetes demonstrating restored functionality when isolated from a diabetogenic environment after a number of days [78]. This suggestion that some beta cell function is recoverable close to diagnosis has perhaps inspired a recent small, phase 2, randomized placebo-controlled trial, using oral Verapamil, a calcium channel inhibitor and approved blood pressure medication, in new onset type 1 diabetes [79, 80].

Interestingly, subjects receiving Verapamil had improved endogenous beta cell function (MMTT stimulated C-peptide AUC), lower insulin requirements, and fewer hypoglycaemic events as compared to individuals receiving placebo added to their standard insulin regimen [79]. Verapamil's mechanism of action seemingly promotes an anti-oxidative, anti-apoptotic and immunomodulatory gene expression profile in human islets [80, 81] to recover and sustain beta cell function in adults with new onset type 1 diabetes for up to 12 months, in addition to an insulin regime.

Conclusions

It is undeniable that the benefits of C-peptide retention have a measurable impact from the point of diagnosis for a person with type 1 diabetes. In the majority of endocrine conditions, the hormone in question is measured as part of usual routine care, in diabetes this is not yet the case. As a robust surrogate marker of endogenous insulin secretion, there is increasing evidence for C-peptide estimates to be used as part of the management pathway in type 1 diabetes, from the point of diagnosis. As such, those with a lower C-peptide at diagnosis are likely to experience less time in glucose range, greater glucose variability, and more hyperglycaemia and would be earmarked for earlier and more intensive support. In addition, this knowledge could also be applicable in demonstrating clinical benefit of new therapies, pushing the idea that the benefit of C-peptide preservation is more holistic and not only important in reducing the complications of diabetes. In parallel with this, more work is needed to define the target level of C-peptide which is required for optimum clinical benefit over a given time. Although progress in the past decade has been significant, the recently outlined differences in the natural history of type 1 diabetes remains a fundamental piece of

the puzzle yet to be fully understood if we are to reach an ultimate goal of preserving beta cell function in all individuals with type 1 diabetes.

C-peptide is the key marker of endogenous insulin secretion, clinically useful in classification, monitoring disease course and as the primary outcome in trials aiming to preserve beta cell function or even delay beta cell destruction. But, to a person with type 1 diabetes C-peptide is more than just a clinically useful molecule. It might be the difference between more or less variable glucose control or hypoglycaemia, impacting every moment they live with type 1 diabetes. As behavioural factors impact glucose control daily, this biologic factor also plays a crucial role. Recognising this would perhaps bring the biggest clinical benefit to people with type 1 diabetes.

References

1. Steiner DF, Oyer PE (1967) The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc Natl Acad Sci U S A 57(2):473–480

2. Steiner DF, Cunningham D, Spigelman L, Aten B (1967) Insulin biosynthesis: evidence for a precursor. Science 157(3789):697–700. https://doi.org/10.1126/science.157.3789.697

3. Steiner DF, Cho S, Oyer PE, Terris S, Peterson JD, Rubenstein AH (1971) Isolation and Characterization of Proinsulin C-Peptide from Bovine Pancreas. Journal of Biological Chemistry 246(5):1365–1374. https://doi.org/10.1016/S0021-9258(19)76983-X

4. Duckworth WC, Hamel FG, Peavy DE (1988) Hepatic metabolism of insulin. Am J Med 85(5A):71–76. https://doi.org/10.1016/0002-9343(88)90399-3

5. Duckworth WC, Bennett RG, Hamel FG (1998) Insulin Degradation: Progress and Potential*. Endocrine Reviews 19(5):608–624. https://doi.org/10.1210/edrv.19.5.0349

6. Rabkin R, Kitaji J (1983) Renal metabolism of peptide hormones. Miner Electrolyte Metab 9(4–6):212–226

7. Polonsky KS, Licinio-Paixao J, Given BD, et al (1986) Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. J Clin Invest 77(1):98–105. https://doi.org/10.1172/JCI112308

8. Licinio-Paixao J, Polonsky KS, Given BD, et al (1986) Ingestion of a Mixed Meal Does Not Affect the Metabolic Clearance Rate of Biosynthetic Human C-Peptide*. The Journal of Clinical Endocrinology & Metabolism 63(2):401–403. https://doi.org/10.1210/jcem-63-2-401

9. Jones AG, Hattersley AT (2013) The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med 30(7):803–817. https://doi.org/10.1111/dme.12159

10. Heding LG (1975) Radioimmunological determination of human C-peptide in serum. Diabetologia 11(6):541–548. https://doi.org/10.1007/BF01222104

11. Madsbad S, Faber OK, Binder C, McNair P, Christiansen C, Transbøl I (1978) Prevalence of residual beta-cell function in insulin-dependent diabetics in relation to age at onset and duration of diabetes. Diabetes 27 Suppl 1:262–264. https://doi.org/10.2337/diab.27.1.s262

12. Dabelea D, Mayer-Davis EJ, Andrews JS, et al (2012) Clinical evolution of beta cell function in youth with diabetes: the SEARCH for Diabetes in Youth study. Diabetologia 55(12):3359–3368. https://doi.org/10.1007/s00125-012-2719-6

13. Shields BM, McDonald TJ, Oram R, et al (2018) C-Peptide Decline in Type 1 Diabetes Has Two Phases: An Initial Exponential Fall and a Subsequent Stable Phase. Diabetes Care 41(7):1486–1492. https://doi.org/10.2337/dc18-0465

14. Davis AK, DuBose SN, Haller MJ, et al (2015) Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care 38(3):476–481. https://doi.org/10.2337/dc14-1952

15. Hao W, Gitelman S, DiMeglio LA, Boulware D, Greenbaum CJ (2016) Fall in C-Peptide During First 4 Years From Diagnosis of Type 1 Diabetes: Variable Relation to Age, HbA1c, and Insulin Dose. Diabetes Care 39(10):1664–1670. https://doi.org/10.2337/dc16-0360

16. Greenbaum CJ, Beam CA, Boulware D, et al (2012) Fall in C-Peptide During First 2 Years From Diagnosis: Evidence of at Least Two Distinct Phases From Composite Type 1 Diabetes TrialNet Data. Diabetes 61(8):2066–2073. https://doi.org/10.2337/db11-1538

17. Leete P, Oram RA, McDonald TJ, et al (2020) Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia 63(6):1258–1267. https://doi.org/10.1007/s00125-020-05115-6

18. Keenan HA, Sun JK, Levine J, et al (2010) Residual insulin production and pancreatic ß-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 59(11):2846–2853. https://doi.org/10.2337/db10-0676

19. Yu MG, Keenan HA, Shah HS, et al (2019) Residual **β** cell function and monogenic variants in long-duration type 1 diabetes patients. J Clin Invest 129(8):3252–3263. https://doi.org/10.1172/JCI127397

20. Rodriguez-Calvo T, Richardson SJ, Pugliese A (2018) Pancreas Pathology During the Natural History of Type 1 Diabetes. Curr Diab Rep 18(11):124. https://doi.org/10.1007/s11892-018-1084-3

21. Leete P, Willcox A, Krogvold L, et al (2016) Differential Insulitic Profiles Determine the Extent of β-Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes 65(5):1362–1369. https://doi.org/10.2337/db15-1615

22. Gepts W, De Mey J (1978) Islet Cell Survival Determined by Morphology An Immunocytochemical Study of the Islets of Langerhans in Juvenile Diabetes Mellitus. Diabetes 27(Supplement_1):251–261. https://doi.org/10.2337/diab.27.1.S251

23. Atkinson MA (2014) Pancreatic biopsies in type 1 diabetes: revisiting the myth of Pandora's box. Diabetologia 57(4):656–659. https://doi.org/10.1007/s00125-013-3159-7

24. Morgan NG, Richardson SJ (2018) Fifty years of pancreatic islet pathology in human type 1 diabetes: insights gained and progress made. Diabetologia 61(12):2499–2506. https://doi.org/10.1007/s00125-018-4731-y

25. Oram RA, Sims EK, Evans-Molina C (2019) Beta cells in type 1 diabetes: mass and function; sleeping or dead? Diabetologia 62(4):567–577. https://doi.org/10.1007/s00125-019-4822-4

26. Yang L, Ji W, Xue Y, Chen L (2013) Imaging beta-cell mass and function in situ and in vivo. J Mol Med (Berl) 91(8):929–938. https://doi.org/10.1007/s00109-013-1056-7

27. Laurent D, Vinet L, Lamprianou S, et al (2016) Pancreatic β-cell imaging in humans: fiction or option? Diabetes Obes Metab 18(1):6–15. https://doi.org/10.1111/dom.12544

28. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS (1986) The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia 29(5):267–274. https://doi.org/10.1007/BF00452061

29. Campbell-Thompson M, Wasserfall C, Kaddis J, et al (2012) Network for Pancreatic Organ Donors with Diabetes (nPOD): Developing a Tissue Biobank for Type 1 Diabetes. Diabetes Metab Res Rev 28(7):608–617. https://doi.org/10.1002/dmrr.2316

30. Komulainen J, Lounamaa R, Knip M, Kaprio EA, Akerblom HK (1996) Ketoacidosis at the diagnosis of type 1 (insulin dependent) diabetes mellitus is related to poor residual beta cell function. Childhood Diabetes in Finland Study Group. Archives of Disease in Childhood 75(5):410–415. https://doi.org/10.1136/adc.75.5.410

31. Carr ALJ, Inshaw JRJ, Flaxman CS, et al (in press) Circulating C-peptide levels in living children and young people and pancreatic beta cell loss in pancreas donors across type 1 diabetes disease duration. Diabetes

32. Lam CJ, Jacobson DR, Rankin MM, Cox AR, Kushner JA (2017) β Cells Persist in T1D Pancreata Without Evidence of Ongoing β-Cell Turnover or Neogenesis. J Clin Endocrinol Metab 102(8):2647–2659. https://doi.org/10.1210/jc.2016-3806

33. Steffes MW, Sibley S, Jackson M, Thomas W (2003) β-Cell Function and the Development of Diabetes-Related Complications in the Diabetes Control and Complications Trial. Diabetes Care 26(3):832–836. https://doi.org/10.2337/diacare.26.3.832

34. Lachin JM, McGee P, Palmer JP, DCCT/EDIC Research Group (2014) Impact of C-peptide preservation on metabolic and clinical outcomes in the Diabetes Control and Complications Trial. Diabetes 63(2):739–748. https://doi.org/10.2337/db13-0881

35. The Diabetes Control and Complications Trial Research Group (1998) Effect of Intensive Therapy on Residual β-Cell Function in Patients with Type 1 Diabetes in the Diabetes Control and Complications Trial. Ann Intern Med 128(7):517–523. https://doi.org/10.7326/0003-4819-128-7-199804010-00001

36. Palmer JP, Fleming GA, Greenbaum CJ, et al (2004) C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes 53(1):250–264. https://doi.org/10.2337/diabetes.53.1.250

37. Buckingham B, Cheng P, Beck RW, et al (2015) CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but do poorly in predicting C-peptide levels in the two years following onset of diabetes. Diabetologia 58(6):1167–1174. https://doi.org/10.1007/s00125-015-3559-y

38. Carr ALJ, Oram RA, Marren SM, McDonald TJ, Narendran P, Andrews RC (2021) Measurement of Peak C-Peptide at Diagnosis Informs Glycemic Control but not Hypoglycemia in Adults With Type 1 Diabetes. Journal of the Endocrine Society 5(10). https://doi.org/10.1210/jendso/bvab127

39. Vantyghem M-C, Raverdy V, Balavoine A-S, et al (2012) Continuous Glucose Monitoring after Islet Transplantation in Type 1 Diabetes: An Excellent Graft Function (β-Score Greater Than 7) Is Required to Abrogate Hyperglycemia, Whereas a Minimal Function Is Necessary to Suppress Severe Hypoglycemia (β-Score Greater Than 3). J Clin Endocrinol Metab 97(11):E2078–E2083. https://doi.org/10.1210/jc.2012-2115

40. Zavaroni I, Deferrari G, Lugari R, et al (1987) Renal Metabolism of C-Peptide in Man*. The Journal of Clinical Endocrinology & Metabolism 65(3):494–498. https://doi.org/10.1210/jcem-65-3-494

41. Henriksen JH, Tronier B, Bülow JB (1987) Kinetics of circulating endogenous insulin, C-peptide, and proinsulin in fasting nondiabetic man. Metabolism 36(5):463–468. https://doi.org/10.1016/0026-0495(87)90044-8

42. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, et al (2008) Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 31(10):1966–1971. https://doi.org/10.2337/dc07-2451

43. Besser REJ, Shields BM, Casas R, Hattersley AT, Ludvigsson J (2013) Lessons From the Mixed-Meal Tolerance Test. Diabetes Care 36(2):195–201. https://doi.org/10.2337/dc12-0836

44. Greenbaum CJ, Harrison LC, on behalf of the Immunology of Diabetes Society (2003) Guidelines for Intervention Trials in Subjects With Newly Diagnosed Type 1 Diabetes. Diabetes 52(5):1059–1065. https://doi.org/10.2337/diabetes.52.5.1059

45. Lynam A, McDonald T, Hill A, et al (2019) Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18–50 years. BMJ Open 9(9):e031586. https://doi.org/10.1136/bmjopen-2019-031586

46. Oram RA, Patel K, Hill A, et al (2016) A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. Diabetes Care 39(3):337–344. https://doi.org/10.2337/dc15-1111

47. Shields BM, Peters JL, Cooper C, et al (2015) Can clinical features be used to differentiate type 1 from type 2 diabetes? A systematic review of the literature. BMJ Open 5(11):e009088. https://doi.org/10.1136/bmjopen-2015-009088

48. Carr ALJ, Perry DJ, Lynam AL, et al (2020) Histological validation of a type 1 diabetes clinical diagnostic model for classification of diabetes. Diabetic Medicine 37(12):2160–2168. https://doi.org/10.1111/dme.14361

49. Oram RA, Jones AG, Besser REJ, et al (2014) The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia 57(1):187–191. https://doi.org/10.1007/s00125-013-3067-x

50. Oram RA, McDonald TJ, Shields BM, et al (2015) Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. Diabetes Care 38(2):323–328. https://doi.org/10.2337/dc14-0871

51. Akbar T, McGurnaghan S, Palmer CN, et al (2017) Cohort Profile: Scottish Diabetes Research Network Type 1 Bioresource Study (SDRNT1BIO). International Journal of Epidemiology 46(3):796–796i. https://doi.org/10.1093/ije/dyw152

52. Jeyam A, Colhoun H, McGurnaghan S, et al (2020) Clinical Impact of Residual C-Peptide Secretion in Type 1 Diabetes on Glycemia and Microvascular Complications. Diabetes Care. https://doi.org/10.2337/dc20-0567

53. Marren SM, Hammersley S, McDonald TJ, et al (2019) Persistent C-peptide is associated with reduced hypoglycaemia but not HbA1c in adults with longstanding Type 1 diabetes: evidence for lack of intensive treatment in UK clinical practice? Diabetic Medicine 36(9):1092–1099. https://doi.org/10.1111/dme.13960

54. Brooks AM, Oram R, Home P, Steen N, Shaw JAM (2015) Demonstration of an Intrinsic Relationship Between Endogenous C-Peptide Concentration and Determinants of Glycemic Control in Type 1 Diabetes Following Islet Transplantation. Diabetes Care 38(1):105–112. https://doi.org/10.2337/dc14-1656

55. Forbes S, McGowan NWA, Duncan K, et al (2015) Islet transplantation from a nationally funded UK centre reaches socially deprived groups and improves

metabolic outcomes. Diabetologia 58(6):1300–1308. https://doi.org/10.1007/s00125-015-3554-3

56. Forbes S, Olateju TO, Lam A, et al (2018) CGM Shows Islet Transplantation Prevents Hypoglycemia, Correcting Time in Range and Reducing Glycemic Variability, Despite Subnormal Beta-Cell Function. Diabetes 67(Supplement 1). https://doi.org/10.2337/db18-143-OR

57. Khan MI, Barlow RB, Weinstock RS (2011) Acute Hypoglycemia Decreases Central Retinal Function in the Human Eye. Vision Res 51(14):1623–1626. https://doi.org/10.1016/j.visres.2011.05.003

58. Jingi AM, Tankeu AT, Ateba NA, Noubiap JJ (2017) Mechanism of worsening diabetic retinopathy with rapid lowering of blood glucose: the synergistic hypothesis. BMC Endocr Disord 17:63. https://doi.org/10.1186/s12902-017-0213-3

59. Bain SC, Klufas MA, Ho A, Matthews DR (2019) Worsening of diabetic retinopathy with rapid improvement in systemic glucose control: A review. Diabetes Obes Metab 21(3):454–466. https://doi.org/10.1111/dom.13538

60. Wewer Albrechtsen NJ, Veedfald S, Plamboeck A, et al (2016) Inability of Some Commercial Assays to Measure Suppression of Glucagon Secretion. In: Journal of Diabetes Research. https://www.hindawi.com/journals/jdr/2016/8352957/. Accessed 11 Nov 2019

61. Mercodia (2013) Mercodia Glucagon ELISA: directions for use. https://cms.mercodia.com/wp-content/uploads/2019/06/10-1271-01-dfu-v-12.0.pdf. Accessed 2 Mar 2022

62. Zenz S, Mader JK, Regittnig W, et al (2018) Impact of C-Peptide Status on the Response of Glucagon and Endogenous Glucose Production to Induced Hypoglycemia in T1DM. J Clin Endocrinol Metab 103(4):1408–1417. https://doi.org/10.1210/jc.2017-01836

63. Gibb FW, McKnight JA, Clarke C, Strachan MWJ (2020) Preserved C-peptide secretion is associated with fewer low-glucose events and lower glucose variability on flash glucose monitoring in adults with type 1 diabetes. Diabetologia 63(5):906–914. https://doi.org/10.1007/s00125-020-05099-3

64. Rickels MR, Evans-Molina C, Bahnson HT, et al (2020) High residual C-peptide likely contributes to glycemic control in type 1 diabetes. https://www.jci.org/articles/view/134057/ga. Accessed 1 Apr 2020

65. Gubitosi-Klug RA, Braffett BH, Hitt S, et al (2021) Residual **β** cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. J Clin Invest 131(3). https://doi.org/10.1172/JCI143011

66. European Medicines Agency (2018) Guideline on clinical investigation of medicinal products in the treatment or prevention of diabetes mellitus.

https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-clinical-investigation-medicinal-products-treatment-prevention-diabetes-mellitus_en.pdf. Accessed 2 Mar 2022

67. Shields BM, McDonald TJ, Oram R, et al (2018) C-Peptide Decline in Type 1 Diabetes Has Two Phases: An Initial Exponential Fall and a Subsequent Stable Phase. Diabetes Care 41(7):1486–1492. https://doi.org/10.2337/dc18-0465

68. Greenbaum CJ, Beam CA, Boulware D, et al (2012) Fall in C-Peptide During First 2 Years From Diagnosis. Diabetes 61(8):2066–2073. https://doi.org/10.2337/db11-1538

69. Davis AK, DuBose SN, Haller MJ, et al (2015) Prevalence of Detectable C-Peptide According to Age at Diagnosis and Duration of Type 1 Diabetes. Diabetes Care 38(3):476–481. https://doi.org/10.2337/dc14-1952

70. Ferrat LA, Vehik K, Sharp SA, et al (2020) A combined risk score enhances prediction of type 1 diabetes among susceptible children. Nat Med 26(8):1247–1255. https://doi.org/10.1038/s41591-020-0930-4

71. Herold KC, Hagopian W, Auger JA, et al (2002) Anti-CD3 Monoclonal Antibody in New-Onset Type 1 Diabetes Mellitus. New England Journal of Medicine 346(22):1692–1698. https://doi.org/10.1056/NEJMoa012864

72. Herold KC, Bundy BN, Long SA, et al (2019) An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N Engl J Med 381(7):603–613. https://doi.org/10.1056/NEJMoa1902226

73. Mortensen HB, Hougaard P, Swift P, et al (2009) New Definition for the Partial Remission Period in Children and Adolescents With Type 1 Diabetes. Diabetes Care 32(8):1384–1390. https://doi.org/10.2337/dc08-1987

74. Akirav E, Kushner JA, Herold KC (2008) Beta-cell mass and type 1 diabetes: going, going, gone? Diabetes 57(11):2883–2888. https://doi.org/10.2337/db07-1817

75. Schölin A, Berne C, Schvarcz E, Karlsson FA, Björk E (1999) Factors predicting clinical remission in adult patients with type 1 diabetes. J Intern Med 245(2):155–162. https://doi.org/10.1046/j.1365-2796.1999.00426.x

76. Nichols CG, Remedi MS (2012) The diabetic β-cell: hyperstimulated vs. hyperexcited. Diabetes Obes Metab 14 Suppl 3:129–135. https://doi.org/10.1111/j.1463-1326.2012.01655.x

77. Shyr ZA, Wang Z, York NW, Nichols CG, Remedi MS (2019) The role of membrane excitability in pancreatic β-cell glucotoxicity. Sci Rep 9(1):6952. https://doi.org/10.1038/s41598-019-43452-8

78. Krogvold L, Skog O, Sundström G, et al (2015) Function of Isolated Pancreatic Islets From Patients at Onset of Type 1 Diabetes: Insulin Secretion Can Be

Restored After Some Days in a Nondiabetogenic Environment In Vitro: Results From the DiViD Study. Diabetes 64(7):2506–2512. https://doi.org/10.2337/db14-1911

79. Ovalle F, Grimes T, Xu G, et al (2018) Verapamil and beta cell function in adults with recent-onset type 1 diabetes. Nat Med 24(8):1108–1112. https://doi.org/10.1038/s41591-018-0089-4

80. Xu G, Chen J, Jing G, Shalev A (2012) Preventing β-cell loss and diabetes with calcium channel blockers. Diabetes 61(4):848–856. https://doi.org/10.2337/db11-0955

81. Xu G, Grimes TD, Grayson TB, et al (2022) Exploratory study reveals far reaching systemic and cellular effects of verapamil treatment in subjects with type 1 diabetes. Nat Commun 13(1):1159. https://doi.org/10.1038/s41467-022-28826-3

CHAPTER 2: HISTOLOGICAL VALIDATION OF A TYPE 1 DIABETES CLINICAL DIAGNOSTIC MODEL FOR CLASSIFICATION OF DIABETES

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Contribution: I completed histological classification of nPOD samples in this study. This required learning immunostaining techniques and an understanding of the histology of type 1 diabetes. During the analysis misclassified cases, originally classified by nPOD, were identified and highlighted to nPOD. I was part of the design and completed all analysis of the data used in this study and was crucial in its interpretation. I created all figures and tables, wrote the first draft and oversaw all subsequent drafts.

All supplementary material for this chapter can be found in Appendix 3.1

Abstract

Aims: Misclassification of diabetes is common due to an overlap in the clinical features of type 1 and type 2 diabetes. Combined diagnostic models incorporating clinical and biomarker information have recently been developed that can aid classification, but they have not been validated using pancreatic pathology. We evaluated a clinical diagnostic model against histologically defined type 1 diabetes.

Methods: We classified cases from the Network for Pancreatic Organ donors with Diabetes (nPOD) biobank as type 1 ($n = 111$) or non-type 1 ($n = 42$) diabetes using histopathology. Type 1 diabetes was defined by lobular loss of insulin-containing islets along with multiple insulin-deficient islets. We assessed the discriminative performance of previously described type 1 diabetes diagnostic models, based on clinical features (age at diagnosis, BMI) and biomarker data [autoantibodies, type 1 diabetes genetic risk score (T1D-GRS)], and singular features for identifying type 1 diabetes by the area under the curve of the receiver operator characteristic (AUC-ROC).

Results: Diagnostic models validated well against histologically defined type 1 diabetes. The model combining clinical features, islet autoantibodies and T1D-GRS was strongly discriminative of type 1 diabetes, and performed better than clinical features alone (AUC-ROC 0.97 vs. 0.95; $P = 0.03$). Histological classification of type 1 diabetes was concordant with serum C-peptide [median < 17 pmol/l (limit of detection) vs. 1037 pmol/l in non-type 1 diabetes; $P < 0.0001$].

Conclusions: Our study provides robust histological evidence that a clinical diagnostic model, combining clinical features and biomarkers, could improve diabetes classification. Our study also provides reassurance that a C-peptide-based definition of type 1 diabetes is an appropriate surrogate outcome that can be used in large clinical studies where histological definition is impossible.

Introduction

Correct classification of diabetes type is crucial for appropriate management reduction of long-term complications. A fundamental difference between type 1 and type 2 diabetes is that the former is characterized by rapid progression to endogenous insulin deficiency due to autoimmune β-cell destruction. This difference forms the basis of differences in their treatment and management (1–3), however, this aetiopathological definition is difficult to apply in clinical practice.

Clinical features are predominately used for classification of diabetes type, with only age at diagnosis and body mass index (BMI) having evidence for clinical utility at onset (4). Rising obesity rates and type 2 diabetes in young people, and the incidence of type 1 diabetes throughout life (5–7) mean that misclassification of diabetes is common, occurring in 7–15% of cases (4). Although measurement of islet autoantibodies can assist classification, they are not perfectly discriminatory as some people with type 1 diabetes do not have islet autoantibodies and although relatively rare, autoantibodies positivity can occur in type 2 diabetes (8). Type 1 diabetes genetic risk scores (T1D-GRS) have recently been shown to assist in discriminating between type 1, type 2 and other forms of diabetes in research settings (9,10). Studies such as the SEARCH for Diabetes in Youth have developed classification criteria that are helpful in guiding diabetes classification at diagnosis and have informed international guidelines (11), but a difficulty with all of these studies is which standard to validate against, and that current guidelines are unable to provide simple criteria that will always ensure correct diagnosis (1–3).

We have shown previously that both clinical features (12) and biomarkers, such as autoantibodies and T1D-GRS, are most discriminative of diabetes type when combined and modelled continuously in diagnostic models that can be made widely available as an app or web calculator (4,9,13). These models were developed and validated on C-peptide-defined type 1 and type 2 diabetes, representing differences in endogenous insulin secretion between the two types. A pilot version of our recently published model is available online (**https://www.diabetesgenes.org/t1dt2d-prediction-model/**). Measurement of C-peptide allows robust diagnosis of type 1 diabetes in long-standing diabetes (> 3 years' duration) and closely relates to treatment requirements (14). A strength of using C-peptide as an outcome is that, irrespective of any assumptions about aetiology, progression to low C-peptide associates very strongly with insulin requirement.

An alternative 'gold standard' would be pancreatic histology, informed by internationally accepted histological criteria (15). Many other human diseases use histology as a gold standard, but this is not available in living people with diabetes due to the dangers of pancreatic biopsy (16). The Network for Pancreatic Organ donors with Diabetes (nPOD) is a unique collection of human pancreata from organ donors with and without diabetes, including those with type 1 and type 2 diabetes, as well as autoantibody-positive donors without diabetes (17). Using the nPOD biobank tissues and associated metadata, we sought to validate the performance of a previously developed clinical diagnostic model against histologically defined insulin deficiency defining type 1 diabetes. It has never been possible to validate diabetes classification against histology, and we aimed to take advantage of the nPOD biobank tissues and

associated metadata to define a histological outcome which we have used to support findings from clinical studies of living patients.

Research design and methods

We assessed the performance of our previously developed diagnostic model based on clinical features (age at diagnosis and BMI) and biomarker data [islet antigen 2 (IA2) and glutamic acid decarboxylase (GAD) antibody status and T1D-GRS] in a histologically defined cohort of type 1 and non-type 1 diabetes from the nPOD biobank. We compared model performance with the performance of individual clinical features and biomarkers.

Study cohort

We identified 221 nPOD diabetes cases with native pancreas available and complete nPOD online pathology. We excluded four cases with known monogenic forms (18) and 11 with secondary causes of diabetes, because the model was designed to discriminate type 1 diabetes from type 2 diabetes. We excluded 53 cases due to incomplete biomarker or clinical information (BMI, age at diagnosis, IA2 and GAD antibody status, T1D-GRS). We categorized diabetes and analysed diagnostic model performance in the remaining 153 cases (Fig. 1). Clinical history, histopathology notes and slide digitization were available through nPOD as described previously (17). A summary of characteristics for this cohort is shown in Table 1.

Table 1 - Characteristics of histologically defined cohort

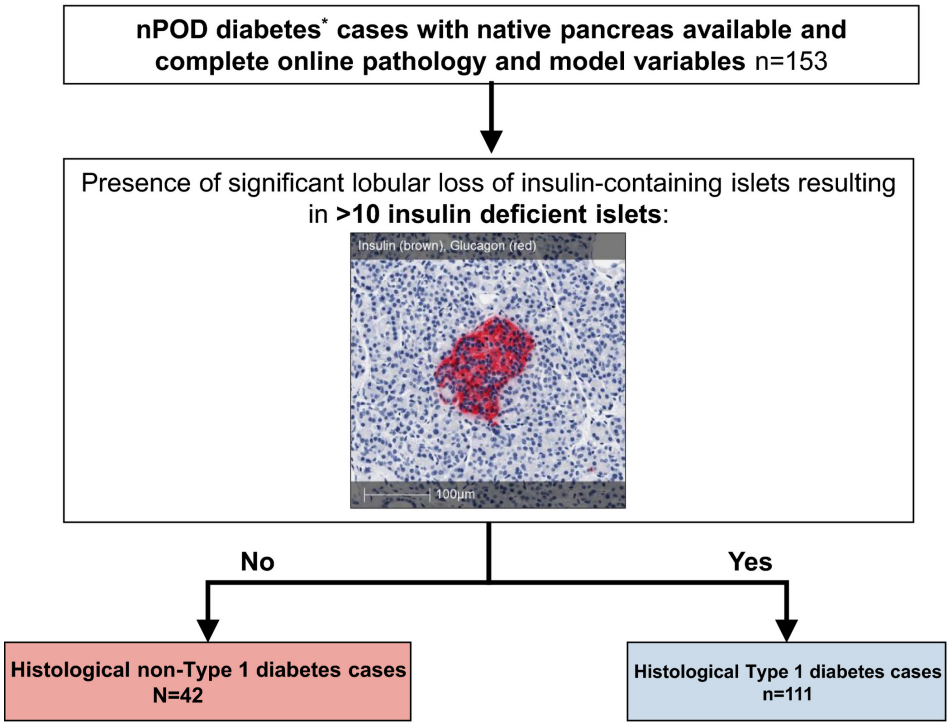
	Non-type 1 diabetes N=42	**Type 1 diabetes** N=111
BMI (kg/m^2), Median [25th;75th]	29.9 [27.5;34.3]	24.3 [22;26.6]
Age At Onset (yrs), Median [25th;75th]	37.5 [26.8;52.3]	11.5 [6.25;17.3]
Diabetes Duration (yrs), Median [25th;75th]	10 [3;1]	12 [6;23]
Age of Death (yrs), Median [25th;75th]	48.2 [40;59.3]	27.6 [19.5;37.1]
Sex, N (%):		
Female	20 (48%)	51 (46%)
Male	22 (52%)	60 (54%)
GRS, Median [25th;75th]	0.23 [0.21;0.26]	0.27 [0.25;0.29]
C-peptide (pmol/l), Median [25th;75th]	1037 [429;2072]	<17* [<17*;<17*]
Antibodies†, N (%):		
0	38 (90%)	56 (51%)
1	4 (10%)	32 (29%)
2	0 (0.0%)	10 (9%)
3	0 (0.0%)	13 (12%)
Race, N (%):		
African American	12 (29%)	11 (10%)

Asian	2 (4.8%)	0 (0.0%)
White European	20 (48%)	91 (82%)
Hispanic/Latino	8 (19%)	9 (8.1%)

BMI (Body Mass Index); GRS (Genetic Risk Score)

*Limit of detection

† Islet autoantibodies counted include GADA, IA-2A and ZnT8A. micro insulin autoantibody (mIAA) is not included in this count as it is not a reliable marker of autoimmunity in persons receiving exogenous insulin.



*Excluding known monogenic forms (18) (n=4) and secondary causes of diabetes (n=11).

Figure 1: Flow diagram of histological cohort identification from nPOD diabetes cases, excluding known monogenic forms and secondary causes of diabetes. All cases included had age at diagnosis, BMI, GADA and IA-2A status and T1D-GRS recorded.

Histological definition of type 1 diabetes and non-type 1 diabetes

We categorized diabetes as type 1 (n = 111) or non-type 1 (n = 42) using visualization of digitized slides via nPOD online pathology database and/or nPOD pancreas material held in Exeter, which were stained for the presence of insulin and/or glucagon using standard immunohistochemical approaches, as described previously (19,20) (Appendix 1.1). Slides were double-stained for insulin/glucagon, or serial sections were stained for insulin and glucagon respectively, where alignment of the two allowed identification of insulin-deficient islets. Histology was reviewed by two independent investigators in Exeter. A minimum of two slices per pancreas section (head, body or

tail) per donor was reviewed. We defined type 1 diabetes histologically by the lobular loss (Appendix 1.2) of insulin-containing islets with the presence of multiple (> 10) insulin-deficient islets (Appendix 1.3). Non-type 1 diabetes was defined as having no insulin-deficient islets across all viewed sections of the pancreas (15). Islets were defined as having > 10 insulin- and/or glucagon-positive cells. As there is no internationally agreed definition of type 2 diabetes, we did not attempt to positively classify type 2 diabetes on histology.

Autoantibody measurement

Autoantibody positivity status was measured by nPOD (Organ Procurement Organizations screening laboratories) using a modified rapid enzyme-linked immunosorbent assay (ELISA) kit (Kronus, Star, ID, USA) with internal calibration on donor serum. Autoantibody-positive samples were re-analysed with an ELISA kit (Kronus, Gainesville, FL, USA), and at the nPOD autoantibody core for GAD antibody, IA2 antibody, micro Insulin Autoantibody and Zinc Transporter 8 Autoantibody by radioligand-binding assay (Denver, CO, USA) (21) as previously described (22).

C-peptide measurement and DNA isolation

Sera were obtained during the donor-screening process and/or at donor organ recovery. Donor C-peptide was determined at the Northwest Lipid Metabolism and Diabetes Research Laboratories (S. Marcovina, University of Washington, Seattle, WA, USA) by a two-site immuno-enzymometeric assay using a Tosoh 2000 auto-analyser (TOSOH, Biosciences, Inc., San Francisco, CA, USA). C-peptide levels are reported in pmol/l with 1000 pmol/l = 3 ng/ml. We did not perform a primary analysis against C-peptide as an outcome because of the interaction between renal failure

(frequent in organ donors) and sample storage time (also less controlled in organ donors). DNA was extracted from frozen spleen where available (17) and analysed for type 1 diabetes genetic susceptibility on a UFDIchip Axiom genotyping array (ThermoFisher Scientific, Waltham, MA, USA) as described below.

T1D-GRS generation

The T1D-GRS was generated using 30 single nucleotide polymorphisms (SNPs) either genotyped directly ($n = 26$) or imputed ($n = 4$, imputation $r^2 > 0.90$) from a custom UFDIchip Axiom genotyping array from ThermoFisher Scientific. In total, the array covers 974 650 unique variants. UFDIchips were processed on an Affymetrix Gene Titan instrument with external sample handling on a BioMek FX dual arm robotic workstation. Genetic data underwent standard quality control procedures at the SNP, sample and plate levels using Axiom™ Analysis Suite 3.0 (ThermoFisher Scientific) set to default stringency thresholds as recommended. Next, discrepancies were assessed for genotyped Human Leukocyte Antigen (HLA) vs. imputed four-digit HLA (Axiom™ HLA Analysis software), as well as for genetic vs. reported sex. Samples that failed QC or were discordant were discarded. Finally, samples were imputed to the Human Reference Consortium (version r1.1) using Michigan Imputation Server (23). The T1D-GRS was calculated on the nPOD cohort as previously described (9,24) and indicates type 1 diabetes risk as a continuous variable.

Combined diagnostic model

We calculated the probability of type 1 diabetes on all 153 included cases using our previously developed diagnostic model (13) (Table **S1**). We assessed performance of

the model against histologically defined type 1 diabetes in the nPOD cohort. We tested the previously developed clinical diagnostic model in four combinations:

1. Clinical features only (age at diagnosis + BMI);
2. Clinical features + T1D-GRS;
3. Clinical features + IA2 antibody + GAD antibody;
4. Clinical features + IA2 antibody + GAD antibody + T1D-GRS.

The primary analysis was to assess the discriminative power and calibration of the diagnostic model in nPOD. We carried out a secondary sensitivity analysis in a white European ancestry subgroup of the cohort diagnosed at between 18 and 50 years of age, in line with the inclusion criteria of the original model development cohort (13) ($N = 31$, type 1 diabetes $n = 19$; Table **S2**).

All procedures were in accordance with federal guidelines for organ donation and approved by the University of Florida Institutional Review Board.

Statistical methods

We assessed discriminative performance by estimating the area under the curve of the receiver operator characteristic (AUC-ROC). We used the integrated discrimination improvement index (IDI) (25) to assess improvements in discrimination slopes when adding in additional features. Calibration was assessed by comparing observed proportions against predicted probabilities using calibration plots and the Brier score, where a score of 0 indicates that the model is completely accurate. We tested for statistical evidence of miscalibration using the Spiegelhalter *z*-test ($P < 0.05$ representing evidence of miscalibration). All AUC-ROC analysis was performed using

the pROC package in R and AUC estimated with DeLong's algorithm. We used a two-tailed DeLong comparison of ROC curves to test for significant improvement in discriminative power against the clinical features only model. Calibration analysis and statistics were performed using the Hmisc (Frank E. Harrell Jr, **https://cran.r-project.org/web/packages/Hmisc/index.html**) and rms (Frank E. Harrell Jr, https://cran.r-project.org/web/packages/rms/index.html) packages in R.

Results

Individual clinical features or biomarkers are discriminative of type 1 diabetes

Age at diagnosis, BMI, autoantibodies (GAD and IA2) and T1D-GRS were all strong individual discriminators of type 1 diabetes when modelled continuously (Fig. **2**). The discrimination varied from an AUC-ROC of 0.71 for autoantibodies to 0.93 for age at diagnosis. This highlights that no single feature in isolation predicted histology perfectly.

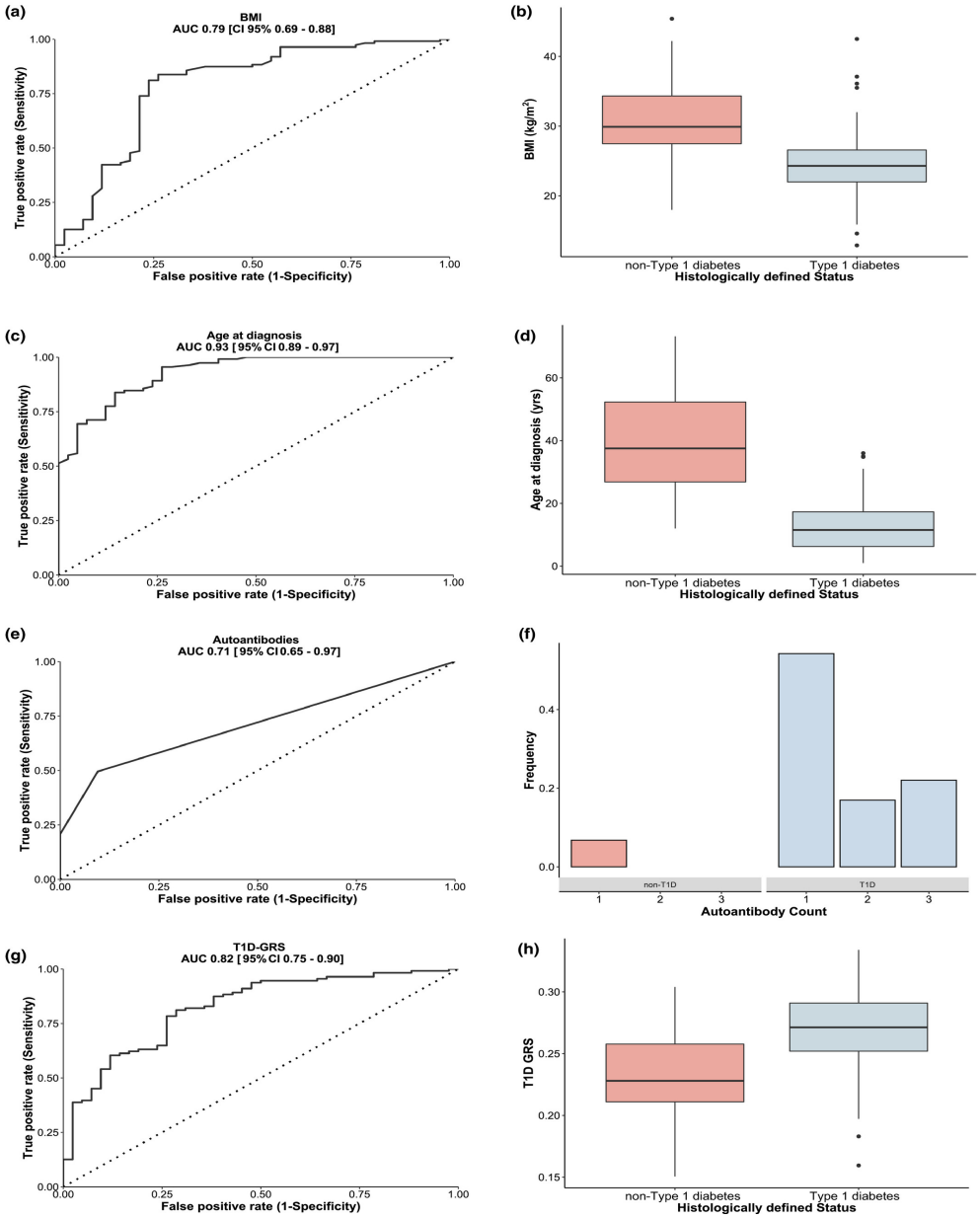


Figure 2: Comparative discrimination of type 1 diabetes and non-type 1 diabetes cases from the nPOD biobank. Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) statistics and distribution are shown for BMI (A-B), age at diagnosis (C-D), autoantibody count (E-F), and T1D-GRS (G-H).

The type 1 diabetes clinical diagnostic model validates well against a histological gold standard

All combinations of the type 1 diabetes clinical diagnostic model tested validated well against a histological definition of type 1 diabetes. Model combination 4, using clinical features continuously with the addition of IA2 and GAD antibody status, as well as T1D-GRS offers better discrimination than a model using clinical features only [AUC-ROC = 0.97, 95% confidence interval (CI) 0.95–1.00 vs. 0.95, 95% CI 0.91–0.98; *P* = 0.03] (Fig. 3). Addition of either IA2 and GAD antibody status or T1D-GRS improved the discrimination slope (IDI = 0.05, 95% CI 0.01–0.08; IDI = 0.07, 95% CI 0.02–0.12) (Fig. **S1**).

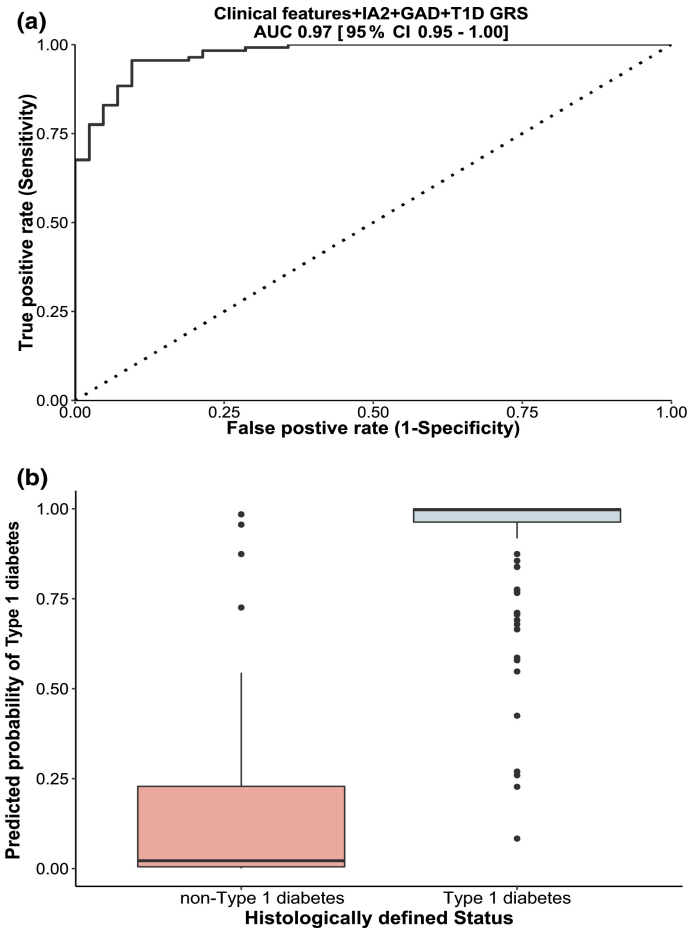


Figure 3: The discriminative ability of diagnostic model 4 combining BMI, age at diagnosis, autoantibody status and T1D-GRS to identify type 1 diabetes cases. Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) statistics (A). A boxplot of model 4 predicted probabilities of type 1 diabetes (B).

The type 1 diabetes clinical diagnostic model calibrates well

The mean overall probabilities of type 1 diabetes in the nPOD cohort for each combination of clinical diagnostic model tested closely reflected the proportion of observed type 1 diabetes cases in the study (111 of 153, 73%) (Fig. **S2**) indicating overall good calibration. We found no evidence of miscalibration across all model combinations as indicated by a low Brier score ($B = 0.06–0.08$) and non-significant Spiegelhalter z-statistics ($Z < 1.76$) (Table **S3**).

Sensitivity analysis in White European subgroup diagnosed in adulthood (18-50 years of age)

Results of a sensitivity analysis, using a white European ancestry subgroup diagnosed at between 18 and 50 years of age, showed equivalent discriminatory power for all variations of the type 1 diabetes clinical diagnostic model ($N = 31$, type 1 diabetes = 19, AUC-ROC > 0.84) (Fig. **S3**). A summary of characteristics for this subgroup is shown in Table **S2**.

Characteristics of cases with discordant model classification compared to histology

The distribution of probabilities of type 1 diabetes generated by model combination 4 are outlined in Fig. **3**(B). This highlights that a clinical diagnostic model will give an output that is a continuous distribution of probabilities, with a small number of type 1 diabetes cases still having low probability of type 1 diabetes and some without type 1 diabetes still identified as having a high probability. We examined the features of cases that had probabilities at the extreme distributions of model combination 4: two cases with histological type 1 diabetes who had a probability of type 1 diabetes < 25%; and three cases with histological non-type 1 diabetes who had a probability of type 1 diabetes > 75%. The characteristics of these cases are outlined in Table **S4**. Serum C-peptide levels in these cases matched the histological classification (two with histological type 1 diabetes had C-peptide < 30 pmol/l, and three with histological non-type 1 diabetes had C-peptide > 1000 pmol/l). Despite our concerns about C-peptide storage and sampling in organ donors, the observed serum C-peptide levels in type 1

vs. non-type 1 diabetes in the whole cohort was significantly different [median < 17 pmol/l (limit of detection) vs. median 1037 pmol/l; $P < 0.0001$) (Table **1**).

Discussion

This is the first study to evaluate a clinical diagnostic model against histological data. We have demonstrated that a model developed previously to classify type 1 diabetes defined by insulin deficiency, is discriminative of type 1 diabetes when using a histological outcome, not possible in routine clinical care. We found that using a combined model performed better than individual clinical features and biomarkers in discriminating type 1 diabetes and non-type 1 diabetes donor cohorts. Our study contributes to the evidence that diagnostic models combining clinical features with at least one clinical biomarker could assist classification of diabetes in clinical practice, and is already available as a beta-version online (**https://www.diabetesgenes.org/t1dt2d-prediction-model/**).

We previously demonstrated that a classification model, which integrated genetic testing combined with multiple continuous clinical variables, was effective at discriminating maturity onset diabetes of the young (MODY) from type 1 diabetes (12). An advantage in identification of MODY is that the outcome, a genetic mutation causing diabetes, is often definitive, but there is less clarity on a standard definition of type 1 diabetes and type 2 diabetes. In developing diagnostic models for diabetes classification, we used progression to insulin deficiency, as measured by serum C-peptide in longstanding diabetes (>3 years duration), as a surrogate marker of type 1 diabetes (9,13). We assumed that insulin deficiency, as defined by serum C-peptide <200 pmol/l >3 years post diagnosis, was an accurate surrogate of type 1 diabetes (14). This study provides evidence that this assumption is valid, by showing that our

model developed on clinical data to predict C-peptide deficiency near perfectly reflects histologically defined insulin deficiency (a robust but rarely used, definition of type 1 diabetes). This result is further reinforced by comparison of C-peptide in type 1 diabetes and non-type 1 diabetes groups which was non-overlapping (Table **1**). Clinically, one strength of a model trained on severe insulin deficiency as an outcome, is that prediction of severe insulin deficiency has a clear treatment implication, the requirement of exogenous implications.

We used histological criteria for type 1 diabetes based on work by Campbell-Thompson *et al* (15). Our criteria focus on insulin deficiency and the presence of insulin-deficient islets as a hallmark of type 1 diabetes that is present in all type 1 diabetes cases. The international consensus definition of type 1 diabetes histology describes various exclusive pathological features in the pancreas. These include the presence of insulitis that is always accompanied by pseudoatrophic islets devoid of β cells (15). However, the proportion of inflamed islets declines with time such that it is seen most readily in short duration type 1 diabetes donors (< 1 year) (26). As the majority of the nPOD donors had a longer duration of disease, and the presence of insulin-deficient islets is evidence of prior insulitis, we have used the detection of insulin-deficient islets as our key histopathological criterion to define type 1 diabetes in this study.

We focused on the positive histological definition of type 1 diabetes rather than defining other diabetes types by histology, and excluded cases that had a diabetes diagnosis of monogenic diabetes or secondary causes of diabetes. The clinical

features of our non-type 1 diabetes group suggest that this group are composed of predominantly type 2 diabetes, however, there is much less consensus on the histology of other diabetes types including type 2 diabetes and our original model was designed with features that discriminate type 1 diabetes from type 2 diabetes, such as age at diagnosis and BMI. In the future it may be possible to develop an approach that additionally classifies type 2 diabetes and less common diabetes types. This will require larger collections of non-type 1 diabetes cases (27) to allow accurate characterisation of type 2 diabetes pancreatic features.

A notable limitation of our study is that the current diagnostic model was developed using data derived primarily from White Europeans between the ages of 18 and 50 years. It is well documented that the incidence and prevalence of type 1 diabetes and type 2 diabetes varies across demographic subgroups (28,29). It is also well accepted that the prior prevalence of type 1 diabetes and type 2 diabetes varies with age, with type 2 diabetes more likely to be diagnosed at older ages and type 1 diabetes more likely to be diagnosed at younger ages. Our cohort included 27% non-white Europeans and diagnosis ages ranging from 1 to 73 years, yet despite this, the model showed good discrimination and calibrated well overall (Table **S3**). Due to the limitations of the sample size in our study, further validation evidence of the model performance is still required in non-white Europeans, in children, and in adults over the age of 50. It is likely that the model will need to be further refined for these age groups.

Our analysis used some features which are unchanged at diagnosis (age at diagnosis and T1D-GRS) but other features that were recorded at the time of organ donation

and could theoretically have been different at the time of diagnosis (autoantibody status, BMI). Despite this, both BMI and autoantibodies were still discriminative. We hypothesise that the discriminative power of these two variables will only be enhanced by ascertainment at the time of diagnosis, further improving model performance. It is possible that, at diagnosis, a model with only 3 variables (for example age at diagnosis, BMI and one of either autoantibodies or T1D-GRS) will perform as well as a 4 variable model. It will be impossible to test this in studies of organ donors but we are currently testing this in a prospective study assessing clinical features and biomarkers at the time of diagnosis (ClinicalTrials.gov identifier: NCT03737799). Our sample size limited our ability to test if a model using all 4 variables was significantly superior to a model using either T1D-GRS or autoantibodies (Fig. **S1**C–F). Existing work suggest a 3 variable model with either autoantibodies or T1D-GRS is as good as a model with 4 variables (9,13). It is likely that the relative benefits of autoantibody testing (a routinely available clinical test that is very discriminative if taken at diagnosis (8)) and T1D-GRS (time-independent and freely available in population biobanks (30)) will see them used differently depending on the setting and availability. We did not have some potentially relevant features at diagnosis, such as the presence of ketoacidosis and pre-diagnosis weight loss, but to date these are not shown to be reliable discriminators of type 1 diabetes (4). However, it will require larger studies with detailed information at diagnosis, across diverse ages and ethnicities, to fully elucidate the most accurate method and combination of features to classify diabetes at diagnosis.

Despite the modest sample size of our study, limited by the numbers of organ donors available worldwide, our study provides robust histological evidence that a model combining clinical features and biomarkers offers improved discrimination of type 1 diabetes, and that progression to C-peptide deficiency is an appropriate surrogate endpoint. Our study therefore provides further evidence for a clinical diagnostic model having utility to identify type 1 diabetes in clinical practice, and for C-peptide as a surrogate outcome for clinical studies where histological classification is not possible. Overall the study strengthens the evidence that a clinical diagnostic model may aid classification in clinical practice.

Acknowledgements

Duality of interest

R.A.O. holds a U.K. Medical Research Council Institutional Confidence in Concept grant to develop a 10-SNP biochip type 1 diabetes genetic test in collaboration with Randox. No other potential conflicts of interest relevant to this article were reported.

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The content and views expressed are those of the authors.

Author Contributions

R.A.O., B.M.S., S.J.R. and A.L.J.C. designed the study. D.J.P. and S.C. analysed genotyping array data and generated genetic risk scores. A.L.J.C. and S.J.R. performed histological analysis. A.L.J.C., S.J.R., C.S.F. and M.L.CT. and I.K. agreed on histological definition of type 1 diabetes. R.A.O. and B.M.S. reviewed and contributed to statistical analysis. A.L.J.C. performed analyses and wrote the first draft. All authors reviewed analysis and reviewed and contributed to final draft.

Ethics Statement

All procedures were in accordance with federal guidelines for organ donation and the University of Florida Institutional Review Board.

Prior Presentation

Parts of this study were presented in abstract form at the Network for Pancreatic Organ Donors Conference, Florida, U.S.A February 19-22 February 2019 and Diabetes UK Professional Conference, Liverpool, U.K 6-8 March 2019.

References

1. Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. Diabetes Care [Internet]. 2020 Jan 1 [cited 2020 Jan 30];43(Supplement 1):S14–31. Available from: https://care.diabetesjournals.org/content/43/Supplement_1/S14

2. Overview | Type 1 diabetes in adults: diagnosis and management | Guidance | NICE [Internet]. [cited 2019 Apr 9]. Available from: https://www.nice.org.uk/guidance/ng17

3. Overview | Type 2 diabetes in adults: management | Guidance | NICE [Internet]. [cited 2019 Apr 9]. Available from: https://www.nice.org.uk/guidance/ng28

4. Shields BM, Peters JL, Cooper C, Lowe J, Knight BA, Powell RJ, et al. Can clinical features be used to differentiate type 1 from type 2 diabetes? A systematic review of the literature. BMJ Open [Internet]. 2015 Nov 1 [cited 2018 Nov 23];5(11):e009088. Available from: https://bmjopen.bmj.com/content/5/11/e009088

5. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. Lancet Diabetes Endocrinol. 2018;6(2):122–9.

6. Thomas NJ, Lynam AL, Hill AV, Weedon MN, Shields BM, Oram RA, et al. Type 1 diabetes defined by severe insulin deficiency occurs after 30 years of age and is commonly treated as type 2 diabetes. Diabetologia [Internet]. 2019 Jul 1 [cited 2019 Aug 1];62(7):1167–72. Available from: https://doi.org/10.1007/s00125-019-4863-8

7. Rogers MAM, Kim C, Banerjee T, Lee JM. Fluctuations in the incidence of type 1 diabetes in the United States from 2001 to 2015: a longitudinal study. BMC Med [Internet]. 2017 Nov 8 [cited 2019 Aug 1];15(1):199. Available from: https://doi.org/10.1186/s12916-017-0958-6

8. Bingley PJ. Clinical Applications of Diabetes Antibody Testing. J Clin Endocrinol Metab [Internet]. 2010 Jan 1 [cited 2019 Jun 21];95(1):25–33. Available from: https://academic.oup.com/jcem/article/95/1/25/2835111

9. Oram RA, Patel K, Hill A, Shields B, McDonald TJ, Jones A, et al. A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. Diabetes Care. 2016 Mar 1;39(3):337–44.

10. Patel KA, Oram RA, Flanagan SE, Franco ED, Colclough K, Shepherd M, et al. Type 1 Diabetes Genetic Risk Score: A Novel Tool to Discriminate Monogenic and Type 1 Diabetes. Diabetes [Internet]. 2016 Jul 1 [cited 2018 Nov

23];65(7):2094–9. Available from: http://hw-f5-diabetes.highwire.org/content/65/7/2094

11. Dabelea D, Pihoker C, Talton JW, D'Agostino RB, Fujimoto W, Klingensmith GJ, et al. Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. Diabetes Care. 2011 Jul;34(7):1628–33.

12. Shields BM, McDonald TJ, Ellard S, Campbell MJ, Hyde CJ, Hattersley AT. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. In: Diabetologia. 2011.

13. Lynam A, McDonald T, Hill A, Dennis J, Oram R, Pearson E, et al. Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18–50 years. BMJ Open [Internet]. 2019 Sep 1 [cited 2019 Oct 1];9(9):e031586. Available from: https://bmjopen.bmj.com/content/9/9/e031586

14. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med [Internet]. 2013 Jul [cited 2019 Apr 8];30(7):803–17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3748788/

15. Campbell-Thompson ML, Atkinson MA, Butler AE, Chapman NM, Frisk G, Gianani R, et al. The diagnosis of insulitis in human type 1 diabetes. Diabetologia. 2013 Nov;56(11):2541–3.

16. Atkinson MA. Pancreatic biopsies in type 1 diabetes: revisiting the myth of Pandora's box. Diabetologia. 2014 Apr;57(4):656–9.

17. Campbell-Thompson M, Wasserfall C, Kaddis J, Albanese-O'Neill A, Staeva T, Nierras C, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): Developing a Tissue Biobank for Type 1 Diabetes. Diabetes Metab Res Rev [Internet]. 2012 Oct [cited 2019 May 21];28(7):608–17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3456997/

18. Sanyoura M, Jacobsen L, Carmody D, del Gaudio D, Alkorta-Aranburu G, Arndt K, et al. Pancreatic Histopathology of Human Monogenic Diabetes Due to Causal Variants in KCNJ11, HNF1A, GATA6, and LMNA. J Clin Endocrinol Metab [Internet]. 2017 Aug 16 [cited 2019 May 29];103(1):35–45. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5761488/

19. Krogvold L, Edwin B, Buanes T, Frisk G, Skog O, Anagandula M, et al. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. Diabetes. 2015 May;64(5):1682–7.

20. Campbell-Thompson ML, Heiple T, Montgomery E, Zhang L, Schneider L. Staining protocols for human pancreatic islets. J Vis Exp JoVE. 2012 May 23;(63):e4068.

21. Wasserfall C, Montgomery E, Yu L, Michels A, Gianani R, Pugliese A, et al. Validation of a rapid type 1 diabetes autoantibody screening assay for community-based screening of organ donors to identify subjects at increased risk for the disease. Clin Exp Immunol. 2016 Jul;185(1):33–41.

22. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab. 2010 Jul;95(7):3360–7.

23. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;48(10):1284–7.

24. Perry DJ, Wasserfall CH, Oram RA, Williams MD, Posgai A, Muir AB, et al. Application of a Genetic Risk Score to Racially Diverse Type 1 Diabetes Populations Demonstrates the Need for Diversity in Risk-Modeling. Sci Rep. 2018 Mar 14;8(1):4529.

25. Pencina MJ, D'Agostino RB, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008 Jan 30;27(2):157–72; discussion 207-212.

26. In't Veld P. Insulitis in human type 1 diabetes. Islets [Internet]. 2011 [cited 2019 Dec 21];3(4):131–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3154446/

27. Morgan NG, Richardson SJ. Fifty years of pancreatic islet pathology in human type 1 diabetes: insights gained and progress made. Diabetologia. 2018;61(12):2499–506.

28. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. N Engl J Med [Internet]. 2017 Apr 13 [cited 2020 Jan 6];376(15):1419–29. Available from: https://doi.org/10.1056/NEJMoa1610187

29. Golden SH, Brown A, Cauley JA, Chin MH, Gary-Webb TL, Kim C, et al. Health Disparities in Endocrine Disorders: Biological, Clinical, and Nonclinical Factors—An Endocrine Society Scientific Statement. J Clin Endocrinol Metab [Internet]. 2012 Sep [cited 2019 Nov 8];97(9):E1579–639. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3431576/

30. Locke JM, Latten MJ, Datta RY, Wood AR, Crockard MA, Lamont JV, et al. Methods for quick, accurate and cost-effective determination of the type 1 diabetes genetic risk score (T1D-GRS). Clin Chem Lab Med CCLM [Internet]. 2019 [cited 2020 Jan 21];0(0). Available from: https://www.degruyter.com/view/j/cclm.ahead-of-print/cclm-2019-0787/cclm-2019-0787.xml

CHAPTER 3: CIRCULATING C-PEPTIDE LEVELS IN LIVING CHILDREN AND YOUNG PEOPLE AND PANCREATIC BETA CELL LOSS IN PANCREAS DONORS ACROSS TYPE 1 DIABETES DISEASE DURATION

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Contribution: I completed some histological analyses of the nPOD and EADB samples, along with others. I was responsible for creating a database containing the relevant nPOD and EADB samples used in this study. I was part of the design and completed all analysis of the data associated with nPOD and EADB samples and was crucial in its interpretation. I created all figures and tables associated with nPOD and EADB data, wrote the first draft and oversaw all subsequent drafts.

All supplementary material for this chapter can be found in Appendix 3.2

Abstract

C-peptide declines in type 1 diabetes although many long-duration patients retain low, but detectable levels. Histological analyses confirm that beta cells can remain following type 1 diabetes onset. We explored the trends observed in C-peptide decline in UK Genetic Resource Investigating Diabetes (UK GRID) cohort (N=4,079), with beta cell loss in pancreas donors from the network for Pancreatic Organ donors with Diabetes (nPOD) biobank and the Exeter Archival Diabetes Biobank (EADB) (combined N=235), stratified by recently reported age at diagnosis endotypes (< 7, 7-12, ≥ 13 years) across increasing diabetes durations. The proportion of individuals with detectable C-peptide declined beyond the first year after diagnosis, but this was most marked in the youngest age group (< 1 year duration: age < 7 years: 18/20 (90%), 7-12 years: 107/110 (97%), ≥ 13 years: 58/61 (95%) versus. 1-5 years post diagnosis: < 7 years: 172/522 (33%), 7-12 years: 604/995 (61%), ≥ 13 years: 225/289 (78%)). A similar profile was observed in beta cell loss, with those diagnosed at younger ages experiencing more rapid loss of islets containing insulin-positive (insulin+) beta cells < 1 year post diagnosis: age < 7 years: 23/26 (88%), 7-12 years: 32/33 (97%), ≥ 13 years: 22/25 (88%) versus. 1-5 years post diagnosis: < 7 years: 1/12 (8.3%) ,7-12 years: 7/13 (54%), ≥ 13 years: 7/8 (88%)). These data should be considered in the planning and interpretation of intervention trials designed to promote beta cell retention and function.

Introduction

Circulating C-peptide, a marker of endogenous insulin secretion from pancreatic beta cells, is known to decline following a diagnosis of type 1 diabetes, but can persist for many years (1–8). It is frequently observed, however, that those diagnosed at the youngest ages have lower levels of C-peptide at diagnosis (2,3,5,6,9). Histological analyses of donor pancreata provide evidence for persistent immunoreactive insulin-positive (insulin+) beta cells; sometimes for many years after diagnosis (7,8,10,11). These findings challenge the dogma that all beta cells are destroyed at, or soon after, onset of type 1 diabetes. The centrepiece of many disease modifying intervention trials is to augment the survival of these residual beta cells, assessed via measures of preserved C-peptide secretion. However, currently there is little understanding of how C-peptide levels relate to absolute beta cell mass, as residual C-peptide alone cannot distinguish between loss of beta cell mass and reduced functionality. It is known that there are clear differences in disease progression between children and adults (3,5), but few studies have explored how this progression varies within children, particularly young children diagnosed under 7 years compared to those diagnosed around puberty (at or over 13 years) (6,11). In this study, we questioned whether trends of C-peptide decline observed in children and young people with type 1 diabetes from the UK Genetic Resource Investigating Diabetes (UK GRID) cohort were similar to trends of beta cell loss in pancreatic donors from the Network of Pancreatic Organ Donors (nPOD) and the Exeter Archival Diabetes Biobank (EADB), across wide ranges of age at diagnosis and durations.

Research design and methods

Three independent resources were used to assess C-peptide levels in the plasma and beta cell loss within the pancreas, respectively: 1) plasma samples from the Genetic Resource Investigating Diabetes (GRID) collection (12), and 2) type 1 diabetes pancreas samples from the Exeter Archival Diabetes Biobank (EADB) (11,13) and Network for Pancreatic Organ Donors (nPOD) biobank (14). We stratified subjects by age at diagnosis (< 7, 7-12, ≥ 13 years) (6), and grouped them by diabetes duration (< 1, 1-5, 5-10, ≥ 10 years).

We report the proportion of individuals from the GRID collection with detectable C-peptide (> 9 pmol/l) and distribution of these levels. We report the proportion of donors from the combined biobanks: EADB and nPOD, retaining islets containing insulin+ beta cells and distribution of beta cell area, expressed as insulin+ area with respect to the sum of insulin+ and glucagon+ area.

Study cohorts

We analysed 4,079 random non-fasting plasma C-peptide measurements from people with clinically-defined type 1 diabetes (on insulin from diagnosis) from the GRID collection, diagnosed ≤ 16 years (12), and 235 native pancreas samples recovered from people with type 1 diabetes, diagnosed < 18 years, from the nPOD biobank (n=111) and EADB (n=124) (Table 1, ESM Table 1). Histopathology notes and slide digitization were available through nPOD as previously described (14).

Table 1 Characteristics of UK GRID cohort and cohort from combined EADB and nPOD pancreas biobanks.

	UK GRID N=4079	**EADB** (n=124) **and nPOD** (n=111) N=235
Age (years), Median [25th;75th]	13 [10;16]	15 [10;22]
Diabetes Duration (years), Median [25th;75th]	5 [2;8]	5 [0.08;12]
Age at diagnosis (years), Median [25th;75th]	8 [4;11]	8 [4.9;13]
Sex, Male, N (%):	2149* (52.7%)	102 (43.4%)
C-peptide (pmol/L), Median [25th;75th]	<9‡ [<9‡, 31]	16.4†‡ [16.4†‡;16.4†‡]
Donors with islets containing insulin+ beta cells, N (%):		
None	-	115 (48.1%)
Present	-	120 (51.9%)

*missing data, n=2

† nPOD only, n=109

‡Limit of detection for UK GRID: 9 pmol/l , for nPOD 16.4 pmol/l

Histological analyses

We studied 235 type 1 diabetes (non-transplant) donors diagnosed <18 years from the combined nPOD and EADB biobanks with native pancreas available or complete nPOD online pathology and age-at-disease-diagnosis information. We examined pancreas sections using either digitised slides via nPOD online pathology database or pancreas material, which was stained for the presence of insulin and/or glucagon using standard immunohistochemical approaches (14). Sections were double-stained

for insulin/glucagon, or serial sections were stained for insulin and glucagon respectively, where alignment of the two stains allowed for identification of the insulin-negative (insulin-) islets. We defined type 1 diabetes histologically by the lobular loss of islets containing insulin+ beta cells with the presence of multiple (>10) insulin- islets in the section(s) studied. Insulin+ and insulin- islet counts were completed either by light microscopy or using high resolution digitised slides (via the Vectra® Polaris™ Automated Quantitative Pathology Imagining system (Akoya)) when appropriately stained sections were available (Appendix 1.4). In some (n=12) older samples from the EADB collection islet count information was collated from historical studies (6,11,13,15). For light microscopy the total number of islets was quantified using the glucagon-stained section with the number of islets with residual beta cells assessed using the serially stained insulin section. In such slides islets were defined as comprising of >10 insulin and/or glucagon positive cells. When digitised slides were available, islets were identified using the Random Forest Classifier Module of HALO V3.0 image analysis software (Indica Labs) and assessed for insulin positivity (Appendix 1.4). In slides assess by the HALO V3.0 image analysis software (Indica Labs), islets were defined as groups of endocrine cells covering an area of ≥ 1000 μm^2 (Appendix 1.3). We identified 120 donors with islets containing insulin+ beta cells from collated recent and historical analyses and expressed the proportions of donors with islets containing insulin+ beta cells across diabetes duration, stratified by age at diagnosis.

100 out of the 120 donors with islets containing insulin+ beta cells, had slides of appropriate quality available for digitization. The Random Forest Classifier Module (Version 3.2.1851.354) was applied to tissue double-stained for insulin/glucagon or

DenseNet AI V2 modules on serial single-stained tissue, within the Indica Labs HALO Image analysis platform (Version 3.2.1851.354), to identify insulin+ area and glucagon+ area (Appendix 1.4) for the sections per donor analysed across a total 38322 identified islets. We define insulin+ area relative to the sum of the insulin+ and glucagon+ area in the total section as: beta cell area with respect to total islet area. We make the assumption that insulin+ and glucagon+ area represents islet area. We report the distribution of beta cell area for these 100 donors across diabetes duration stratified by age at diagnosis.

In an additional sub-analysis, we selected 87 donors from nPOD that had been processed using the HALO Image analysis platform to identify beta cell area and who had random C-peptide measurements taken at the time of organ donation, without documented renal disease/failure or on dialysis, to assess if those with detectable C-peptide also had islets containing insulin+ beta cells.

C-peptide measurement

Plasma was obtained from 5,565 non-fasted blood samples from UK GRID patients, collected using the anticoagulant Acid Citrate Dextrose (ACD). Samples were excluded with C-peptide > 500 pmol/l (n=75), if time from blood draw to freeze > 72 hrs (n=1378) or data was incomplete (n=33). Samples were stored at -80°C. C-peptide was measured using the Diasorin Liaison C-peptide kit insert (product 316171, issued 24-02-2012) where the lower limit of the assay is 9 pmol/l, with a coefficient of variation of < 20%. C-peptide levels in nPOD donors were measured as described (14). Due to the variable limits of detection of C-peptide in nPOD, we chose the minimum limit of

detection (16.4 pmol/l) as the limit of detection for nPOD C-peptide in our sub-analysis, where detectable C-peptide is defined as ≥16.4 pmol/l. C-peptide levels are reported in pmol/l (1000 pmol/l = 3 ng/ml).

Results

Patterns of beta cell loss mirror patterns of C-peptide decline in children and young people

C-peptide levels were detectable in some individuals across all age at diagnosis groups and diabetes durations. This was least common in those diagnosed < 7 years (Figure 1a, ESM Table 2), (detectable C-peptide: age < 7 years: 254/1666 (15%), 7-12 years: 838/1887 (44%), ≥ 13 years: 325/526 (62%)). In all age at diagnosis groups, the number of individuals with detectable C-peptide declined beyond the first year after diagnosis, but this trend was most marked in those diagnosed at younger ages (detectable C-peptide < 1 year duration: age < 7 years: 18/20 (90%), 7-12 years: 107/110 (97%), ≥ 13 years: 58/61 (95%) versus. detectable C-peptide 1-5 years post diagnosis: < 7 years: 172/522 (33%),7-12 years: 604/995 (61%), ≥ 13 years: 225/289 (78%)).

Across all diabetes durations, similar trends were observed in the proportions of individuals retaining islets containing insulin+ beta cells in the sections of pancreas studied. Although present in all groups irrespective of age at diagnosis or disease duration, fewer individuals diagnosed < 7 years retained islets containing insulin+ beta cells (Figure 1b, ESM Table 2), (retaining islets containing insulin+ beta cells: < 7 years: 30/86 (35%), 7-12 years: 50/89 (56%), ≥ 13 years: 41/61 (67%)). There was a more precipitous drop off in the number of individuals retaining islets containing insulin+ beta cells post 1 year diagnosis in those diagnosed at younger ages compared to those diagnosed older (retaining islets containing insulin+ beta cells < 1

year post diagnosis: age < 7 years: 23/26 (88%), 7-12 years: 32/33 (97%), ≥ 13 years: 22/25 (88%) versus. retaining islets containing insulin+ beta cells 1-5 years post diagnosis: < 7 years: 1/12 (8.3%) ,7-12 years: 7/13 (54%), ≥ 13 years: 7/8 (88%)).

The absolute levels of detectable C-peptide declined in all age groups across all diabetes durations (Figure 1c), and this mirrored the decline in beta cell area (as fraction of insulin+ and glucagon+ area), across the groups (Figure 1d, ESM Table 3).

Children diagnosed < 7 years had lower absolute levels of C-peptide and less insulin+ beta cells close to diagnosis

C-peptide decreased in all age groups over time (ESM Table 4). In those with detectable levels, C-peptide was markedly lower soon after diagnosis in children diagnosed < 7 years compared to those diagnosed ≥ 13 years (< 1 year post diagnosis: median (IQR) < 7 years: 61.5 (45.4-110.8) pmol/l vs. ≥ 13 years: 199.5 (114.3-282.3) pmol/l; $p=1x10^{-4}$) (Figure 1c). Similarly, among children diagnosed < 7 years who retained islets containing insulin+ beta cells close to diagnosis, as judged by beta cell area, was lower (< 1 year post diagnosis: median (IQR) < 7 years: 15% [6.7%,27%] vs. ≥ 13 years: 31% [12%,42%] p=0.025 (Figure 1d, ESM Table 3). This compares with a median beta cell area of 70.4% [64.0%, 79.1%] in 44 <18y donors without diabetes (median age of donors 9 years [4.6, 12.9]).

Approximately 5% of children diagnosed < 7 years retained detectable C-peptide 10 years post diagnosis

Across all age groups, a proportion of children retained C-peptide > 10 years post diagnosis and a similar proportion retained islets containing insulin+ beta cells over this time (Figure 1a, ESM Table 2). In long duration disease (≥10 years), children originally diagnosed < 7 years were more likely to be insulin deficient at the time of organ donation than those who were older at diagnosis (detectable C-peptide ≥ 10 years post diagnosis: < 7 years: 21/489 (4.3%), 7-12 years: 25/249 (10%), ≥ 13 years: 12/107 (11%)), and they were also less likely to retain islets with insulin+ beta cells (retaining islets containing insulin+ beta cells ≥ 10 years post diagnosis: < 7 years: 3/34 (8.8 %), 7-12 years: 4/26 (15%), ≥ 13 years: 2/13 (15%)) (Figure 1b, ESM Table 2).

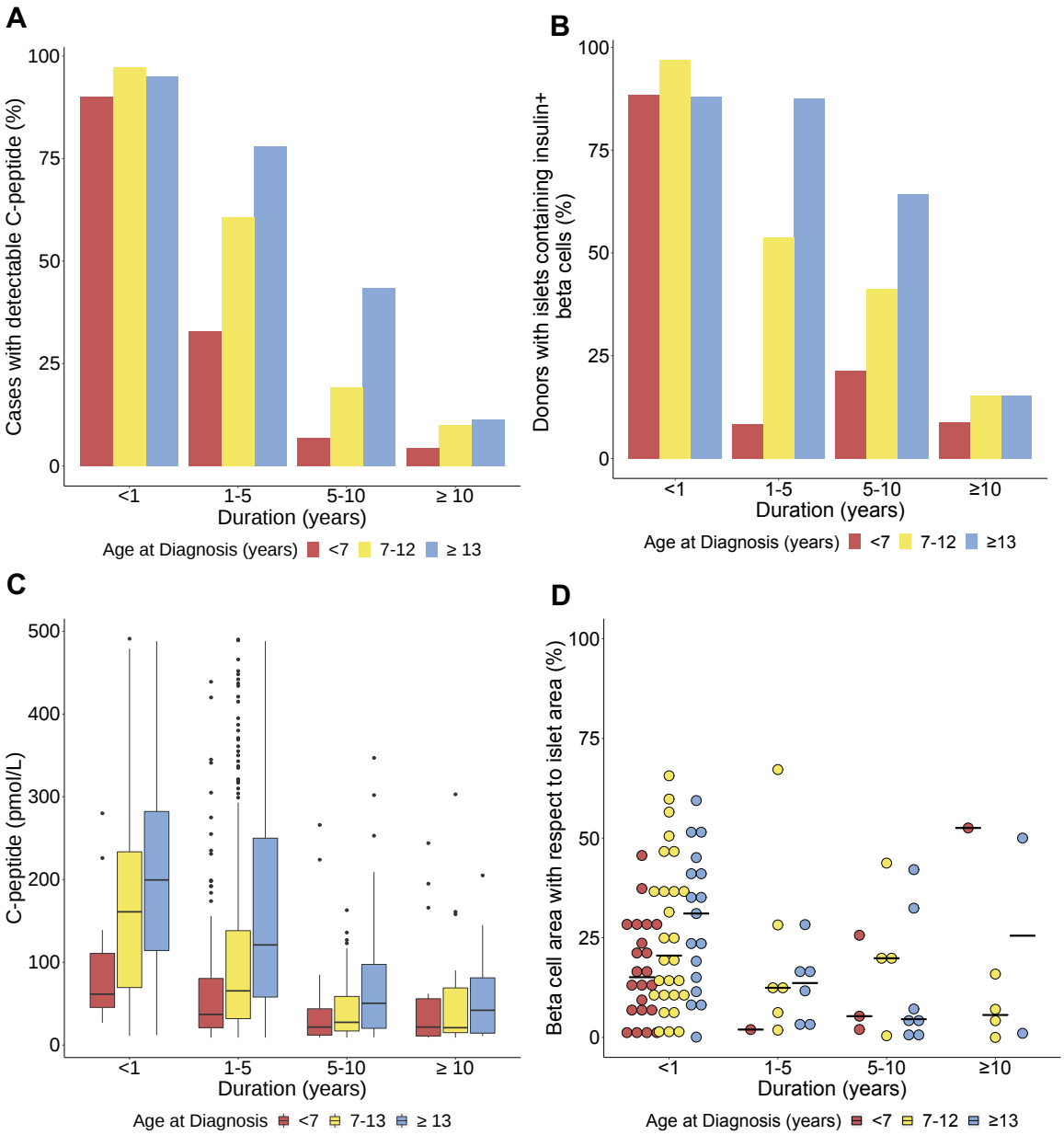

Figure 1 Comparison of proportions of individuals with detectable C-peptide (n=1417/4079) (a), proportions of donors retaining islets containing insulin+ beta cells (n=120/235) (b), absolute levels of detectable C-peptide (n=1417) (c) and within donor beta cell area, expressed as insulin+ area relative to the sum of the insulin+ and glucagon+ area (n=100) (d) stratified by age at diagnosis (< 7, 7-12, ≥ 13 years) and grouped by diabetes duration (<1, 1-5, 5-10, ≥ 10 years). Lines represent median and bars represent interquartile range. Proportions of donors with detectable C-peptide from UK GRID cohort (a) and donors with insulin+ beta cells from nPOD and EADB

(b) are outlined in more detail in ESM Table 2. A summary of donors with available beta cell area (d) is outlined in ESM Table 3.

In nPOD pancreas donors with detectable C-peptide, the majority also had presence of insulin+ islets

Among a subset of nPOD donors (n=87), 17 had detectable C-peptide with 13 of these donors (76%) having presence of insulin+ beta cells, as determined by a > 0% beta cell area (ESM Table 5). There was a significant difference in presence or absence of insulin+ islets between the detectable/undetectable C-peptide groups (81.6% agreement, $p=1.5x10^{-6}$). The characteristics of 4 donors with detectable C-peptide but with no insulin+ beta cells in sections analysed are outlined in ESM Table 6. In these 4 donors, the C-peptide level was low (<100 pmol/l) and in 2 of the donors the histopathology notes state that, in some curated sections, islets containing insulin+ beta cells were seen but were rare (ESM Table 6).

Discussion

We report that trends in C-peptide decline in living children and young people with type 1 diabetes are similar to the trends of loss of islets containing insulin+ beta cells within sections of donor pancreata, across all ages and disease durations. Our results support the proposition that C-peptide levels are a reliable, inexpensive and practical marker of retention of islets containing insulin+ beta cells in children and young adults with type 1 diabetes. Our results are consistent with those of other studies showing higher C-peptide levels in people diagnosed at older ages, but decline over time (2,3,5,9). Our study also supports the findings of Aida et al who demonstrated a significant correlation between beta cell volume and fasting serum C-peptide levels in Japanese patients with adult-onset type 1 diabetes (16). Our study is the first to provide a comparison of pancreatic histology with an independent clinical cohort, examining patterns of C-peptide loss according to age at diagnosis and duration in children with type 1 diabetes. Our study is also the largest to assess such disease progression trends in very young children (< 7 years). We demonstrate that, when compared with those who are older at diagnosis, children diagnosed < 7 years progress more rapidly towards total C-peptide loss and have minimal beta cell retention.

These data confirm that trialling a safe immunotherapy close to diagnosis to inhibit or halt the autoimmune destruction, as in recent clinical trials (17), is worthwhile to preserve pancreatic mass. The rapid depletion of C-peptide and beta cells in children diagnosed < 7 years, when comparing < 1 years and 1-5 years duration, emphasizes

that early intervention close to (or before) diagnosis may be most time critical in those progressing to disease in very early life. Our results highlight that among children there are differences in progression which should be considered in the planning and interpretation of intervention trials designed to promote beta cell retention and function.

We find that a small proportion of children retain some residual beta cells over > 10 years duration and a similar proportion retain C-peptide over this period. These proportions do not change markedly between disease durations of 5 or 10 years, in keeping with the concept that there are two phases of C-peptide decline: a rapid fall in the first 7 years after diagnosis, followed by a more stable phase (2). Our results are likely to be an underestimate given a higher limit of detection of C-peptide (9 pmol/l), compared to contemporary assays (2). It must be noted that the UK GRID cohort, included only those individuals with type 1 diabetes, and as such in this study we do not have access to a non-type 1 diabetes population for comparison of C-peptide levels. However, it is well established that levels of residual C-peptide in long duration type 1 diabetes are low and detectable using ultrasensitive assays (18,19).

We acknowledge that in histological analyses we have not been able to assess beta cell area for all 120 donors with islets containing insulin+ beta cells, calculating this for 100 such samples. Of the 20 samples we were unable to calculate beta cell area in, 12 were derived from the EADB biobank; a 50-year-old archival biobank mainly comprising of non-systematically collected autopsy samples from younger children very close to diagnosis. We were unable to include

these archival sections due to deterioration of glass slides/ staining intensity which impacted on scan quality, and the rarity of material available from these donors precluded re-staining. In addition, we must emphasise that the standard error around the proportion estimates in the histological analyses are large, as influenced by the sample numbers. We also acknowledge that there is little information on the anatomical location of the sampled pancreas in the histological analyses of the EADB donors. However, as sampling was random across the 235 donors, we think it is very unlikely that systematic sampling bias might explain our observations.

A further limitation of this study is its cross-sectional design and the dissociated biobanks used. Extensive, within donor, analysis is difficult in this setting, since there are no large systematic studies of C-peptide in clinical type 1 diabetes cohorts in whom post-death pancreas samples are available. Despite this, we are able to demonstrate that 81% (17/21) of donors from the nPOD biobank with detectable C-peptide also had islets containing insulin+ beta cells in the sections studied. 4 donors had detectable C-peptide and no islets containing insulin+ beta cells in the sections we were able to assess. It is reasonable to assume that, due to the nature of sampling, such islets could be present elsewhere in the pancreas. This is illustrated in 2 of the 4 donors studied, since the histopathology reports held by nPOD describe rare islets containing insulin+ beta cells in the sections they curate. In addition, it should be noted that C-peptide levels in nPOD organ donors may be influenced by end-of-life circumstances and must therefore be interpreted with caution. In donors with undetectable C-peptide but who retain insulin+ beta cells, acute glucotoxicity (20) and sample degradation may have contributed to false negative C-peptide results. Additionally, we accept that

limited clinical data were available and that, in particular, no information was accessible on rates of diabetic ketoacidosis in the UK GRID cohort, which is known to be an independent predictor of C-peptide decline (20).

Despite these caveats, our data suggest that progressive loss of beta cells is the main contributory factor to the decline in endogenous insulin secretion observed in children and young people diagnosed with type 1 diabetes. Our results add weight to the proposal that intervention trials should be powered separately for each age at diagnosis group and highlight that consideration of age at diagnosis is very important in the interpretation of outcomes. Interventions that delay diagnosis in “at-risk” individuals are likely to improve clinical outcomes by promoting the retention of beta cells and maintaining a higher C-peptide secretion rate.

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Author Contributions

REJB, SJR, ALJC and JRJI designed the study. REJB and JRJI performed analysis of C-peptide data. CSF, BCW, PL, ALJC, BH, TW, DP, MP, LAR and SJR performed histological assessments and analysis. ALJC performed analysis of histological data and wrote the first draft. RAO and NGM helped with data interpretation and revision of manuscript. JAT, LSW and DBD provided access to the data from the GRID study and contributed to scientific discussion. All authors reviewed analysis and reviewed and contributed to final draft. REJB and SJR are responsible for the integrity of the work as a whole.

Disclosure Statement

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Ethics Statement

All procedures in nPOD were in accordance with federal guidelines for organ donation and the University of Florida Institutional Review Board. All EADB samples were used with ethical permission from the West of Scotland Research Ethics Committee ((ref: 20/WS/0074; IRAS project ID: 283620). Plasma samples were obtained from the

Genetics Resource Investigating Diabetes or GRID Study (Rec Reference 00/5/44) which encompassed the UK Nephropathy Family Study (Rec Reference 00/5/65).

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The content and views expressed are those of the authors.

Prior Presentation

Parts of this work were presented at the European Association for the Study of Diabetes conference (online) 21st-25th September 2020 and the Network for Pancreatic Organ Donors Conference (online) 22nd-24th February 2021.

Data Availability

Further information about the data is available from the corresponding author upon request.

References

1. Dabelea D, Mayer-Davis EJ, Andrews JS, Dolan LM, Pihoker C, Hamman RF, et al. Clinical evolution of beta cell function in youth with diabetes: the SEARCH for Diabetes in Youth study. Diabetologia [Internet]. 2012 Dec [cited 2020 Mar 9];55(12):3359–68. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4492685/

2. Shields BM, McDonald TJ, Oram R, Hill A, Hudson M, Leete P, et al. C-Peptide Decline in Type 1 Diabetes Has Two Phases: An Initial Exponential Fall and a Subsequent Stable Phase. Diabetes Care. 2018;41(7):1486–92.

3. Davis AK, DuBose SN, Haller MJ, Miller KM, DiMeglio LA, Bethin KE, et al. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care. 2015 Mar;38(3):476–81.

4. Hao W, Gitelman S, DiMeglio LA, Boulware D, Greenbaum CJ. Fall in C-Peptide During First 4 Years From Diagnosis of Type 1 Diabetes: Variable Relation to Age, HbA1c, and Insulin Dose. Diabetes Care [Internet]. 2016 Oct [cited 2018 Sep 7];39(10):1664–70. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5033079/

5. Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, et al. Fall in C-Peptide During First 2 Years From Diagnosis: Evidence of at Least Two Distinct Phases From Composite Type 1 Diabetes TrialNet Data. Diabetes [Internet]. 2012 Aug 1 [cited 2020 Mar 9];61(8):2066–73. Available from: https://diabetes.diabetesjournals.org/content/61/8/2066

6. Leete P, Oram RA, McDonald TJ, Shields BM, Ziller C, Roep BO, et al. Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia [Internet]. 2020 Jun 1 [cited 2020 Oct 5];63(6):1258–67. Available from: https://doi.org/10.1007/s00125-020-05115-6

7. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, et al. Residual insulin production and pancreatic ß-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes. 2010 Nov;59(11):2846–53.

8. Yu MG, Keenan HA, Shah HS, Frodsham SG, Pober D, He Z, et al. Residual **β** cell function and monogenic variants in long-duration type 1 diabetes patients. J Clin Invest [Internet]. 2019 Aug 1 [cited 2022 Mar 21];129(8):3252–63. Available from: https://www.jci.org/articles/view/127397

9. Besser REJ, Shields BM, Casas R, Hattersley AT, Ludvigsson J. Lessons From the Mixed-Meal Tolerance Test. Diabetes Care [Internet]. 2013 Feb [cited 2020 Jun 17];36(2):195–201. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3554273/

10. Rodriguez-Calvo T, Richardson SJ, Pugliese A. Pancreas Pathology During the Natural History of Type 1 Diabetes. Curr Diab Rep [Internet]. 2018 Oct 6 [cited 2020 Feb 24];18(11):124. Available from: https://doi.org/10.1007/s11892-018-1084-3

11. Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, et al. Differential Insulitic Profiles Determine the Extent of β-Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes [Internet]. 2016 May 1 [cited 2018 Sep 7];65(5):1362–9. Available from: http://diabetes.diabetesjournals.org/content/65/5/1362

12. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009 Jun;41(6):703–7.

13. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS. The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia. 1986 May;29(5):267–74.

14. Campbell-Thompson M, Wasserfall C, Kaddis J, Albanese-O'Neill A, Staeva T, Nierras C, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): Developing a Tissue Biobank for Type 1 Diabetes. Diabetes Metab Res Rev [Internet]. 2012 Oct [cited 2019 May 21];28(7):608–17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3456997/

15. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol [Internet]. 2009 [cited 2021 Sep 27];155(2):173–81. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2249.2008.03860.x

16. Aida K, Fukui T, Jimbo E, Yagihashi S, Shimada A, Oikawa Y, et al. Distinct Inflammatory Changes of the Pancreas of Slowly Progressive Insulin-dependent (Type 1) Diabetes. Pancreas [Internet]. 2018 Oct [cited 2021 Mar 10];47(9):1101–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6143218/

17. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, et al. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N Engl J Med. 2019 Aug 15;381(7):603–13.

18. Oram RA, Jones AG, Besser REJ, Knight BA, Shields BM, Brown RJ, et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia. 2014 Jan;57(1):187–91.

19. Oram RA, McDonald TJ, Shields BM, Hudson MM, Shepherd MH, Hammersley S, et al. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. Diabetes Care. 2015 Feb;38(2):323–8.

20. Komulainen J, Lounamaa R, Knip M, Kaprio EA, Akerblom HK. Ketoacidosis at the diagnosis of type 1 (insulin dependent) diabetes mellitus is related to poor

residual beta cell function. Childhood Diabetes in Finland Study Group. Arch Dis Child [Internet]. 1996 Nov 1 [cited 2021 Mar 10];75(5):410–5. Available from: https://adc.bmj.com/content/75/5/410

CHAPTER 4: MEASUREMENT OF PEAK C-PEPTIDE AT DIAGNOSIS INFORMS GLYCEMIC CONTROL BUT NOT HYPOGLYCEMIA IN ADULTS WITH TYPE 1 DIABETES

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Contribution: I was part of the design and completed the analysis of the data used in this study and was crucial in its interpretation. I created all figures and tables, wrote the first draft and oversaw all subsequent drafts.

Abstract

Context: High-residual C-peptide in longer-duration type 1 diabetes (T1D) is associated with fewer hypoglycemic events and reduced glycemic variability. Little is known about the impact of C-peptide close to diagnosis.

Objective: Using continuous glucose monitoring (CGM) data from a study of newly diagnosed adults with T1D, we aimed to explore if variation in C-peptide close to diagnosis influenced glycemic variability and risk of hypoglycemia.

Methods: We studied newly diagnosed adults with T1D who wore a Dexcom G4 CGM for 7 days as part of the Exercise in Type 1 Diabetes (EXTOD) study. We examined the relationship between peak stimulated C-peptide and glycemic metrics of variability and hypoglycemia for 36 CGM traces from 23 participants.

Results: For every 100 pmol/L-increase in peak C-peptide, the percentage of time spent in the range 3.9 to 10 mmol/L increased by 2.4% (95% CI, 0.5-4.3), $P = .01$) with a reduction in time spent at level 1 hyperglycemia (> 10 mmol/L) and level 2 hyperglycemia (> 13.9 mmol/L) by 2.6% (95% CI, –4.9 to –0.4, $P = .02$) and 1.3% (95% CI, –2.7 to –0.006, $P = .04$), respectively. Glucose levels were on average lower by 0.19 mmol/L (95% CI, –0.4 to 0.02, $P = .06$) and SD reduced by 0.14 (95% CI, –0.3 to –0.02, $P = .02$). Hypoglycemia was not common in this group and no association was observed between time spent in hypoglycemia ($P = .97$) or hypoglycemic risk (P

= .72). There was no association between peak C-peptide and insulin dose–adjusted glycated hemoglobin A_{1c} (P = .45).

Conclusion: C-peptide is associated with time spent in the normal glucose range and with less hyperglycemia, but not risk of hypoglycemia in newly diagnosed people with T1D.

Introduction

Residual endogenous insulin production, as measured by serum C-peptide, is invariably present at the time of diagnosis with type 1 diabetes (T1D) (1). These C-peptide levels are variable and fall exponentially in the first 7 years (2–5), with those diagnosed in adulthood more likely to retain significant levels of C-peptide years post diagnosis (1,6,7).. Evidence originally from the Diabetes Control and Complications Trial (8–10) and more recent studies (11–15) indicates that persistent detectable C-peptide is associated with reduced frequency and severity of self-reported hypoglycemia and fewer long-term microvascular complications. This has led to the adoption of mixed-meal stimulated C-peptide as a primary outcome measure of intervention trials that prevent or delay β-cell destruction (16).

Recently, increased use of flash glucose monitoring and continuous glucose monitoring (CGM) have highlighted the impact of persistent C-peptide on glycemic variability and hypoglycemia (12,14,15,17–19). Most data have been derived from studies of adults with long duration T1D or post islet transplantation where C-peptide persistence is associated with lower glycated hemoglobin A_{1c} (HbA_{1c}) and/or insulin dose, fewer low-glucose events, decreased variation, and more time spent in range (3.9-10 mmol/L) (11,12,14,15,19). Less is known about the impact of C-peptide close to diagnosis. This is important because this has the potential to inform the most effective approach to supporting the newly diagnosed patient, and to identify early benefits of C-peptide preservation. A single study in newly diagnosed children demonstrated that the level of preserved peak C-peptide correlates with more time in

the range of 3.9 to 7.8 mmol/L and less variability (18). This study found no association between peak C-peptide and hypoglycemia (detected by CGM) in contrast to a study of adults with long-duration T1D, in which such an association was demonstrated (12). No studies have looked at the impact of C-peptide on glucose control as measured by CGM in adults newly diagnosed with T1D.

In the present study we aimed to use CGM data from adults with recent-onset T1D to assess and describe the impact of variation in endogenous insulin secretion close to diagnosis on glycemic variability and hypoglycemia.

Methods

We performed a secondary analysis of peak (90-minute) mixed-meal tolerance test (MMTT) C-peptide and glycemic metrics of variability and hypoglycemia from a Dexcom G4 CGM measured as part of the Exercise in Type 1 Diabetes (EXTOD) Study (ISRCTN91388505) (20). EXTOD was a pilot study undertaken to explore whether exercise can preserve β-cell function in adults newly diagnosed with T1D. It aimed to assess uptake, intervention adherence, dropout rates, and the rate of loss of β-cell function in a usual care group and exercise intervention group over 12 months (20,21). The EXTOD study was approved by the Birmingham East, North and Solihull Research Ethics Committee (No. 0/H1206/4), UK. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Study Cohort

Participants were recruited between November 2011 and January 2014, from 19 UK National Health Service (NHS) hospitals. Eligible participants had a clinical diagnosis of T1D, were older than 16 years at diagnosis, and were self-administering their insulin as part of a multiple-dose (basal/bolus) injection regimen. Participants included in the EXTOD pilot study were adults aged 18 to 60 years, diagnosed with T1D for less than 3 months, had C-peptide greater than 200 pmol/L at 90 minutes following meal stimulation, had controlled blood pressure, were not pregnant or planning pregnancy, and were able to increase exercise levels and not on therapy that affect heart rate (β-blocker, calcium channel antagonist).

A total of 507 adults with new-onset T1D were identified; of these 214 were assessed for eligibility for the EXTOD pilot study. No participants were excluded from taking part because of low C-peptide. Eighty-six were eligible for face-to-face screening; of these, 15 participants were recruited into a distinct but linked study exploring barriers to exercise in newly diagnosed T1D, and 58 participants were randomly assigned to control (usual care) or intervention (exercise consultation every month + usual care) in a 1:1 ratio for 12 months (Fig. 1).

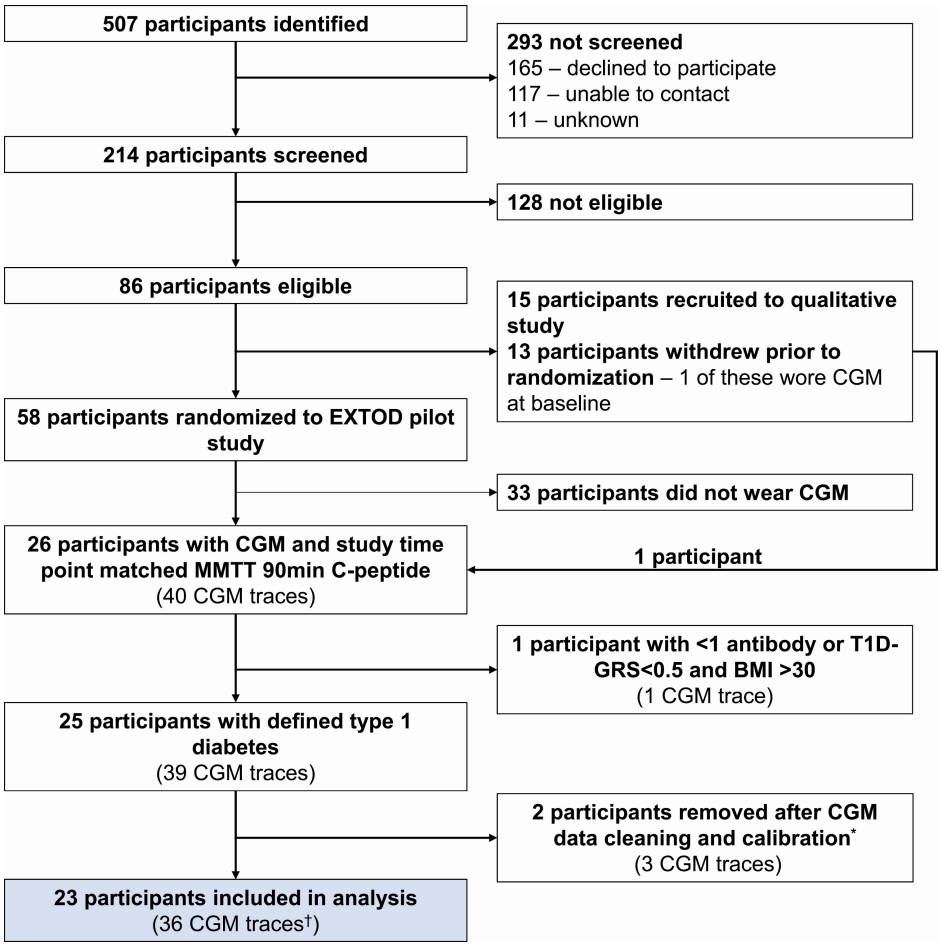

Figure 1. Flow of participants included in this secondary analysis and breakdown of number of corresponding continuous glucose monitoring (CGM) traces analyzed by study time point. *Calibration is dependent on the sensor used in this study (Dexcom G4). Calibration excluded 1) whole traces if 2 blood glucose calibrations were not

completed at the start of sensor wearing, 2) a day of wear if the mean absolute relative difference of the sensor glucose and blood glucose calibration that day is greater than 20% or if fewer than 2 blood glucose calibrations were completed that day. †Breakdown of CGM traces by study time point: baseline n = 21 CGM traces, 6 months n = 9 CGM traces, 12 months n = 6 CGM traces.

In this study we performed a secondary analysis of peak MMTT C-peptide and glycemic metrics of variability and hypoglycemia from 26 eligible participants of the EXTOD study who had consented to wearing a CGM at any study time point. We included 23 participants in this secondary analysis with defined T1D by 1 or more autoantibodies or a T1D genetic risk score (T1D-GRS) greater than the 50th percentile for T1D, and a body mass index (BMI) of less than 30, a validated CGM trace, and a 90-minute MMTT C-peptide matched to the study time point of CGM wear. A total of 36 CGM traces (4101 hours) were analyzed from 23 participants (see Fig. 1).

Procedures

β-Cell function was assessed at baseline (pre randomization), and at 6 and 12 months post randomization using a 240-mL Fortisip MMTT with blood taken for C-peptide at –10, 0, 15, 30, 60, 90, and 120 minutes. Blood was immediately centrifuged and the plasma stored at –80 °C until analysis. Patients at each study visit had an option to wear a Dexcom G4 CGM with the aim of assessing the feasibility of using this as an outcome. The CGM was worn blinded with participants using usual care to monitor their blood glucose during the study. C-peptide was measured using a direct electrochemiluminescence immunoassay at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust as previously described (22). The limit of the C-peptide assay is 3.3 pmol/L. Antibodies were

measured at the Research Laboratories of the School of Clinical Sciences, University of Bristol (Southmead Hospital, Bristol, UK). Insulin doses were used in calculation of the insulin dose–adjusted HbA_{1c} ($IDAA_{1c}$) as previously described (23). We generated a T1D-GRS using a KASP genotyping assay (LGC Genomics) of 10 single-nucleotide variations as previously described (24).

CGM Processing

All CGM processing and analysis were performed in R statistical software version 3.6.1 (Foundation for Statistical Computing) (Appendix 2). Forty CGM traces from 26 participants went through processing. The CGM was expected to be worn for a minimum of 7 days, with the majority of the participants meeting this goal, with data cut off at 8 days if participants exceeded 7 days. As part of the processing, we calibrated CGM traces against an updated self-monitoring of blood glucose value every 12 hours as required by the Dexcom G4 sensor. Calibration excluded 1) whole traces if 2 blood glucose calibrations were not completed at the start of sensor wear, which are required to start the Dexcom G4 sensor (n = 1); 2) a whole day of wear from traces if the mean absolute relative difference (MARD) of blood glucose calibration reading and sensor glucose reading was greater than 20%, or if fewer than 2 blood glucoses calibrations were completed that particular day. The majority of traces after calibration had more than 95% of data remaining. Traces from participants were excluded if 12 hours or less of data remained after calibration (n = 2). We generated CGM-derived metrics of glycemic variability and hypoglycemia in accordance with, and using definitions of hypoglycemic/hyperglycemic episodes from, the International Consensus on Use of Continuous Glucose Monitoring (25) (Appendix 2).. We

validated the CGM processing and analysis against results from manual processing completed by 2 individuals.

Statistical analysis

Statistical analysis was performed in R statistical software version 3.6.1 (Foundation for Statistical Computing) using the nlme and afex packages. We assessed the association between MMTT C-peptide level and the consensus glycemic metrics of variability and hypoglycemia using repeated-measures, mixed-effects models, with glycemic metric as the outcome, C-peptide level (as picomole per liter [pmol/L]) as the predictor, with patient identification as the random effect. We report the modeling coefficients for 100-pmol/L change in C-peptide for clinical interpretation. Residual plots were examined for normality to ensure model assumptions were met. Significance was tested at the level of .05. Many clinical variables were not normally distributed, so data are presented as median and interquartile range (IQR).

Results

Participants and Characteristics

Twenty-one participants' first point of CGM wear was at baseline, and 2 participants' first point of CGM wear was at 6 months post trial randomization. Twenty-one participants wore a CGM at baseline, 10 at 6 months, and 6 at 12 months post trial randomization, totaling 36 CGM traces with a study time point–matched MMTT 90-minute C-peptide (Fig. 1). One participant was not randomly assigned in the primary EXTOD study. The characteristics of these participants at first wear of CGM are shown in Table 1. Participants had a median duration of disease of 2.4 months (IQR, 1.2-2.4 months), with the majority having a duration of symptoms of less than 1 month (median [IQR] 8 days [range, 4-12 days] with 14% presenting in diabetic ketoacidosis. Participants had a median peak MMTT C-peptide of 865 pmol/L (IQR, 684-1120 pmol/L) and an HbA_{1c} of 67.5 mmol/mol (IQR, 48.2-76.2 mmol/mol) at their first wearing of CGM. Participants were all of White European descent with a BMI of (median [IQR] 23.5 [22.2-26.4]) and median insulin dose of 0.25 U/kg (IQR, 0.15-0.45 U/kg). Baseline characteristics of our sample were similar to the remaining participants recruited to the EXTOD study (20).

Table 1 Characteristics of participants included in analysis from the EXTOD pilot study at first wear of CGM (N=23 participants).

	N=23*
Age (years)	27.2 [23.4;36.5]
Duration of diabetes (months)	2.40 [1.20;2.40]
Sex	
Female	10 (44%)
Male	13 (57%)
Ethic Origin:	
White - British	22 (96%)
Other White Background	1 (4.4%)
BMI (kg/m^2)	23.5 [22.2;26.4]
HbA$_{1c}$ (mmol/mol)	67.5 [48.2;76.2]
Insulin dose (U/kg)	0.25 [0.15;0.45]
Peak (90min) MMTT C-peptide (pmol/l)	865 [684;1120]
Type 1 Diabetes Genetic Risk Score (T1D-GRS)	0.64 [0.55;0.76]
Presentation of diabetes:	
Duration of symptoms pre-diagnosis (days)	8.00 [4.00;12.0]
DKA	3 (14%)
Hyperglycemia without acidosis	19 (86%)
GADA positive titre	19 (83%)
IA 2A positive titre	13 (57%)
ZnT8A positive titre	12 (52%)
Randomisation Arm:	
Usual care	9 (39%)
Intervention	13 (57%)
Not randomized	1 (4%)

BMI (body mass index); HbA$_{1c}$ (glycated hemoglobin A$_{1c}$); DKA (diabetic ketoacidosis); GAD(glutamic acid decarboxylase antibody); IA 2A (tyrosine

phosphatase-related islet antigen 2 antibody); ZnT8 (Zinc transporter 8 antibody)

*Data presented: Median [25th;75th], n (%)

Glycemic Characteristics

The glycemic characteristics of these participants during each time of CGM wear are shown in Table 2. Most participants had tight glucose control, with median percentage of time spent in the range of 3.9 to 10 mmol/L of 68% (IQR, 55%-76% mmol/L). Average glucose was 8.3 mmol/L (median [IQR], 7.1-9.3 mmol/L) with low levels of hypoglycemia (percentage of time spent in hypoglycemia; median [IQR], 0.0% [0.0%-0.6%]), little to no time in level 1 low events (percentage of time spent 3-< 3.9 mmol/L; median [IQR], 0.6% [0.2%-1.7%]), and even fewer level 2 low events (percentage of time spent < 3 mmol/l; median [IQR], 0.0% [0.0%-1.0%]). Overall, glycemia was considered stable (< 36%) (26) in these participants (coefficient of variation; median [IQR], 32% [26%-36%]).

Table 2 Metrics of glycemic variability and hypoglycemia for participants for CGM wear at any study time point (N=36 CGM traces, 23 participants).

	N=36*
Percentage expected wear	97.5 [83.9;105]
Percentage of good data remaining post-calibration	97.4 [92.7;100]
Number of days of good data	6.35 [5.26;7.18]
Average Glucose (mmol/l)	8.27 [7.12;9.30]
Standard Deviation (mmol/l)	2.60 [1.97;3.42]
Coefficient of Variation (CV) (%)	32.0 [26.0;36.0]
Mean Amplitude Glycemic Excursion (MAGE)	4.99 [4.02;6.63]
Estimated HbA_{1c} (mmol/mol)	51.4 [43.2;57.7]
Time spent > 10 mmol/l (level 1 elevated) (%)	22.9 [9.43;37.3]
Time spent level 1 hyperglycemia (>10 mmol/l ≥ 15 minutes) (%)	25.2 [9.56;38.5]
Time spent > 13.9 mmol/l (level 2 elevated) (%)	5.98 [1.59;13.2]
Time spent level 2 hyperglycemia (>13.9 mmol/l ≥ 15 minutes) (%)	2.62 [0.28;8.70]
High Blood Glucose Index (HBGI)	7.43 [3.28;10.4]
Time spent 3.9-10 mmol/l (%)	68.3 [55.1;76.2]
Time spent 3-<3.9 mmol/l (level 1 low) (%)	0.63 [0.23;1.70]
Time spent <3 mmol/l (level 2 low) (%)	0.00 [0.00;1.00]
Time spent in hypoglycemia (%)	0.00 [0.00;0.58]
Low Blood Glucose Index (LBGI)	1.96 [1.28;2.64]

HbA_{1c} (glycated hemoglobin A_{1c})

*Data presented: Median [25th;75th]

C-Peptide at Diagnosis Is Associated With Less Glucose Variability, More Time in Range, and Less Hyperglycemia

For every 100-pmol/L increase in peak C-peptide, glucose levels were on average lower by 0.2 mmol/L (95% CI, –0.4 to 0.02, $P = .06$) (Fig. 2A, Table 3). In addition, there was a reduced SD in glucose of 0.1 (95% CI, –0.3 to –0.02, $P = .02$) (Fig. 2B, see Table 3). Percentage of time spent in the range of 3.9 to 10 mmol/L increased by 2.4% (95% CI, 0.5-4.3, $P = .01$) (Fig. 2C, see Table 3) with a reduction in the amount of time (percentage) spent at level 1 (> 10 mmol/L) elevated glucose levels by 2.6% (95% CI, –4.9 to –0.4, $P = .02$) (Fig. 2D, see Table 3) and level 2 (> 13.9 mmol/l) elevated glucose levels by 1.3% (95% CI, –2.7 to –0.006], $P = .04$) (see Table 3). Coefficients for all metrics of glycemic variability are outlined in Table 3, and all followed the same direction in a reduction of variability for every 100-pmol/L change in C-peptide.

Table 3 Associations from repeated measures mixed effects regression modelling of glycemic variability and hypoglycemia metrics with peak MMTT C-peptide (N=36 CGM traces, 23 participants).

	Coefficient for 100 pmol/l change in C-peptide N=36*	**p-value†**
Average glucose (mmol/l)	-0.19 [-0.39,0.015]	0.06
Standard deviation (mmol/l)	-0.14 [-0.25,-0.023]	0.02
Coefficient of Variation (CV) (%)	-1.00 [-2.16,0.15]	0.08
Mean Amplitude Glycemic Excursion (MAGE)	-0.34 [-0.63,-0.050]	0.02
Estimated HbA_{1c} (mmol/mol)	-0.14 [-0.25,-0.023]	0.06
Time spent > 10 mmol/l (level 1 elevated) (%)	-2.64 [-4.87,-0.41]	0.02
Time spent level 1 hyperglycemia (> 10 mmol/l excursion ≥ 15 minutes) (%)	-3.53 [-6.64,-0.42]	0.02
Time spent > 13.9 mmol/l (level 2 elevated) (%)	-1.33 [-2.66,-0.0057]	0.04
Time spent level 2 hyperglycemia (>13.9 mmol/l excursion ≥ 15min) (%)	0.92 [-2.02,0.19]	0.09
High Blood Glucose Index (HBGI)	-0.71 [-1.27,-0.14]	0.01
Time spent 3.9-10 mmol/l (%)	2.39 [0.51,4.26]	0.01
Time spent 3-<3.9 mmol/l (level 1 low) (%)	-0.015 [-0.22,0.19]	0.88
Time spent <3 mmol/l (level 2 low) (%)	-0.028 [-0.17,0.11]	0.66
Time spent in hypoglycemia (%)	0.0052 [-0.27,0.26]	0.97
Low Blood Glucose Index (LBGI)	-0.062 [-0.41,0.29]	0.72

HbA_{1c} (glycated heamoglobin A_{1c})

*Data presented: Coefficient [95% CI]

† Kenward-Roger approximation for degrees of freedom

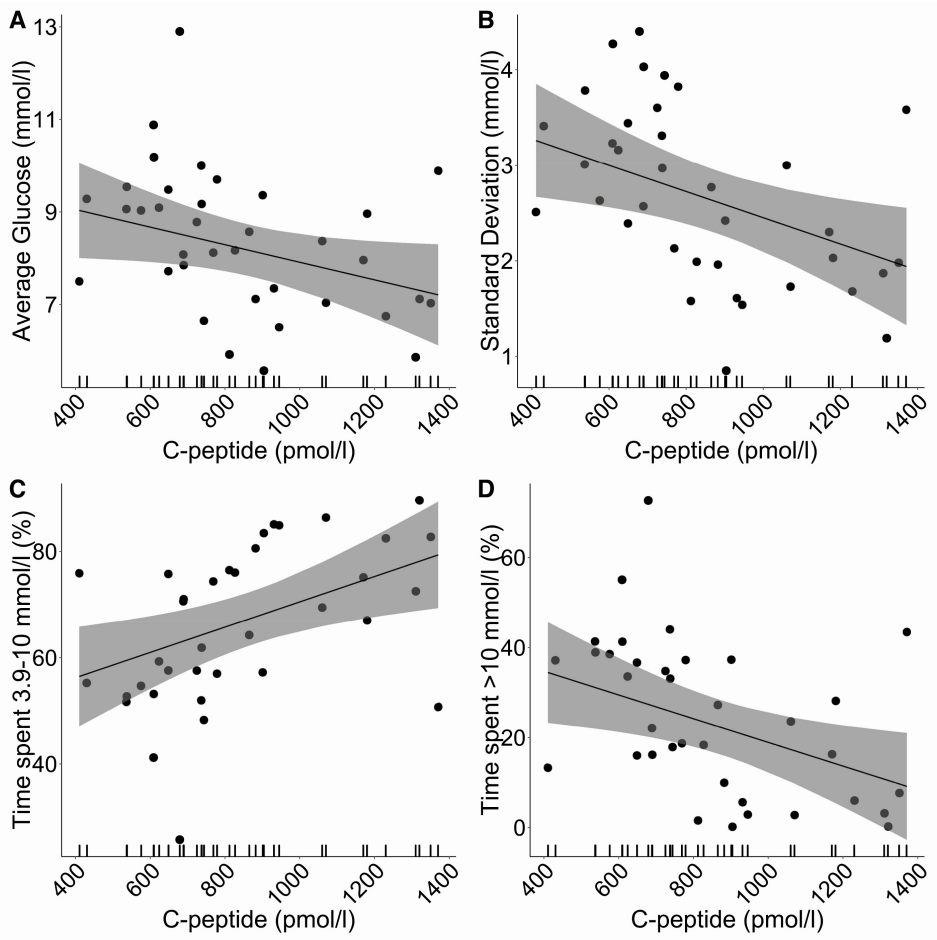

Figure 2. Distribution of 4 key glycemic metrics: A, average glucose; B, SD; C, percentage of time in range 3.9 to 10 mmol/L; and D, percentage of time spent at greater than 10 mmol/L with peak mixed-meal tolerance test (MMTT) C-peptide. The line represents repeated-measures, mixed-effects regression modeling between glycemic metric and peak MMTT C-peptide with 95% CI shown as a shaded bar.

C-Peptide at Diagnosis Is not Associated With Hypoglycemia

In this cohort hypoglycemic events were rare during all times of CGM wear (Fig. 3, see Table 2). There was no association with peak C-peptide and percentage time spent in hypoglycemic ranges ($P = .97$) or hypoglycemic risk, as measured by low blood glucose index ($P = .72$) (Fig. 3, see Table 3).

There was also no association between peak C-peptide and insulin dose ($P = .71$), HbA_{1c} ($P = .36$), or $IDAA_{1c}$, $P = .45$) (Table 4).

Table 4 Associations for clinical measures with peak MMTT C-peptide (N=36 observations, 23 participants).

	Coefficient for 100 pmol/l change in C-peptide N=36*	**p-value†**
IDA1CC‡	-0.091 [-0.34,0.16]	0.45
Insulin dose	-0.005 [-0.033,0.023]	0.71
HbA_{1c} ‡ (mmol/mol)	-1.02 [-3.37,1.32]	0.36

*Data presented: Coefficient [95% CI]

†Kenward-Roger approximation for degrees of freedom

‡Missing for 1 participant (1 observation)

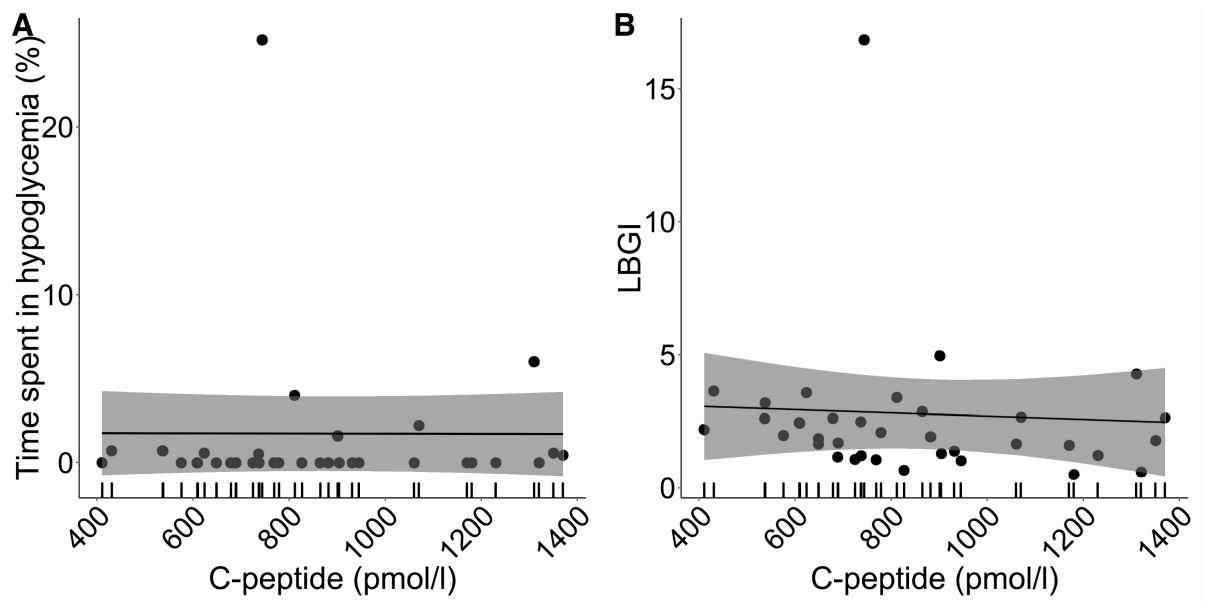

Figure 3. Distribution of hypoglycemic metrics: percentage of time spent in A, hypoglycemia, and B, low blood glucose index (LBGI) with peak mixed-meal tolerance test (MMTT) C-peptide. The line represents repeated-measures, mixed-effects regression modeling between glycemic metric and peak MMTT C-peptide with 95% CI shown as a shaded bar.

Discussion

We report that higher levels of C-peptide at diagnosis are associated with lower glycemic variability, more time in range, and less hyperglycemia, but not with hypoglycemia or HbA_{1c}. Variations in the high levels of residual C-peptide present at the time of diagnosis with T1D are associated with key clinical outcomes and could potentially inform the most effective approach to supporting the newly diagnosed patient.

Our findings support and enhance the understanding of the benefits of preserved C-peptide in patients with newly diagnosed T1D. Our results are consistent with the one other study in newly diagnosed participants by Buckingham et al comparing CGM-measured glucose variability with MMTT C-peptide; however, Buckingham's study was conducted in a largely pediatric cohort and did not compare C-peptide with CGM metrics as the primary analysis outcome (18).. The study by Buckingham et al also found lower glucose variability is associated with higher levels of C-peptide, with increased time spent in range (3.9-7.8 mmol/L) and decreased variation for higher levels of C-peptide, with no associations demonstrated with hypoglycemia. Both studies estimated C-peptide at peak following meal stimulation with similar values in the range of 100 to 1500 pmol/L. We did not find an association of C-peptide with HbA_{1c} and insulin dose in our study, in contrast with the study by Buckingham and colleagues, as well as studies in new-onset T1D (27) in which immunomodulation has resulted in some preservation in C-peptide being associated with lower HbA_{1c} and insulin doses. It is possible that the lower numbers in our study prevented us from

observing an impact on HbA_{1c} and insulin dose; another possibility is that alterations of HbA_{1c} and insulin dose may be influenced by study protocol or clinical care.

Studies exploring the benefits of preserved C-peptide in long-duration T1D demonstrate a similar impact on time in range and glucose variability, but also commonly show protection from hypoglycemia (11,12,28) in addition to lower HbA_{1c} (13,15) and lower insulin doses (11). However, we and others (18) do not find an association between variations in C-peptide level present at diagnosis and hypoglycemia. It is possible that the C-peptide levels at diagnosis, in addition to other factors related to a short duration of T1D, may offer more protection from hypoglycemia than in long-duration T1D, when endogenous insulin secretion is much lower or absent.

We therefore propose that the impact on glucose control associated with preserved C-peptide appears to vary across duration of disease in people with T1D (Fig. 4). Early after diagnosis when stimulated C-peptide values can reach higher than 1500 pmol/L, relatively higher levels of C-peptide reduce hyperglycemia and glucose variability, but not hypoglycemia. Later in the natural history, when stimulated C-peptide values are around 500 pmol/L or lower, higher values in this range reduce hypoglycemia and potentially also HbA_{1c} and insulin dose. The benefits of less glycemic variability and greater time in range are present across the spectrum. Furthermore, preserved C-peptide in longer-duration T1D is associated, presumably through consistent tight glucose control, with fewer microvascular complications (5,13). Our study, combined with others (18) highlights that hypoglycemia is rare around the time of diagnosis.

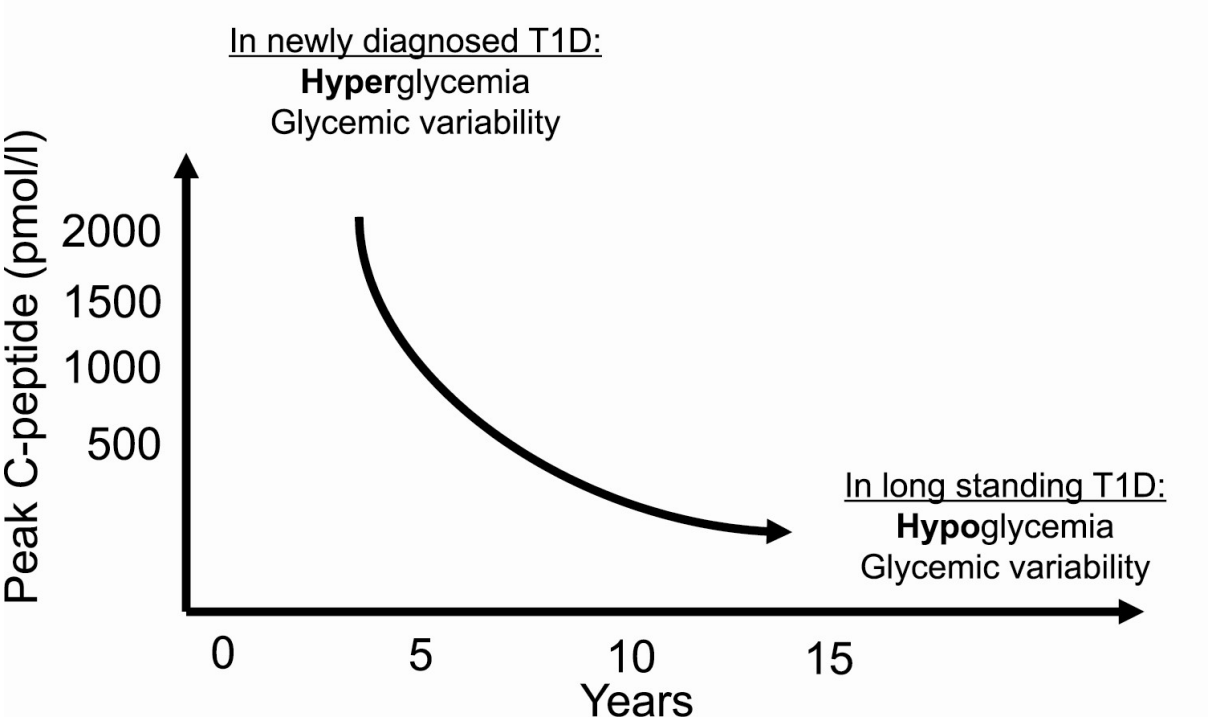

Figure 4. Summary of the impact variation in C-peptide level has on glucose control in people with type 1 diabetes (T1D), across diabetes duration. In newly diagnosed T1D, C-peptide variation impacts do not affect hypoglycemia, as demonstrated in longer-duration T1D. C-peptide variation affects glycemic variability near to diagnosis of T1D and at long-duration disease.

The strengths of this study include the careful cleaning and interpretation of the CGM data. To ensure a high standard of accuracy in our CGM data for analysis, we developed in-house CGM processing that compared the MARD of each self-monitored blood glucose calibration and the 15-minute later CGM sensor glucose reading, since interstitial glucose trails blood glucose by 5 to 20 minutes (29–31). We used the assumption that an entire day of glucose readings had a systematic error if the self-monitored blood glucose calibration reading and CGM sensor glucose reading had a MARD of greater than 20%, and therefore removed it from the CGM trace before analysis and generation of CGM-derived glycemic metrics. We developed in-house CGM analysis to generate these metrics in accordance with the International

Consensus on Use of Continuous Glucose Monitoring (25). Our in-house processing and analysis were validated against manual processing and analysis conducted by 2 people. Furthermore, the CGM data obtained from the participants were blinded at the time of wear, ensuring measured sensor glucose was not highly influenced by patient reactivity. Also, MMTT-measured C-peptide, obtained according to protocol, offered reduced variation of C-peptide levels, which is more likely with randomly measured C-peptide. Since misclassification of diabetes is common at diagnosis, occurring in 7% to 15% of cases (32), we used a specific criteria for defining T1D that included clinical diagnosis and either positive autoantibodies or T1D-GRS in addition to BMI in our definition.

A notable limitation of this study is that this is a retrospective analysis of data collected as part of a randomized controlled trial, using CGM data and peak MMTT C-peptide from participants involved in the EXTOD study, a randomized exercise trial. Participants who enroll in exercise trials may not be wholly representative of the T1D population because of their levels of activity and the effect exercise may have on blood glucose. This may have affected the average glucose metrics that we demonstrate in this cohort. Our sample size was limited to the consent rate to CGM monitoring during the study, and a high dropout rate of CGM monitoring over the 12 months of study. This may have affected the power to detect associations with C-peptide and CGM metrics that describe glycemic variability. Nevertheless, it is reassuring that we observed the same directional associations in all CGM metrics that describe glycemic variability and hyperglycemia with peak MMTT C-peptide, with no associations observed with the CGM metrics that describe hypoglycemia and hypoglycemia risk.

As we previously highlighted, the higher C-peptide levels present close to diagnosis may exceed a threshold needed to protect from hypoglycemia (minimal islet transplant function has been shown to protect from hypoglycemia (17)), which would explain the low rate of hypoglycemia commonly found post diagnosis and the lack of association found with postdiagnosis C-peptide by us and others.

Notwithstanding these limitations, our findings are important because they suggest that the benefits of C-peptide retention have a measurable impact from the point of diagnosis for a person with T1D. Our findings also highlight that metabolic or physiological differences between individuals may have more of an impact on glycemic variability than previously thought, with C-peptide playing a part in defining the manifestation of their T1D.

As we propose in Fig. 4, our results add to findings from previous studies of longer-duration diabetes, offering a more complete picture of the impact that variation in C-peptide levels has on glucose control in people with T1D. We suggest that managing newly diagnosed patients, informed by a current estimate of their C-peptide reserve, will influence how they are managed. Those with a lower C-peptide are likely to experience less time in glucose range, greater glucose variability, and more hyperglycemia and would be earmarked for earlier and more intensive support. Diabetes is currently the only endocrine condition for which the hormone in question is not measured as part of routine care. We suggest there is now increasing evidence to start doing so.

Acknowledgements

Ethics Statement

The study that the data used in the paper comes from received ethical opinion approval from Birmingham, East, North and Solihull Research Ethics committee in February 2010 (reference number 10/H1206/4). The study was sponsored by the University of Birmingham.

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The content and views expressed are those of the authors.

Disclosure Summary

The authors have nothing to disclose.

Prior presentation

Parts of this work we presented at the American Diabetes Association conference (online) 25-29th June 2021

Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References

1. Palmer JP. C-peptide in the Natural History of Type 1 Diabetes. *Diabetes Metab Res Rev* 2009;25(4):325–328.

2. Shields BM, McDonald TJ, Oram R, Hill A, Hudson M, Leete P, Pearson ER, Richardson SJ, Morgan NG, Hattersley AT. C-Peptide Decline in Type 1 Diabetes Has Two Phases: An Initial Exponential Fall and a Subsequent Stable Phase. *Diabetes Care* 2018;41(7):1486–1492.

3. Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC, Lachin JM, McGee P, Palmer JP, Pescovitz MD, Krause-Steinrauf H, Skyler JS, Sosenko JM. Fall in C-Peptide During First 2 Years From Diagnosis. *Diabetes* 2012;61(8):2066–2073.

4. Dabelea D, Mayer-Davis EJ, Andrews JS, Dolan LM, Pihoker C, Hamman RF, Greenbaum C, Marcovina S, Fujimoto W, Linder B, Imperatore G, D'Agostino R. Clinical evolution of beta cell function in youth with diabetes: the SEARCH for Diabetes in Youth study. *Diabetologia* 2012;55(12):3359–3368.

5. McGee P, Steffes M, Nowicki M, Bayless M, Gubitosi-Klug R, Cleary P, Lachin J, Palmer J, DCCT/EDIC Research Group. Insulin secretion measured by stimulated C-peptide in long-established Type 1 diabetes in the Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications (EDIC) cohort: a pilot study. *Diabet. Med.* 2014;31(10):1264–1268.

6. Davis AK, DuBose SN, Haller MJ, Miller KM, DiMeglio LA, Bethin KE, Goland RS, Greenberg EM, Liljenquist DR, Ahmann AJ, Marcovina SM, Peters AL, Beck RW, Greenbaum CJ, T1D Exchange Clinic Network. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. *Diabetes Care* 2015;38(3):476–481.

7. Barker A, Lauria A, Schloot N, Hosszufalusi N, Ludvigsson J, Mathieu C, Mauricio D, Nordwall M, Schueren BV der, Mandrup-Poulsen T, Scherbaum WA, Weets I, Gorus FK, Wareham N, Leslie RD, Pozzilli P. Age-dependent decline of β-cell function in type 1 diabetes after diagnosis: a multi-centre longitudinal study. *Diabetes, Obesity and Metabolism* 2014;16(3):262–267.

8. Steffes MW, Sibley S, Jackson M, Thomas W. β-Cell Function and the Development of Diabetes-Related Complications in the Diabetes Control and Complications Trial. *Diabetes Care* 2003;26(3):832–836.

9. Lachin JM, Orchard TJ, Nathan DM, DCCT/EDIC Research Group. Update on cardiovascular outcomes at 30 years of the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care* 2014;37(1):39–43.

10. Effect of Intensive Therapy on Residual β-Cell Function in Patients with Type 1 Diabetes in the Diabetes Control and Complications Trial. *Ann Intern Med* 1998;128(7):517–523.

11. Marren SM, Hammersley S, McDonald TJ, Shields BM, Knight BA, Hill A, Bolt R, Tree TI, Roep BO, Hattersley AT, Jones AG, Oram RA. Persistent C-peptide is associated with reduced hypoglycaemia but not HbA1c in adults with longstanding Type 1 diabetes: evidence for lack of intensive treatment in UK clinical practice? *Diabetic Medicine* 2019;36(9):1092–1099.

12. Gibb FW, McKnight JA, Clarke C, Strachan MWJ. Preserved C-peptide secretion is associated with fewer low-glucose events and lower glucose variability on flash glucose monitoring in adults with type 1 diabetes. *Diabetologia* 2020;63(5):906–914.

13. Jeyam A, Colhoun H, McGurnaghan S, Blackbourn L, McDonald TJ, Palmer CNA, McKnight JA, Strachan MWJ, Patrick AW, Chalmers J, Lindsay RS, Petrie JR, Thekkepat S, Collier A, MacRury S, McKeigue PM. Clinical Impact of Residual C-Peptide Secretion in Type 1 Diabetes on Glycemia and Microvascular Complications. *Diabetes Care* 2020. doi:10.2337/dc20-0567.

14. Brooks AM, Oram R, Home P, Steen N, Shaw JAM. Demonstration of an Intrinsic Relationship Between Endogenous C-peptide Concentration and Determinants of Glycemic Control in Type 1 Diabetes Following Islet Transplantation. *Diabetes Care* 2014. doi:10.2337/dc14-1656.

15. Rickels MR, Evans-Molina C, Bahnson HT, Ylescupidez A, Nadeau KJ, Hao W, Clements MA, Sherr JL, Pratley RE, Hannon TS, Shah VN, Miller KM, Greenbaum CJ, T1D Exchange β-Cell Function Study Group. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. *J. Clin. Invest.* 2020;130(4):1850–1862.

16. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. *Diabetes* 2004;53(1):250–264.

17. Vantyghem M-C, Raverdy V, Balavoine A-S, Defrance F, Caiazzo R, Arnalsteen L, Gmyr V, Hazzan M, Noël C, Kerr-Conte J, Pattou F. Continuous Glucose Monitoring after Islet Transplantation in Type 1 Diabetes: An Excellent Graft Function (β-Score Greater Than 7) Is Required to Abrogate Hyperglycemia, Whereas a Minimal Function Is Necessary to Suppress Severe Hypoglycemia (β-Score Greater Than 3). *J Clin Endocrinol Metab* 2012;97(11):E2078–E2083.

18. Buckingham B, Cheng P, Beck RW, Kollman C, Ruedy KJ, Weinzimer SA, Slover R, Bremer AA, Fuqua J, Tamborlane W, for the Diabetes Research in Children Network (DirecNet) and Type 1 Diabetes TrialNet Study Groups. CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but

do poorly in predicting C-peptide levels in the two years following onset of diabetes. *Diabetologia* 2015;58(6):1167–1174.

19. Forbes S, Olateju TO, Lam A, Casey J, Campbell J, Reid L, Oram RA, Malcolm AJ, Shapiro AMJ, Senior PA. CGM Shows Islet Transplantation Prevents Hypoglycemia, Correcting Time in Range and Reducing Glycemic Variability, Despite Subnormal Beta-Cell Function. *Diabetes* 2018;67(Supplement 1). doi:10.2337/db18-143-OR.

20. Narendran P, Jackson N, Daley A, Thompson D, Stokes K, Greenfield S, Charlton M, Curran M, Solomon TPJ, Nouwen A, Lee SI, Cooper AR, Mostazir M, Taylor RS, Kennedy A, Andrews RC. Exercise to preserve β-cell function in recent-onset Type 1 diabetes mellitus (EXTOD) - a randomized controlled pilot trial. *Diabet Med* 2017;34(11):1521–1531.

21. Lascar N, Kennedy A, Jackson N, Daley A, Dowswell G, Thompson D, Stokes K, Greenfield S, Holder R, Andrews R, Narendran P. Exercise to preserve beta cell function in recent-onset type 1 diabetes mellitus (EXTOD)--a study protocol for a pilot randomized controlled trial. *Trials* 2013;14:180.

22. McDonald TJ, Perry MH, Peake RWA, Pullan NJ, O'Connor J, Shields BM, Knight BA, Hattersley AT. EDTA improves stability of whole blood C-peptide and insulin to over 24 hours at room temperature. *PLoS One* 2012;7(7):e42084.

23. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, Bjoerndalen H, de Beaufort C, Chiarelli F, Danne T, Schoenle EJ, Åman J. New Definition for the Partial Remission Period in Children and Adolescents With Type 1 Diabetes. *Diabetes Care* 2009;32(8):1384–1390.

24. Oram RA, Patel K, Hill A, Shields B, McDonald TJ, Jones A, Hattersley AT, Weedon MN. A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. *Diabetes Care* 2016;39(3):337–344.

25. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, Garg S, Heinemann L, Hirsch I, Amiel SA, Beck R, Bosi E, Buckingham B, Cobelli C, Dassau E, Doyle FJ, Heller S, Hovorka R, Jia W, Jones T, Kordonouri O, Kovatchev B, Kowalski A, Laffel L, Maahs D, Murphy HR, Nørgaard K, Parkin CG, Renard E, Saboo B, Scharf M, Tamborlane WV, Weinzimer SA, Phillip M. International Consensus on Use of Continuous Glucose Monitoring. *Diabetes Care* 2017;40(12):1631–1640.

26. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, Owens DR. Toward Defining the Threshold Between Low and High Glucose Variability in Diabetes. *Diabetes Care* 2016. doi:10.2337/dc16-1769.

27. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA. Anti-CD3 Monoclonal

Antibody in New-Onset Type 1 Diabetes Mellitus. *New England Journal of Medicine* 2002;346(22):1692–1698.

28. Gubitosi-Klug RA, Braffett BH, Hitt S, Arends V, Uschner D, Jones K, Diminick L, Karger AB, Paterson AD, Roshandel D, Marcovina S, Lachin JM, Steffes M, Palmer JP. Residual β cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. *J Clin Invest* 2021;131(3). doi:10.1172/JCI143011.

29. Boyne MS, Silver DM, Kaplan J, Saudek CD. Timing of Changes in Interstitial and Venous Blood Glucose Measured With a Continuous Subcutaneous Glucose Sensor. *Diabetes* 2003;52(11):2790–2794.

30. Stout PJ, Peled N, Erickson BJ, Hilgers ME, Racchini JR, Hoegh TB. Comparison of Glucose Levels in Dermal Interstitial Fluid and Finger Capillary Blood. *Diabetes Technology & Therapeutics* 2001;3(1):81–90.

31. Cengiz E, Tamborlane WV. A Tale of Two Compartments: Interstitial Versus Blood Glucose Monitoring. *Diabetes Technol Ther* 2009;11(Suppl 1):S-11-S-16.

32. Shields BM, Peters JL, Cooper C, Lowe J, Knight BA, Powell RJ, Jones A, Hyde CJ, Hattersley AT. Can clinical features be used to differentiate type 1 from type 2 diabetes? A systematic review of the literature. *BMJ Open* 2015;5(11):e009088.

CHAPTER 5: GLYCEMIC VARIABILITY AND HYPOGLYCEMIA IS INCREASED THE DAY AFTER FREE-LIVING EXERCISE IN PEOPLE WITH TYPE 1 DIABETES

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Contribution: I was part of the design and completed the analysis of the data used in this study and was crucial in its interpretation. I created all figures and tables I wrote the first draft and oversaw all subsequent drafts.

All supplementary material for this chapter can be found in Appendix 3.3

Abstract

Objective*:* We assessed the impact of two modes of home-based exercise, as compared to a free-living non-exercise control period, on glycemic variability and hypoglycemia- two key barriers to exercise for people with type 1 diabetes, using intermittently scanned continuous glucose monitoring (isCGM).

Design*:* Ten adults with type 1 diabetes performed three 14-day interventions: home-based high-intensity interval exercise (HIIE), home-based moderate-intensity continuous exercise (MICE), free-living non-exercise control period (CON). Exercise periods consisted of 6 unsupervised non-time-controlled sessions, interspaced by 48h. isCGM data was collected using the Abbott FreeStyle Libre. We compared post- HIIE/MICE glycemia to the average CON period, matched for time of day and day of the week. We evaluated glycemic variability and hypoglycemia at 0-4h, overnight (00:00-06:00) and next day (06:00-24:00) after exercise.

Results: HIIE and MICE increased glucose coefficient of variation (CV) and time in level 1 hypoglycemia (L1H) during 0-4h post-exercise. Only MICE increased glycemic variability overnight post-exercise. Both HIIE and MICE increased glycemic variability and L1H the next day after exercise.

Conclusions: Home-based HIIE and MICE increase glycemic variability and hypoglycemia within 4 hours after starting exercise sessions, have less effect on glycemia overnight, but increase glycemic variability and hypoglycemia the day after

exercise. Assessment of the glycemic impact of exercise in free-living environments, when carefully matched for non-exercise days, may improve the management of blood glucose in type 1 diabetes and reduce barriers to exercise.

Introduction

Physical Activity (PA) is a key component in the management of type 1 diabetes. [1,2] People living with type 1 diabetes are recommended to undertake 150 minutes of moderate to vigorous intensity PA a week, spread over at least 3 days, with no more than 2 consecutive days without activity.[1] However, despite advances in guidance around exercise,[3] rates of inactivity in the type 1 diabetes population are higher than those found in the general population. [4,5]

A number of common exercise barriers, (lack of time, work commitments, bad weather, lack of access to facilities and lack of motivation) [4,6] can be attributed to the low levels of PA observed in people with type 1 diabetes. However, four of the six top ranking barriers to PA for people with type 1 diabetes are 'diabetes specific': fear of hypoglycemia/ hypoglycemia, loss of control/ glycemic variability, limited health professional support or advice and inadequate knowledge of glycemic management surrounding exercise. [4]

Laboratory based studies of the 24-hours post-exercise have found no difference between moderate-intensity continuous exercise (MICE) and high-intensity interval exercise (HIIE) for glucose time in range (TIR) (3.9-10mmol/L), incidence and time in hypoglycemia and glycemic variability. [7,8] A recent study by Brockman et al. [9] found that both MICE and resistance exercise had limited impact on 24-hour post-exercise glycemia, with MICE only having an effect on glycemic variability in nocturnal periods, when compared to a 45 minute sedentary control completed by the same individuals.

Such findings seemingly contradict the glycemic variability and hypoglycemia-related barriers to exercise commonly expressed by patients. [4]

Very few studies have assessed the impact of exercise on glycemia in free-living environments. To the best of our knowledge, Riddell et al. [10] is the only home-based study to date to assess the effect of exercise on glycemia for up to 24-hours, with the key finding that exercise days resulted in greater TIR than sedentary days, with no difference between the exercise modes of home-based HIIE or home-based MICE compared to sedentary control days. Again, this finding is contradictory or may mask the exercise associated variability patients express.

Understanding the impact of exercise on glycemia in the context of everyday life is a crucial step toward reducing the key barriers to exercise in people with type 1 diabetes. In this study, we used time-matched exercise and non-exercise glucose traces to explore the effect of two modes of home-based exercise, specifically on glycemic variability and hypoglycemia as these are two of the main barriers to exercise people with type 1 diabetes report.

Materials and methods

The study was approved by the NHS west midlands Edgbaston research ethics committee (18/WM/0203) and was registered on clinicaltrials.gov (NCT03598400) and conformed to the Declaration of Helsinki.[11] All participants provided written informed consent.

Study cohort

We recruited sixteen adults (men n = 6, women n = 10) living with type 1 diabetes on basal-bolus insulin treatment administered via multiple daily injections (MDI) (n = 8) or continuous subcutaneous insulin infusion (CSII) (n = 8) through adverts on type 1 diabetes social media groups. Inclusion criteria were; aged between 18-55 years, diagnosed with type 1 diabetes > 6 months and body mass index (BMI) $\leq$ 32 kg.m^2. Exclusion criteria were; pregnancy, disability preventing participation in an exercise regime, angina, autonomic neuropathy, medication that affects heart rate, major surgery planned within 6 weeks of the study, uncontrolled blood pressure, significant history of hyperglycemia, history of severe hypoglycemia requiring third party assistance within the last 3 months, severe non-proliferative and unstable proliferative retinopathy. Participant eligibility was confirmed during an initial meeting, which included a 12-lead resting electrocardiogram. Eleven adults proceeded with the study after five dropped out before the interventions started.

Study Design

Participants completed a counterbalanced crossover experiment, consisting of three 14-day intervention periods: home-based HIIE, home-based MICE and a free-living non-exercise control period (CON). Participants had a choice of the order in which they completed the three intervention periods. Exercise sessions were unsupervised and performed on days; 1, 3, 6, 8, 10 and 13 of the 14-day period, leaving at least 48 hours between sessions. The timing of exercise sessions was not controlled, but participants were asked to complete sessions at a similar time of day within and between interventions, and bouts were not performed after an overnight fast. Exercise sessions in both HIIE and MICE intervention periods were mostly completed in the late afternoon-evening (median [IQR] HIIE 17:39 [15:32,20:53], MICE 17:07 [15:18,18:41]). Participants were asked to refrain from any form of structured exercise other than the prescribed sessions during the intervention periods. During the CON period, participants were instructed to perform no structured exercise (e.g. playing sport, going to the gym or running), but could continue any habitual physical activity (e.g. walking to work or shops). Glycemia was assessed throughout the 14-day periods using an Abbott Freestyle Libre (Abbott Diabetes Care, CA, USA) isCGM, inserted subcutaneously into the interstitial fluid of the upper arm prior to each intervention. Participants were unblinded to the isCGM. Participants recorded insulin doses and dietary intake throughout the intervention periods.

Exercise Session Monitoring

Participants were provided with a Polar H10 heart rate (HR) monitor (Polar, Kempele, Finland) to wear during each exercise session. During HIIE and MICE sessions,

participants were asked to attain specific HR targets, described in the following sections below. Participants received instant visual feedback on their HR during sessions, using the compatible polar application.[12] Following exercise, HR data was automatically uploaded to a cloud storage site (www.flow.polar.com), enabling the research team to monitor exercise time and compliance with session completion and HR targets.

Home-Based High-Intensity Interval Exercises (HIIE)

The HIIE protocol was based on the previous work of Scott et al. [8] which has previously been shown to improve cardiorespiratory fitness and reduce insulin requirement in people with type 1 diabetes. Participants were instructed to complete a low-intensity (approx. 50% predicted HR_{max} (220-age)) warm up for 3-minutes prior to starting each HIIE session. All HIIE sessions had a duration of 12-minutes, with participants completing six 1-minute high-intensity intervals, interspersed with 1-minute rest intervals. Intervals used bodyweight exercises, with each interval divided into two different bodyweight exercises performed for 30 seconds with no rest between exercises. Participants were able to choose from a selection of 18 exercise pairs detailed in an exercise workbook. Participants were advised to achieve ≥80% of predicted HR_{max} during the intervals. A session was deemed compliant if participants completed at least 1 interval with a heart rate ≥80% of predicted HR_{max}. [13]

Home-Based Moderate-Intensity Continuous Exercises (MICE)

MICE sessions consisted of 30 minutes of continuous exercise of the participant's choosing (e.g. walking/jogging, cycling, swimming etc). Participants were asked to

attain and maintain a HR of 60-70% of predicted HR_{max} during the exercise session. A session was deemed compliant if mean HR (HR_{mean}) was between 60 and 70% HR_{max}.

Insulin Dose

Participants using MDI self-reported insulin doses using either the smart phone application (LibreLink) or the reader linked to the isCGM. This data was then automatically uploaded to the cloud system (LibreView) alongside the interstitial glucose data. Participants using CSII were asked to provide information from their pump report online for the dates they were participating in the study. Basal and bolus (units) insulin were recorded and total daily dose relative to bodyweight (TDD/kg) was also calculated (basal dose + bolus dose / bodyweight(kg) = TDD/kg (U/kg)). Mean daily insulin dose (U/kg) was calculated per individual for each 14-day intervention and across the total study period.

Dietary Intake

Dietary Intake was assessed using the MyFitnessPal application on smartphone (MyFitnessPal, CA, USA). Participants were asked to maintain their habitual diet and report their dietary intake as accurately as possible. A day was considered complete and valid if the calorie intake recorded was ≥500 kcal and ≤5000kcal. [14] If participants recorded less than 50% valid days (<7 days) in an intervention period, then their dietary data was excluded. [15] Mean Calorie (kcal), carbohydrate (grams), fat (grams) and sugar (grams) intake were calculated per individual for each 14-day intervention and across the total study period.

CGM Processing

All isCGM processing, analysis and visualisation was performed in R statistical software version 4.0.4 (Foundation for Statistical Computing, Vienna, Austria). Thirty 14-day isCGM traces from 10 participants went through processing.

We evaluated metrics of glycemic variability and hypoglycemia for 0-4 hours post-exercise, overnight (00:00-06:00) and next day (06:00-24:00) post-exercise for HIIE and MICE and the respectively matched control. We also evaluated the absolute difference in metrics of glycemic variability and hypoglycemia between exercise mode and respectively matched control for 0-4 hours post-exercise, overnight (00:00-06:00) and next day (06:00-24:00) post-exercise in the period 0-4 hours post-exercise for each individual, in order to compare effects on glycemia between each exercise mode.

For each individual, average traces for the CON period were generated for each day of the week, roll matched by nearest time, within 1 hour, using the *datatable* [16] package. IsCGM traces from each HIIE and MICE session were aligned to the average CON trace for each individual, matched by day of the week and time of day using the same roll matching technique as described above. A one-day example of matching an exercise session trace to the average CON trace for the same day of the week for one individual is demonstrated in Figure 1A. Traces were time-restricted with respect to time of exercise session to assess periods 0-4 hours post-exercise, overnight (00:00-06:00) and next day (06:00-24:00) after the day of exercise. As the isCGM has reading intervals of minimum 15 minutes, we included the 2 readings before the start of

exercise in the period 0-4 hours post-exercise to ensure all glucose readings during exercise sessions were captured.

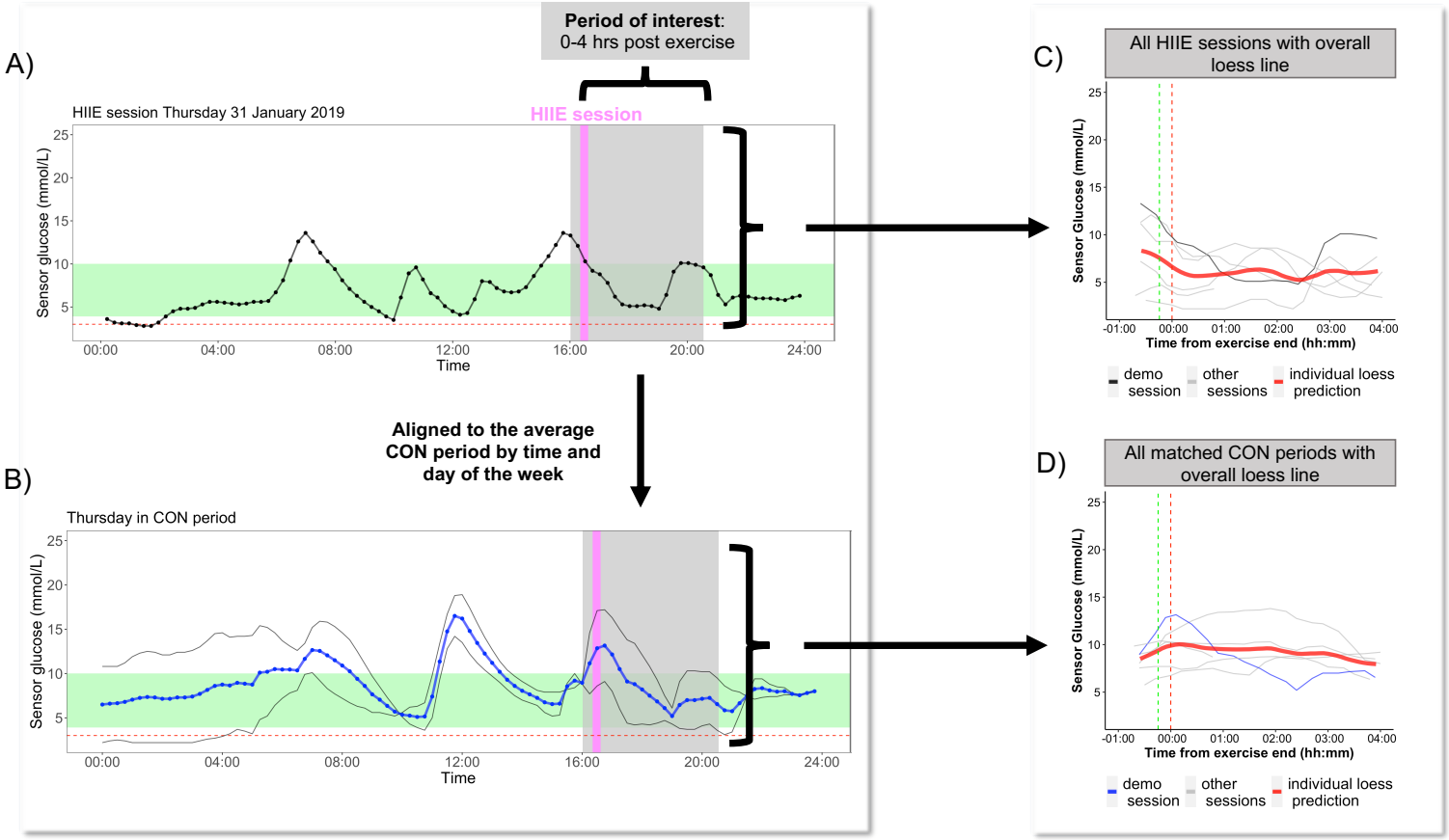


Figure 1 Example of the process to generate an average non-exercise control (CON) glucose trace, time and day of the week-matched to an exercise trace for one individual. In this example the time restriction of a period of 0-4 hours post exercise is visualized. We demonstrate how one exercise session (A) is matched to the average CON period, denoted by the blue line, on the same day of the week (B). This matching process is completed for all 6 exercise sessions (C) and matched CON sessions (D), with the individual's loess estimation of ambulatory glucose profile overlayed in red. Each individual's loess estimation of ambulatory glucose profile is outlined in Supplementary Figure 1. In the main analysis (Figure 5) the overall loess estimation for all individuals is displayed.

We generated isCGM derived metrics of glycemic variability and hypoglycemia in accordance with, and using definitions of hypo/hyperglycemic episodes from, the International Consensus on Use of Continuous Glucose Monitoring.17 Coefficient of variation (CV) (Appendix 2.1) was used as the primary measure of glycemic variability. [17] Level 1 hypoglycemia (L1H) is defined as glucoses in the range of 3-<3.9 mmol/L, Level 2 hypoglycemia (L2H) is defined as glucoses <3 mmol/L. A clinically defined hypoglycemic event is defined as a beginning when glucose <3mmol/L for at least 15 minutes and ending when glucose ≥ 3.9 mmol/L for 15 minutes.[17] Level 1 elevated glucose was defined as readings >10 mmol/L and level 2 elevated glucose was defined as readings >13.9 mmol/L. Hyperglycemic episodes were defined when glucose was at elevated levels ≥ 15 minutes.[17] Other glycemic metrics were generated and reported in accordance with consensus guidelines.[17] R code used to generate CGM metrics is available on GitHub and has been used in previous studies.[18,19]

Ambulatory glucose profiles for the 0-4 hours post-exercise period for HIIE, MICE and respectively matched control were visualised for each individual by a locally weighted smoothed (loess) regression of all isCGM traces for that individual within the 0-4 hour period of interest using the *stats* [20] package. Turning points after the start of exercise were identified in the loess estimated ambulatory glucose profile for each individual to assess immediate glucose peaks/nadirs associated with exercise. The loess regression of all individuals was also completed to show the overall trend. A demonstration of the loess estimated ambulatory glucose profile for HIIE and respectively matched control, in the 0-4 hours post-exercise period, for one individual is outlined in Figure 1B.

Statistical analysis

Statistical analysis was performed in R statistical software version 4.0.4 (Foundation for Statistical Computing, Vienna, Austria) using the *gtsummary*[21] and *stats*[20] packages. Significance was tested at the level of 0.05. Many clinical variables were not normally distributed, and so, data are presented as median and interquartile range. Paired analysis of isCGM characteristics between HIIE and MICE periods and respectively matched control was conducted using Wilcoxon signed-rank exact test. When ties were present, Wilcoxon signed-rank with continuity correction was used. As this is a crossover trial, period effects for key outcomes were assessed by Freidman rank-sum test (related data). between the 14-day intervention periods and between the order (1,2 or 3) each 14-day intervention period was completed. No significant effects were identified between the order each period was completed (Supplementary Table 1). Related analysis of insulin and dietary data between the 14-day intervention periods was conducted by Freidman rank-sum tests. Glucose changes observed by ambulatory glucose profile for HIIE and MICE were compared in paired analysis by using Wilcoxon signed-rank exact test. When ties were present, Wilcoxon signed-rank with continuity correction was used.

Results

Participants and characteristics

Eleven participants completed three 14-day intervention periods. Ten (4 male, 6 female) participants were included in analysis, with one participant, who completed the study protocol, excluded from analysis due to a technical issue with the CGM sensor (Figure 2). Nine out of the ten participants completed interventions consecutively, one participant had a 14-day gap between intervention two and three. The characteristics of participants included in analysis are outlined in Table 1. Of the participants included, 6 were on MDI and 4 were on CSII. The majority of participants wore an isCGM for the expected minimum of 14 days in each intervention period, with minimal sensor drop out (>90% data attained) (Supplementary Table 2). All participants were adults with a median age 23 years (IQR 21;32) and duration of type 1 diabetes of 9.2 years (2.5;13) (median IQR). Participants were all white European with a BMI 26 kg/m^2 (23;28) (median IQR). Across the study period participants had a mean daily insulin dose of 0.5 (0.4;0.5) U/kg (median IQR) mean daily calorie intake of 1,431 kcals (1,382;1,766) (median IQR) and mean daily carbohydrate intake of 186g (154;191) (median IQR) (Table 1). No significant difference was observed in mean daily insulin dose or dietary intake between intervention periods (Supplementary Table 3).

Table 1 Characteristics of participants included in analysis (N=10)

Characteristic	N = 10[1]
Age (years)	23 (21, 32)
Sex (Male)	4 (40%)
Height (cm)	167 (159, 175)
Mass (Kg)	76 (70, 83)
BMI (Kg/m^2)	26 (23, 28)
Type 1 diabetes duration (years)	9.2 (2.5, 13)
MDI/CSII	
MDI	6 (60%)
CSII	4 (40%)
Predicted Max HR (bpm)	198 (189, 199)
80% HR max (bpm)	158 (151, 159)
Mean Daily insulin dose (U/kg)	0.5 (0.4, 0.5)
Unknown	*3*
Mean Daily Calories (kcal)	1,457 (1,382, 1,766)
Unknown	*1*
Mean Daily CHO (g)	189 (158, 200)
Unknown	*1*
Mean Daily Fat (g)	54 (47, 73)
Unknown	*1*
Mean Daily Sugar (g)	43 (36, 53)
Unknown	*1*

[1]n (%); Median (IQR)

BMI (Body Mass Index); MDI (multiple daily injections); CSII (continuous subcutaneous insulin infusion); HR (Heart rate); CHO (carbohydrate)

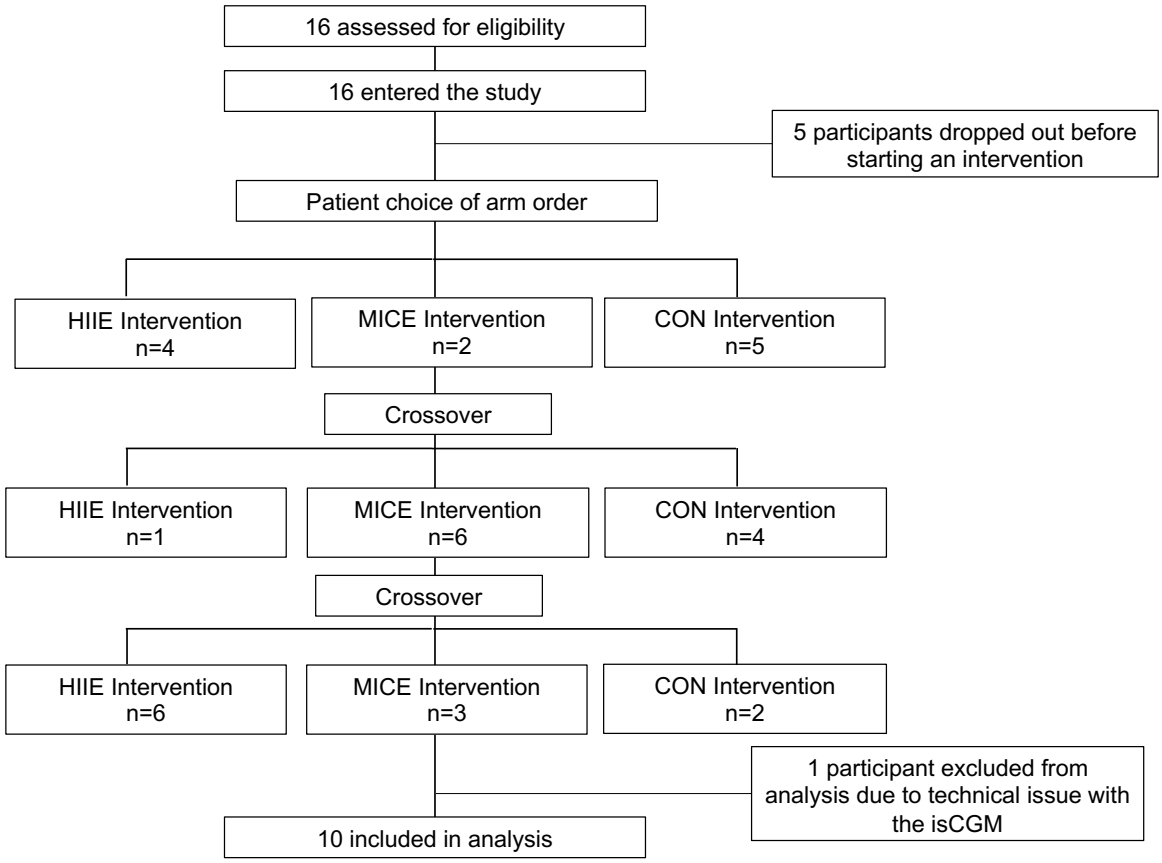


Figure 2 Consort diagram showing participant allocation at each stage of the study.

Glycemic characteristics

The glycemic characteristics of these participants, as derived through isCGM, across each 14-day intervention period are outlined in detail in Supplementary Table 2. Coefficient of Variation (CV) was ≥36% across each intervention period (38% (CON) 40% (HIIE) and 37% (MICE), p=0.3), with participants spending 55% (CON), 58% (HIIE), and 58% (MICE) (p=0.3) time in range in the respective intervention periods. Average glucose was approximately 9mmol/l across each intervention period (9.4 mmol/l (CON), 9.6 mmol/l (HIIE), 9.1 mmol/l (MICE), p=0.9). Time spent in

hypoglycemia was similar between intervention periods, with minimal time spent in L2H (p=0.8) and clinically defined hypoglycemia (p=0.9) and moderate time spent in L1H (p=0.9).

Both HIIE and MICE increased glycemic variability and hypoglycemic risk within 4 hours after exercise

When compared to control days, HIIE and MICE increased glycemic variability (Figure 3, Table 2) and time spent in L1H (Figure 4, Table 2) within 4 hours after exercise (CV: p=0.006 (HIIE), p=0.02 (MICE) and Time L1H: p= 0.02 (HIIE), p=0.01 (MICE)). Minimal time was spent in L2H and clinically defined hypoglycemia within the 4 hours post-exercise (Table 2). Average glucose, Time spent in range (3.9-10mmol/L) and time spent elevated (>10mmol/>13.9mmol/L) were similar to matched control periods (p>0.05) (Supplementary Table 4). The absolute difference in the glycemic metrics between each exercise and matched control period, assessed 0-4h post-exercise, were similar between exercise mode (Supplementary Table 5).

Table 2 Metrics of glycemic variability, represented by CV, and hypoglycemia for participants 0-4 hours post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respectively matched control (CON) periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
CV (%)	37 (30, 41)	25 (22, 26)	0.006	34 (31, 37)	26 (24, 28)	0.02
Time spent 3-<3.9 mmol/L, level 1 low, (%)	3.0 (0.5, 3.9)	0.0 (0.0, 0.0)	0.02	1.4 (0.9, 2.6)	0.0 (0.0, 0.0)	0.01
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.2	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Figure 3 Distribution of Coefficient of Variation (CV) for each time period (0-4 hours, overnight (00:00-06:00), next day (06:00-24:00) post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respective matched control (CON) periods at same time of day, on the same day of the week.

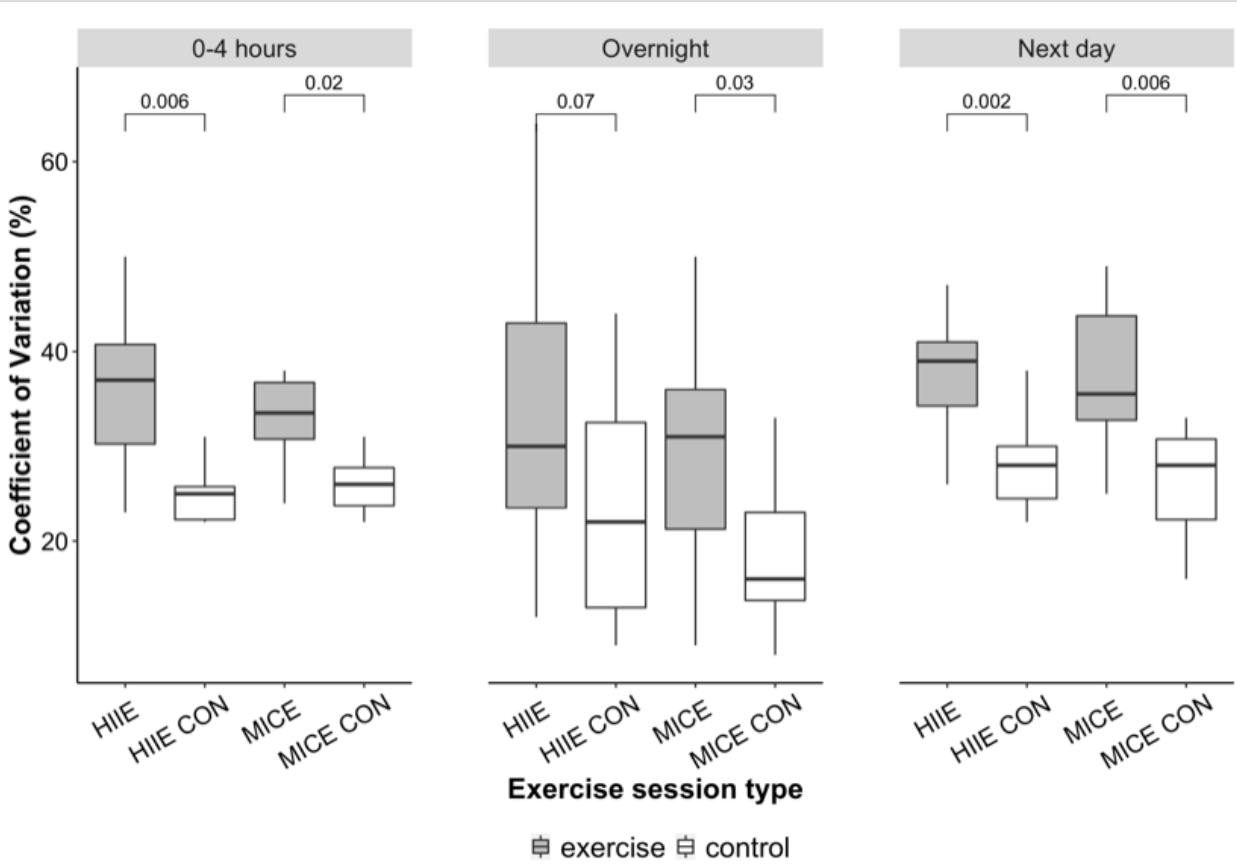
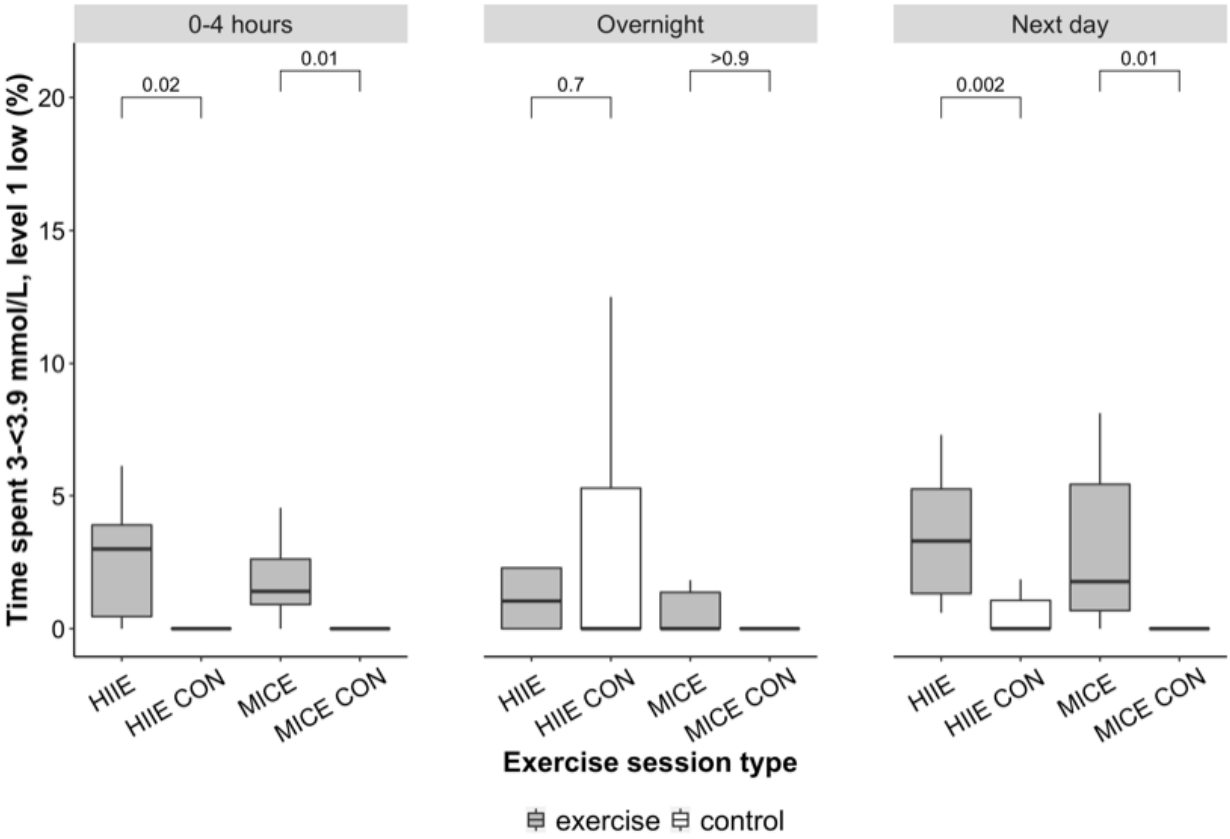


Figure 4 Distribution of time spent (%) in level 1 hypoglycemia for each time period (0-4 hours, overnight (00:00-06:00), next day (06:00-24:00)) post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respective matched control (CON) periods at same time of day, on the same day of the week.



A drop of 2-3 mmol/l in glucose level was observed in the loess regressed ambulatory glucose profiles within the first hour, for the majority of individuals, in both HIIE and MICE. Most participants recovered to a level >9mmol/l within 90 minutes (Figure 5, Table 3). Each individual's ambulatory glucose profile, as derived from the within person loess regression, is outlined in Supplementary Figure 1.

Table 3 Summary of the loess estimated (average) ambulatory glucose profiles for individuals in the 0-4 hours post-exercise for HIIE and MICE sessions.

Characteristic	HIIE, N = 10[1]	MICE, N = 10[1]	p-value[2]
Average duration of exercise session (minutes)	14 (13, 15)	31 (30, 33)	0.002
Average starting glucose (mmol/L)	9.3 (7.9, 11)	9.8 (8.6, 11)	0.3
Average change in starting glucose to first trough (mmol/L)	-1.9 (-2.6, -0.9)	-2.6 (-3.4, -1.5)	0.3
Average glucose at first trough (mmol/L)	7.0 (6.2, 9.0)	7.1 (5.3, 8.7)	0.5
Average time to first trough (minutes)	47 (43, 52)	54 (40, 60)	>0.9
Average change in starting glucose to first peak (mmol/L)	-0.4 (-1.6, 0.3)	-0.01 (-0.8, 1.3)	0.6
Average glucose at first peak (mmol/L)	8.9 (8.5, 9.9)	10 (7.8, 11)	0.6
Average time to first peak (minutes)	62 (17, 100)	80 (32, 149)	0.4

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

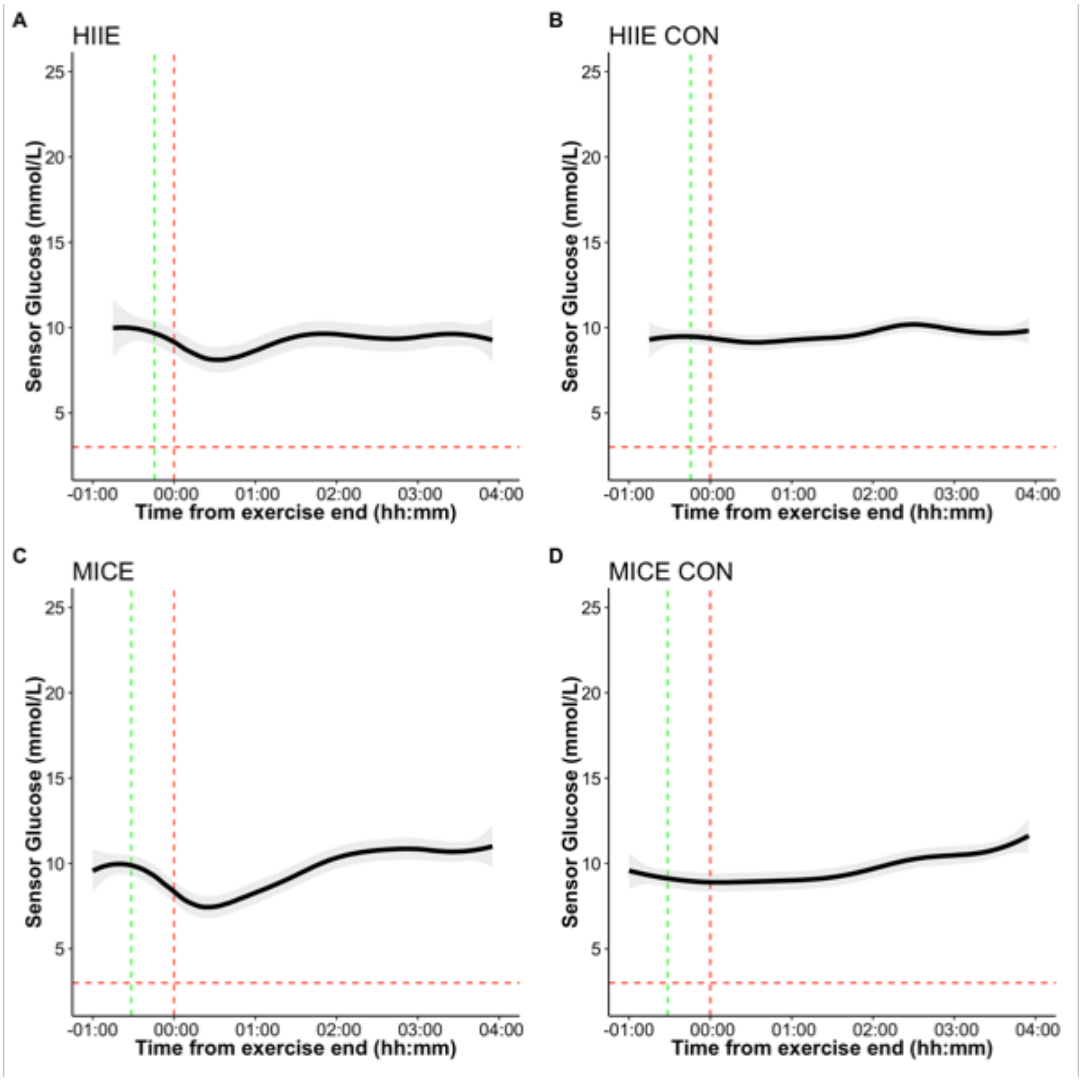


Figure 5 Loess regression estimate of overall ambulatory glucose profile for all individuals in the time 0-4 hours post-exercise for: high-intensity interval exercise (HIIE) (A) and moderate-intensity interval exercise (MICE) (C) sessions, compared respectively matched control (CON) (B,D). Horizonal dashed red line represents 3 mmol/L. Vertical dashed lines represents the average start (green) and end (red) of an exercise session. Shaded ribbon represents standard error.

MICE increased glycemic variability in overnight periods after exercise

We found less of an impact of exercise on glycemic variability and hypoglycemia overnight. Our results were suggestive that exercise impacted glycemic variability

overnight with MICE increasing CV in overnight periods (p=0.03), however CV post HIIE sessions was more overlapping with control periods compared to other post-exercise periods assessed (p=0.07) (Figure 3, Table 4). Neither HIIE or MICE influenced the amount of time spent in L1H overnight, as compared to control overnight periods (p=0.7 (HIIE), p > 0.9 (MICE) (Figure 4, Table 4)) with no time spent L2H and clinically defined hypoglycemia overnight (Table 4). Average glucose, time spent in range (3.9-10mmol/L) and time spent elevated (>10mmol/>13.9mmol/L) were similar to matched control overnight periods (p>0.05) (Supplementary Table 6). The absolute difference in the glycemic metrics between each exercise and matched control period, assessed overnight post-exercise, were similar between exercise mode (Supplementary Table 7).

Table 4 Metrics of glycemic variability, represented by CV, and hypoglycemia for participants during overnight sleep periods (00:00-06:00) post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respectively matched control (CON) periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
CV (%)	30 (24, 43)	22 (13, 33)	0.07	31 (21, 36)	16 (14, 23)	0.03
Time spent 3-<3.9 mmol/L, level 1 low, (%)	1.2 (0.0, 9.3)	0.0 (0.0, 5.3)	0.7	0.0 (0.0, 1.4)	0.0 (0.0, 0.0)	>0.9
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	>0.9
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	>0.9

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Both HIIE and MICE increased glycemic variability and hypoglycemic risk the next day after exercise

Both HIIE and MICE increased glycemic variability (Figure 3, Table 5) and time spent in L1H (Figure 4, Table 5) the next day after exercise, as compared to control (CV: p=0.002 (HIIE), p=0.006 (MICE) and Time L1H: p=0.002 (HIIE), p=0.01 (MICE)). Overall, little time was spent in L2H and clinically defined hypoglycemia the next day post-exercise (Table 5). HIIE marginally lowered average glucose during the next day post-exercise as compared to control, as opposed to MICE which nominally increased time spent >13.9mmol/L and in defined hyperglycemia (Supplementary Table 8). Time spent in range (3.9-10mmol/L) was similar to matched control periods (p>0.05)

(Supplementary Table 8). The absolute difference in the glycemic metrics between each exercise and matched control period, assessed the next day post-exercise, were similar between exercise mode (Supplementary Table 9).

Table 5 Metrics of glycemic variability and hypoglycemia for participants during the next day (06:00-24:00) post HIIE and MICE exercise session, as compared to the respective matched CON periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
CV (%)	39 (34, 41)	28 (25, 30)	0.002	36 (33, 44)	28 (22, 31)	0.006
Time spent 3-<3.9 mmol/L, level 1 low, (%)	3.3 (1.3, 5.3)	0.0 (0.0, 1.1)	0.002	1.8 (0.7, 5.4)	0.0 (0.0, 0.0)	0.01
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.5)	0.0 (0.0, 0.0)	0.3	0.0 (0.0, 0.4)	0.0 (0.0, 0.0)	0.2
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.6	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.2

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Discussion

We report that home-based HIIE and MICE increase glycemic variability and hypoglycemia in the 4 hours after exercise, with a modest effect on glycemic variability overnight, but with increased glycemic variability and hypoglycemia the day after exercise in people with type 1 diabetes. By comparing the same time of day that exercise sessions were completed to that in non-exercise control periods, we highlight the immediate and prolonged effect of exercise in free-living environments, particularly the impact the day after exercise. Our results suggest that developing focused clinical guidance around these time periods post-exercise, may result in better glycemic control for people with type 1 diabetes and reduce barriers to exercise.

Riddell et al.[10] was the first home-based study to assess post-exercise glycemia across a 24-hour period, finding that on days of structured exercise, time in range was increased when compared to sedentary days in the same assessment period, as well as moderately increasing L1H in the 12 hours post-exercise. Riddell et al. additionally highlighted mean glucose and hyperglycemia in the first 4 hours after exercise was lower than similar time periods on sedentary days, recovering to levels similar to that of sedentary days by 16-20 hours after exercise. Our study is supportive of the finding of increased L1H in the short-term. However, our findings suggest that this increased time spent in L1H is mainly due to the drop in glucose that occurs in the short-term after exercise (within the first 4 hours) since we find a significant increase in the time spent in L1H in the first 0-4 hours post-exercise but no difference overnight. It is possible with the study by Riddell et al., the 12 hours period they assess extends into

overnight periods, which could explain the increasing modesty of exercise effect they observe in the discrete 4-hour periods post-exercise they examine. Importantly, like Riddell et al. we found that neither exercise mode increased time in spent in serious, clinically defined hypoglycemia in any time period post-exercise (0-4 hours, overnight, next day). Unlike Riddell et al. however, we find no difference between time spent in range in the exercise periods we examine and non-exercise periods. In our study, we use discrete 14-day exercise periods and non-exercise control periods, with participants completing the same type of exercise within each exercise period. As Riddell et al. used sedentary days occurring within the same assessment periods as exercise, this could have influenced the results obtained in their study.

Brockman et al.[9] is a recent laboratory-based study in which post-exercise glycemia was assessed for 24-hours after an acute bout of resistance and aerobic exercise, as compared to one non-exercise session**.** Unlike in our current study, resistance exercise and MICE were found to have no impact on 24-hour glycemic outcomes. In contrast to Brockman et al. however, our study used multiple exercise sessions, with matched non-exercise control, to assess the 24-hour impact on glycemia, in addition to assessing this in free-living. This difference in study design may account for differences observed in results.

In people without diabetes, it is well known that even minimal exercise increases insulin sensitivity up until the next day.[22,23] Anecdotally, people with type 1 diabetes express difficulty managing blood glucose the day after a bout of exercise. Our study is the first to show that the next day after exercise has increased variability and

hypoglycemia, as compared to days of non-exercise, in people with type 1 diabetes. We hypothesise that the difficulties patients express may be emphasised by daytime external factors, such as food-intake and insulin administration, since we see weaker evidence of an effect overnight when these external factors are minimised. Further work could be undertaken to understand if this effect is particularly emphasised by meal times. Our results highlight that immediately after exercise and the next day are periods where patients could benefit from focused guidance for management of glucose, where we demonstrate strong evidence for increased glycemic variability and hypoglycemia, two key barriers to exercise people with type 1 diabetes experience.[4] Although there is weaker evidence for increased overnight glycemic variability post exercise, management strategies on an individual basis in this overnight time period could be considered.

It must be highlighted that HIIE protocols may result in different glycemic responses.[7,8,24–26] In this study we used the same HIIE protocol as used by Scott et al[13]. Scott et al. found that the group mean blood glucose concentration immediately post-exercise and 1-hour post-exercise was not different from baseline. In our study by examining ambulatory glucose profiles for up to 4 hours post-exercise we observe the first glucose nadir occurring between 40 minutes-1 hour, with a rise following this to reach a peak within 90 minutes. It is possible that in the study by Scott et al., glucose nadirs may not have been captured, since 2 discrete self-monitored blood glucose (SMBG) readings were obtained: 12 minutes post-exercise (approximate duration of HIIE session) and 1-hour post-exercise. isCGM, as used in our study, is advantageous over SMBG because it enables greater granularity in ambulatory

glucose profiles by obtaining glucose readings every 15 minutes for any time-restricted period.

A key strength of our study is its free-living aspect, enabling an insight into the "real-life" impact of exercise. In order to do this, our study involved the careful cleaning and interpretation of isCGM data through in house processing, and direct matching of control periods to exercise periods, by time and within day of the week, to minimise biases that could be introduced through usual day-to-day variability in type 1 diabetes. Importantly, the average of 2 control traces on the same day of the week were used as the control comparison in order to obtain a more robust estimate of an individual's non-exercise control day. Further, we used instructional videos to standardize the at-home prescribed exercise and confirmed start and end time of exercise by heart rate monitor timestamps. An additional strength of our study is that our control comparison of non-exercise was derived from an independent 14-day habitual period, to maximise detection of true exercise effect. Furthermore, we extend the assessment of glycemic variability and hypoglycemia within overnight periods and crucially the next day post-exercise, where anecdotally patients report difficulty with glycemic control.

A notable limitation of our study is that participants had a choice in the order in which intervention periods were completed, in order to maximise study retention, and was therefore not randomised. In addition, there was no designated wash-out period between interventions. Reassuringly however, we found no significant difference in 14-day glycemic outcomes between the order at which intervention periods were completed or the type of intervention period itself. Furthermore, participants that enrol

in exercise trials may not be wholly representative of the type 1 diabetes population, due to their levels of activity and the overall effect exercise may have on blood glucose. This may have impacted the average glucose metrics that we demonstrate in this cohort. We acknowledge that the samples size in this study is small. We do however highlight that multiple exercise sessions were completed by each individual which improves the robustness of the within person glycemic trends we observe post-exercise. It should be noted however that these repeated bouts of exercise could have influenced glycemia within exercise intervention periods through increasing insulin sensitivity. In addition, the time-of-day exercise was completed could influence glycemia. Although participants had the choice in time of exercise, most opted for late afternoon/evening exercise.

Notwithstanding these limitations, our findings are important in furthering the knowledge of post-exercise glycemia in type 1 diabetes, suggesting that both MICE and HIIE exercise modes may increase glycemic variability and hypoglycemia shortly after exercise and for prolonged periods. HIIE exercise has been proposed as safer alternative to MICE exercise, however our results suggest there is little difference in their effect on glycemia up until the next day after exercise. Our findings are crucial to improving glycemic variability and hypoglycemia-related barriers to exercise commonly expressed by patients and could potentially inform management protocols of post-exercise blood glucose in people with type 1 diabetes.

Acknowledgments

Author Contributions

MC, RCA, ALJC, SOS, SNS, PN, TSP and JP designed the study. ALJC developed analysis techniques and performed analysis. JP and ALJC wrote the first draft. LAF and BMS reviewed and contributed to statistical analysis. PN and RAO contributed to the understanding of analysis. JP, NH, SOS, SNS, BW, TSP, RZ, and JB recruited study participants, oversaw data collection and performed preliminary analyses. All authors reviewed analysis and reviewed and contributed to final draft. MC and RCA are responsible for this work as a whole.

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Disclosure Summary

The authors have nothing to disclose.

Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

Ethics Statement

The study that the data used in the paper comes from received ethical opinion approval from the NHS west midlands Edgbaston Research Ethics Committee (18/WM/0203).

Prior presentation

Parts of this work were presented at Diabetes UK, London, 28th March-1st April and the Advanced Technologies and Therapeutics Conference, Paris, 27-30th April 2022.

References

1. Colberg SR, Sigal RJ, Yardley JE, et al. Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. Diabetes Care 2016;39(11):2065–2079; doi: 10.2337/dc16-1728.

2. Chetty T, Shetty V, Fournier PA, et al. Exercise Management for Young People With Type 1 Diabetes: A Structured Approach to the Exercise Consultation. Frontiers in Endocrinology 2019;10:326; doi: 10.3389/fendo.2019.00326.

3. Riddell MC, Gallen IW, Smart CE, et al. Exercise Management in Type 1 Diabetes: A Consensus Statement. The Lancet Diabetes & Endocrinology 2017;5(5):377–390; doi: 10.1016/S2213-8587(17)30014-1.

4. Brennan MC, Brown JA, Ntoumanis N, et al. Barriers and Facilitators of Physical Activity Participation in Adults Living with Type 1 Diabetes: A Systematic Scoping Review. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 2021;46(2); doi: 10.1139/apnm-2020-0461.

5. Plotnikoff RC, Taylor LM, Wilson PM, et al. Factors Associated with Physical Activity in Canadian Adults with Diabetes. Med Sci Sports Exerc 2006;38(8):1526–1534; doi: 10.1249/01.mss.0000228937.86539.95.

6. Trost SG, Owen N, Bauman AE, et al. Correlates of Adults' Participation in Physical Activity: Review and Update. Med Sci Sports Exerc 2002;34(12):1996–2001; doi: 10.1097/00005768-200212000-00020.

7. Lee AS, Johnson NA, McGill MJ, et al. Effect of High-Intensity Interval Training on Glycemic Control in Adults With Type 1 Diabetes and Overweight or Obesity: A Randomized Controlled Trial With Partial Crossover. Diabetes Care 2020;43(9):2281–2288; doi: 10.2337/dc20-0342.

8. Scott SN, Cocks M, Andrews RC, et al. High-Intensity Interval Training Improves Aerobic Capacity Without a Detrimental Decline in Blood Glucose in People With Type 1 Diabetes. The Journal of Clinical Endocrinology & Metabolism 2019;104(2):604–612; doi: 10.1210/jc.2018-01309.

9. Brockman NK, Sigal RJ, Kenny GP, et al. Afternoon Aerobic and Resistance Exercise Have Limited Impact on 24-h CGM Outcomes in Adults with Type 1 Diabetes: A Secondary Analysis. Diabetes Res Clin Pract 2021;177:108874; doi: 10.1016/j.diabres.2021.108874.

10. Riddell MC, Li Z, Beck RW, et al. More Time in Glucose Range During Exercise Days than Sedentary Days in Adults Living with Type 1 Diabetes. Diabetes Technol Ther 2021;23(5):376–383; doi: 10.1089/dia.2020.0495.

11. Rickham PP. Human Experimentation: Code of Ethics of W.M.A. Br Med J 1964;2(5402):177; doi: 10.1136/bmj.2.5402.177.

12. Anonymous. Polar Global. n.d. Available from: https://www.polar.com/en [Last accessed: 2/14/2022].

13. Scott SN, Shepherd SO, Andrews RC, et al. A Multidisciplinary Evaluation of a Virtually Supervised Home-Based High-Intensity Interval Training Intervention in People With Type 1 Diabetes. Diabetes Care 2019;42(12):2330–2333; doi: 10.2337/dc19-0871.

14. Carter MC, Burley VJ, Nykjaer C, et al. Adherence to a Smartphone Application for Weight Loss Compared to Website and Paper Diary: Pilot Randomized Controlled Trial. J Med Internet Res 2013;15(4):e32; doi: 10.2196/jmir.2283.

15. Burke LM, Hawley JA, Wong SHS, et al. Carbohydrates for Training and Competition. J Sports Sci 2011;29 Suppl 1:S17-27; doi: 10.1080/02640414.2011.585473.

16. Matt Dowle and Arun Srinivasan. Data.Table: Extension of `data.Frame`. R. 2021.

17. Danne T, Nimri R, Battelino T, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care 2017;40(12):1631–1640; doi: 10.2337/dc17-1600.

18. Carr ALJ, Oram RA, Marren SM, et al. Measurement of Peak C-Peptide at Diagnosis Informs Glycemic Control but Not Hypoglycemia in Adults With Type 1 Diabetes. Journal of the Endocrine Society 2021;5(10); doi: 10.1210/jendso/bvab127.

19. Carr ALJ. CGMProcessing. R. 2021. https://github.com/alicelouisejane/CGMprocessing

20. R Core Team. R: A Language and Environment for Statistical Computing. R. R Foundation for Statistical Computing: Vienna, Austria; n.d.

21. Daniel D. Sjoberg, Michael Curry, Margie Hannum, et al. Gtsummary: Presentation-Ready Data Summary and Analytic Result Tables. R. 2021.

22. Bird SR and Hawley JA. Update on the Effects of Physical Activity on Insulin Sensitivity in Humans. BMJ Open Sport Exerc Med 2017;2(1):e000143; doi: 10.1136/bmjsem-2016-000143.

23. Newsom SA, Everett AC, Hinko A, et al. A Single Session of Low-Intensity Exercise Is Sufficient to Enhance Insulin Sensitivity Into the Next Day in Obese Adults. Diabetes Care 2013;36(9):2516–2522; doi: 10.2337/dc12-2606.

24. Riddell MC, Pooni R, Yavelberg L, et al. Reproducibility in the Cardiometabolic Responses to High-Intensity Interval Exercise in Adults with Type 1 Diabetes. Diabetes Res Clin Pract 2019;148:137–143; doi: 10.1016/j.diabres.2019.01.003.

25. Aronson R, Brown RE, Li A, et al. Optimal Insulin Correction Factor in Post-High-Intensity Exercise Hyperglycemia in Adults With Type 1 Diabetes: The FIT Study. Diabetes Care 2019;42(1):10–16; doi: 10.2337/dc18-1475.

26. Martín-San Agustín R, Laguna Sanz AJ, Bondia J, et al. Impact of High Intensity Interval Training Using Elastic Bands on Glycemic Control in Adults with Type 1 Diabetes: A Pilot Study. Applied Sciences 2020;10(19):6988; doi: 10.3390/app10196988.

CHAPTER 6: DISCUSSION

Summary

The work presented in this thesis demonstrates that C-peptide is a robust marker of functional beta cells in clinical settings and how estimates of a patient's beta cell function could benefit classification and clinical management, in combination with newly afforded insights from continuous glucose monitoring technology. I demonstrate that C-peptide provides an accurate definition of type 1 diabetes that can be used in diagnostic model development, through the first validation of a diagnostic model against histologically defined insulin deficiency. Furthermore, by exploring the similarities in the trends of decline of C-peptide in living children and young adults with type 1 diabetes and decline in beta cells in pancreatic donors, I strengthen the evidence that C-peptide levels can be used as an inexpensive and practical marker of retention of islets containing insulin-positive beta cells, especially in an intervention trial setting. In addition to this, I show how the trends of decline vary by age at diagnosis, with the very young (<7 years) progressing to near-total loss of C-peptide and beta cells rapidly, emphasising the importance of early intervention within this group.

Through the development of a robust pipeline for the analysis of Continuous Glucose Monitoring data, I demonstrate the benefits evident for the preservation of C-peptide in type 1 diabetes, close to diagnosis. I demonstrate that variations in the high levels of residual C-peptide present at the time of diagnosis with type 1 diabetes associates with key clinical outcomes of glyceamic variability but not hypoglycaemia. I argue that

there is potential for C-peptide to become part of a clinical management pathway for a person with type 1 diabetes, depending on their point from diagnosis.

In addition to how C-peptide, a biological factor, may impact the lived experience of type 1 diabetes, I explore how behavioural aspects, such as exercise, could have an impact. Using the same CGM analysis pipeline, I demonstrate the effect that two modes of home-based exercise have on glycaemic variability and hypoglycaemia, and discuss how a better understanding of post-exercise glycaemia could reduce the barriers to exercise that people with type 1 diabetes report.

This chapter provides an overview of the main findings from this thesis and discusses conclusions, implications and limitations along with potential areas for future research.

Chapter title: Histological validation of a type 1 diabetes clinical diagnostic model for classification of diabetes

Summary

This chapter explores the classification uses of C-peptide. Using pancreas samples that have been classified as having type 1 diabetes or non-type 1 diabetes using the current histological gold standard of disease, I test the performance of a clinical diagnostic model that has been previously developed using C-peptide level as a surrogate outcome of disease to classify type 1 diabetes [1]. I demonstrate that classification models combining clinical features and biomarkers out-perform classification by singular features and, through validation within a histologically defined cohort, demonstrate that C-peptide is a robust, appropriate surrogate outcome that can be used in large clinical studies where histological definition is impossible.

Implications

This study is the first to evaluate a clinical diagnostic model against histological data, providing robust histological evidence that a clinical diagnostic model, combining clinical features and biomarkers, could improve diabetes classification. Moreover, this study provides reassurance that progression to C-peptide deficiency (<200 pmol/l >3 years post diagnosis [2]), is an appropriate surrogate endpoint within a clinical setting where histological classification is not possible.

Limitations

A notable limitation of this study is that the diagnostic model was developed using data derived primarily from White Europeans between the ages of 18 and 50 years. Our histological cohort included 27% non-white Europeans and diagnosis ages ranging from 1 to 73 years. It is well documented that the incidence and prevalence of type 1 diabetes and type 2 diabetes varies across demographic subgroups [3, 4]. It is also well accepted that the prior prevalence of type 1 diabetes and type 2 diabetes varies with age, with type 2 diabetes more likely to be diagnosed at older ages and type 1 diabetes more likely to be diagnosed at younger ages. Despite showing good discrimination and calibration, this may have limited the performance of the model in this cohort, with further validation needed of the model performance in non-white Europeans, in children, and in adults over the age of 50. Additionally, T1D-GRS [5] used within the combined model has its own limitations, the first being that it was discovered and validated in European ancestry cohorts. It is known that the genetics of type 1 diabetes differ within specific ancestry populations, with Onengut-Gumuscu et al recently demonstrating, by GWAS of type 1 diabetes in African-Ancestry participants, some newly associated variants that are rare in white Europeans [6]. Additionally, the T1D-GRS used in this study is a now an outdated version of the polygenic risk score. Combining 30 single nucleotide polymorphisms (SNPs) the T1D-GRS, herein referred to as T1D-GRS1, included the susceptible HLA haplotypes DR3-DQ2 and DR4-DQ8 and the resistant haplotype DR15-DQ6, but missed many other DR-DQ haplotypes important in type 1 diabetes genetic risk or protection. Work by Sharp et al. [7] has seen the development of an improved type 1 diabetes genetic risk score, termed T1D-GRS2, which combines 67 SNPs with improved capture of HLA

DR-DQ risk, HLA interactions and additional non-HLA SNPs, yielding better discrimination of type 1 diabetes when compared to the T1D GRS1. However, the T1D GRS2 was still developed in cohorts of only European ancestry.

Subsequent and Future Work

In subsequent work the StartRight study, which aimed to assess clinical features and biomarkers at the time of diagnosis in adults (ClinicalTrials.gov identifier: NCT03737799), has demonstrated in a prospective longitudinal analysis that autoantibodies are helpful in classification of type 1 diabetes (Eason et al. unpublished). This study is expected to complete in December 2022, however these results are encouraging and support recent recommendations for routine islet autoantibody assessment in adult-onset type 1 diabetes [8]. Using data from this study, it will now also be possible to retest the clinical diagnostic model to investigate if 4 variables are significantly superior to a model using clinical features and either T1D-GRS or autoantibodies as discussed in Chapter 2, which was impossible within our study of organ donors. It is hypothesised, with the subsequent findings from the StartRight study however, that a 3 variable model will perform just as well as a 4-variable model. In addition, there are approved manuscript proposals to look at combined variable clinical prediction models in the SEARCH for Diabetes in Youth study (SEARCH), a U.S based cohort of individuals diagnosed with type 1 diabetes at <20 years with diverse ancestry, as well as investigation of the T1D-GRS2, in a subset of individuals with C-peptide as an endpoint, investigating genetic prediction of total beta-cell loss.

Additionally, development of genetic risk scores for use in diverse populations customized for ethnicity and race in ongoing. Initial data from Perry et al [9] and others suggest that the T1D-GRS2 will be discriminative in Hispanics, but possibly less so in Africans, although unpublished work from Sharp and collegues suggests that the score works well in South Asian populations.

Future work should seek to validate the combined clinical diagnostic model discussed in Chapter 2, in non-white Europeans, children, and in adults over the age of 50. Additionally, validation of polygenic risk scores for type 1 diabetes in specific ancestry populations and subpopulations is necessary to further inform the best use of these risk scores in non-White ancestries and mixed populations. Combining these new risk scores in models with clinical features and autoantibodies may then offer improved discrimination of type 1 diabetes in mixed populations. Furthermore, validation of the combined clinical diagnostic should be completed using the data collected by the StartRight study to assess the efficacy of a 3-variable model vs. a 4-variable model combining clinical features with autoantibody status and/or genetic risk score is significantly different.

There is also scope to assess the efficacy of different classification algorithms for determining diabetes type. The clinical diagnostic model discussed in Chapter 2 was developed using logistic regression, however many classification algorithms exist from simple to more complex machine learning algorithms. This is currently under development within the polygenic risk score environment, assessing the performance

of machine; particularly deep learning algorithms, in generating improved T1D-GRS for classification and prediction.

Chapter title: Circulating C-peptide levels in living children and young people and pancreatic beta cell loss in pancreas donors across type 1 diabetes

Summary

This chapter builds upon recent work describing type 1 diabetes endotypes that highly correlate with age at diagnosis. Stratifying by age at diagnosis endotypes, I explore the similarities in the trends of decline in C-peptide levels in living children and young people with type 1 diabetes within the UK Genetic Resource Investigating Diabetes (UK GRID) cohort and the trends of decline in beta cells in pancreatic donors from nPOD and Exeter Archival Diabetes Biobank (EADB). This study examines these trends for durations of disease over 10 years and is the first to provide a comparison of pancreatic histology with an independent clinical cohort assessing these trends of loss. A centrepiece of this chapter is what these findings mean in the context of intervention trials.

Implications

A key finding of this work is that among children there are differences in progression of disease, with the youngest of children (<7 years) experiencing rapid depletion of C-peptide and beta cells after 1 year of disease. The data highlights that trialling a safe immunotherapy close to diagnosis is most time-critical in those progressing to disease in very early life. These findings have implications in the planning and interpretation of intervention trials designed to promote beta cell retention and function.

Limitations

Most limitations within this study stems from the histological analyses, mainly due to the nature of the samples. Complete analysis of beta cell area could not be completed in 20 donors, due to lack of adequate pancreatic material for this particular analysis or rarity of material available from some donors of from the 50-year-old archival EADB biobank which precluded re-staining. Additionally due to the circumstances of curation of the EADB biobank (non-systematically collected autopsy samples) there is little information on the anatomical location of the sampled pancreas in the EADB donors. Regional heterogeneities in the distribution and composition of islets have been well studied in rodents finding that the density of beta cell mass in the body and tail regions is higher than in the head region [10, 11]. Similarly, within the head region of the human pancreas, there has been reports that >50% of islet cell volume is represented by pancreatic polypeptide (PP) cells [12–14], which is likely to account for these regional differences in beta cell mass. In a study of type 2 diabetes pancreas, the distribution of islets was found to be >2-fold higher in the tail region than in the head and body regions [15]. Although regional information could be useful in this study, it is unlikely that systematic sampling bias explains the observations described as sampling was random across the donors included in the study.

A further limitation of this study is its cross-sectional design and the dissociated nature of the biobanks used. However, there are no systematic large studies currently available of C-peptide in clinical type 1 diabetes cohorts in whom post-death pancreas samples are available in which the questions asked in this study could be replicated. However, using the samples from nPOD in which at organ-donation C-peptide is

available, we demonstrated evidence to support out primary findings. it should be noted however, that C-peptide levels in nPOD organ donors may be influenced by end-of-life circumstances and must therefore be interpreted with caution. In donors with undetectable C-peptide but who retain insulin+ beta cells, acute glucotoxicity [16] and sample degradation may have contributed to false negative C-peptide results. Furthermore, limited clinical data were available within the UK GRID cohort, in particular, no information was accessible on rates of diabetic ketoacidosis, which as mentioned, is known to be an independent predictor of C-peptide decline [16].

Subsequent and Future Work

The heterogeneity of type 1 diabetes in children driven by underlying endotypes is a fairly recent hypothesis [17, 18]. Within the field there has been some contention that the differences observed are in fact endotypes and the role that age of diagnoses plays within the hypothesis. Subsequent work has set out to confirm the initial findings from Leete et al., focusing mainly on the composition of the immune cell infiltrate, a hallmark of new onset type 1 diabetes known as insulitis [19]. Using a new comprehensive, automated, analysis methodology of multiple islets across a large number of donors with recent-onset type 1 diabetes, this work has substantiated previous conclusions that type 1 diabetes can be subclassified into two endotypes (T1DE1 and T1DE2) (Wyatt et al., unpublished). In addition, donors identified as having T1DE1 endotype, which is characterised by higher inflammation and more rapid loss of beta cells, had a lower age of diagnosis as compared to those identified as T1DE2. The implications of these confirmative findings may have substantial impact on the landscape of intervention trials. In both current and future trials, the

interpretation of results should be within the context of these age at diagnosis endotypes. Many intervention trials have recruited/ are recruiting children at or over the age of puberty (around 13 years) which, as suggested by the aforementioned findings, mainly captures the less aggressive T1DE2 endotype. If we are to see an effective intervention or even a preventative therapeutic, one would speculate that children of younger ages must be recruited into these trials to ensure efficacy within the T1DE1 endotype and to determine just how early this intervention may need to be to be effective.

Chapter title: Measurement Of Peak C-Peptide At Diagnosis Informs Glycemic Control But Not Hypoglycemia In Adults With Type 1 Diabetes

Summary

In this chapter I describe the impact that variation in endogenous insulin secretion close to diagnosis, as measured by stimulated C-peptide, has on glycaemic variability and hypoglycaemia, using Continuous Glucose Monitoring (CGM) data from adults enrolled in the Exercise for Type 1 Diabetes (EXTOD) study with recent onset type 1 diabetes. I propose how these finding enhance what is known about the impact that variation in C-peptide levels have on glucose control in people with type 1 diabetes across duration of disease. I suggest that the clinical impact of C-peptide on a person with type 1 diabetes varies depending on the point from diagnosis; as C-peptide reserve declines with time, and therefore how current estimates of a patient's C-peptide reserve could influence their clinical management.

Implications

The findings from this study demonstrate that variations in the high levels of residual C-peptide present at the time of diagnosis with type 1 diabetes are associated with lower variability measures, but not hypoglycemia or HbA_{1c}, which is observed at longer duration disease. Clinically this is important as this could potentially inform the most effective approach to supporting the newly diagnosed patient, adding further understanding to the glucose patterns observed in this time period, rather than accounting these only to behaviour. Additionally, these findings add to the discussions

within intervention trials surrounding what level of preserved C-peptide offers the greatest clinical benefit.

Limitations

A notable limitation of this study is that this is a retrospective analysis of data collected as part of a randomized controlled trial, using CGM data and peak MMTT C-peptide from participants involved in the EXTOD study, a randomized exercise trial. Participants who enrol in exercise trials may not be wholly representative of the type 1 diabetes population because of their levels of activity and the effect exercise may have on blood glucose which, in turn, may impact the average glucose metrics reported. In addition, sample size was limited to the consent rate to CGM monitoring during the study, and a high dropout rate of CGM monitoring over the 12 months of study. This may have affected the power to detect associations with C-peptide and CGM metrics that describe glycaemic variability. As discussed in Chapter 4, it is unlikely that this is behind the low rates of hypoglycaemia in this cohort. Similar to islet transplant patients, the higher C-peptide levels present close to diagnosis may exceed a threshold needed to protect from hypoglycaemia (minimal islet transplant function has been shown to protect from hypoglycaemia [20]), and as such hypoglycaemia is uncommon at diagnosis [21].

Subsequent and Future Work

Teplizumab is the first trialled therapeutic for type 1 diabetes which has shown success in preventing disease progression in those at risk and in promoting beta cell retention and function [22–25]. However, there is still no consensus on what a “successful”

therapeutic is in this context. With the understanding that C-peptide is influential on glucose control from the point of diagnosis it is important to identify what level of C-peptide confers the greatest benefit to the patient. The Trial Outcome Markers Initiative in Type 1 Diabetes (TOMI-T1D) group, part of the Critical path (C-path) institute, has various work packages directed at unpicking this. Preliminary analysis of data within this initiative is promising in identifying a level of C-peptide that remains beneficial to a patient, through the stabilisation of HbA_{1c} (unpublished, discussions with Professor Colin Dayan, Dr Peter Senior and Dr Peter Taylor as part of the TOMI work package 5 group).

In addition, data from this study and data from the EXTOD study, described in Chapter 4, are being used to develop a better outcome measure that defines "benefit to the patient". Termed the CHAllenge Of Stability (CHAOS) index, this measure is based off the day-to-day predictability of glucose, aiming to better capture the "rollercoaster" of type 1 diabetes over that of standard measures. In doing so this measure also potentially captures aspects of glucose control that influence quality of life often expressed by people with type 1 diabetes (unpublished, discussions with Professor Colin Dayan, Dr Peter Senior and Dr Peter Taylor as part of the TOMI work package 5 group). Although, anecdotally, individuals with type 1 diabetes often report that high levels of glycaemic variability negatively impact mood and quality of life (QoL), there is no current evidence to support this direct relationship [26, 27]. Conversely, hypoglycaemia and the fear of such has been shown to have a profound impact on QoL and diabetes self-care behaviours [28, 29] suggesting this fear of hypoglycaemia may be a driver in glycaemic variability and suboptimal glucose control [29–32].

Future analyses will assess the relationship of C-peptide and quality of life measures, such as fear of hypoglycaemia, with the CHAOS index.

Chapter title: Glycemic variability and hypoglycemia is increased the day after free-living exercise in people with type 1 diabetes

Summary

This chapter focuses on the impact of that a behavioural factor: exercise, has on glycaemic control in people with type 1 diabetes, expanding on the recurrent theme throughout this thesis of the impact of a biological factor: residual C-peptide. Building upon the skills I have refined in Chapter 4; I build upon the CGM analysis pipeline, developing a way of interrogating CGM data in the context of exercise. Utilising intermittently scanned continuous glucose monitoring, I explore the effect of two modes of home-based exercise on glycaemic variability and hypoglycaemia, as these are two of the main barriers to exercise people with type 1 diabetes express.

Implications

This study crucially demonstrates the independent impact of free-living exercise on glycaemic variability and hypoglycaemia by carefully comparing to non-exercise days. Through demonstrating these effects in clinically relevant time windows (4 hours post exercise, overnight and the next day) the findings from this study suggest that developing focused clinical guidance around these time periods post-exercise and accounting for “everyday life” may improve the management of blood glucose in type 1 diabetes and reduce barriers to exercise.

Limitations

This study was an analysis of data from participants enrolled in an exercise trial. As discussed in Chapter 4, participants that enrol in exercise trials may not be wholly representative of the type 1 diabetes population, due to their levels of activity and the overall effect exercise may have on blood glucose. This may have impacted the average glucose metrics that are demonstrated in this cohort.

Additionally, the samples size in this study is small. However, multiple exercise sessions were completed by each individual which improves the robustness of the within person glycaemic trends observed post-exercise. It should be noted however that these repeated bouts of exercise could have influenced glycaemia within the exercise intervention periods through increasing insulin sensitivity. Furthermore, this study focused on exercise in free-living, as such minimal control was had over the completion of the exercise bout. Time-of-day exercise was completed could therefore influence glycemia however, although participants had the choice in time of exercise, most opted for late afternoon/evening exercise. Additionally, participants had a choice in the order in which intervention periods were completed, in order to maximise study retention, and was therefore not randomised. Further to this there was no designated wash-out period between interventions. Reassuringly however, we found no significant difference in 14-day glycaemic outcomes between the order at which intervention periods were completed or the type of intervention period itself.

Another notable limitation could be found in the calculation of maximum heart rate (HR_{max}) used to determine a valid high intensity session or moderate intensity session. As is standard practice in most clinical settings HR_{max} is calculated using the Fox

equation of $HR_{max}=220-age$ [33] however this equation has been reported to have a standard deviation of between 10 and 12 bpm [34] , as well as significantly over and underestimating HR_{max} in younger and older adults, respectively [35, 36]. As the participants in this study were mainly young adults, this could potentially lead to an underestimation of exercise intensity, altering expected glucose changes. However, the gold standard measurement of HR_{max} by graded exercise testing is less accessible in clinical practice and recent study has suggested that the Fox equation may represent the best option for a general population [37], where graded exercise testing is not possible.

Subsequent and Future Work

Subsequent work has focused on the use of the analysis methods developed in Chapter 4 and Chapter 5 for the analysis of CGM data from other exercise studies. In particular, this analysis pipeline has been applied CGM data collected as part of EXTOD education study [38], a randomised control piolet trial testing a structured education programme for safe and effective exercise in type 1 diabetes. Analysis is directed at the comparison of pre and post intervention within clinically relevant time windows (4 hours post exercise, overnight and the next day).

Future work is needed to confirm and expand on the findings in this chapter in larger cohorts. It is hoped that the analysis methods in this chapter that demonstrate careful matching for non-exercise days would be adopted by future studies in this area with the pipeline code being available in the future on GitHub [39].

Conclusions of Thesis

This thesis expands on the evidence that C-peptide is a robust surrogate marker of functional beta cells in clinical settings. Importantly, highlighted within this thesis is the heterogeneity of beta cell loss in type 1 diabetes, demonstrating that the youngest children are a group in which possible intervention therapies would be most time-critical. Additionally, the work in this thesis shows how measures of C-peptide reserve are clinically relevant, having a measurable impact from the point of diagnosis, and should be integrated into management.

Through the development of methods of CGM data analysis, this thesis has offered newly afforded insights not only to the impact that biological factors (C-peptide) have on glycaemic control in type 1 diabetes, but also that of behavioural factors (exercise). These findings offer a better understanding into the factors that influence the “roller coaster” of type 1 diabetes, which could enable improved management strategies that are personalised to the patient.

References

1. Lynam A, McDonald T, Hill A, et al (2019) Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18–50 years. BMJ Open 9(9):e031586. https://doi.org/10.1136/bmjopen-2019-031586

2. Jones AG, Hattersley AT (2013) The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med 30(7):803–817. https://doi.org/10.1111/dme.12159

3. Mayer-Davis EJ, Lawrence JM, Dabelea D, et al (2017) Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. New England Journal of Medicine 376(15):1419–1429. https://doi.org/10.1056/NEJMoa1610187

4. Golden SH, Brown A, Cauley JA, et al (2012) Health Disparities in Endocrine Disorders: Biological, Clinical, and Nonclinical Factors—An Endocrine Society Scientific Statement. J Clin Endocrinol Metab 97(9):E1579–E1639. https://doi.org/10.1210/jc.2012-2043

5. Oram RA, Patel K, Hill A, et al (2016) A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. Diabetes Care 39(3):337–344. https://doi.org/10.2337/dc15-1111

6. Onengut-Gumuscu S, Chen W-M, Robertson CC, et al (2019) Type 1 Diabetes Risk in African-Ancestry Participants and Utility of an Ancestry-Specific Genetic Risk Score. Diabetes Care 42(3):406–415. https://doi.org/10.2337/dc18-1727

7. Sharp SA, Rich SS, Wood AR, et al (2019) Development and Standardization of an Improved Type 1 Diabetes Genetic Risk Score for Use in Newborn Screening and Incident Diagnosis. Diabetes Care dc181785. https://doi.org/10.2337/dc18-1785

8. Holt RIG, DeVries JH, Hess-Fischl A, et al (2021) The management of type 1 diabetes in adults. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 64(12):2609–2652. https://doi.org/10.1007/s00125-021-05568-3

9. Perry DJ, Wasserfall CH, Oram RA, et al (2018) Application of a Genetic Risk Score to Racially Diverse Type 1 Diabetes Populations Demonstrates the Need for Diversity in Risk-Modeling. Sci Rep 8(1):4529. https://doi.org/10.1038/s41598-018-22574-5

10. Trimble ER, Renold AE (1981) Ventral and dorsal areas of rat pancreas: islet hormone content and secretion. American Journal of Physiology-Endocrinology and Metabolism 240(4):E422–E427. https://doi.org/10.1152/ajpendo.1981.240.4.E422

11. Aguayo-Mazzucato C, Sanchez-Soto C, Godinez-Puig V, Gutiérrez-Ospina G, Hiriart M (2006) Restructuring of Pancreatic Islets and Insulin Secretion in a

Postnatal Critical Window. PLOS ONE 1(1):e35. https://doi.org/10.1371/journal.pone.0000035

12. Orci L, Malaisse-Lagae F, Baetens D, Perrelet A (1978) PANCREATIC-POLYPEPTIDE-RICH REGIONS IN HUMAN PANCREAS. The Lancet 312(8101):1200–1201. https://doi.org/10.1016/S0140-6736(78)92181-5

13. RAHIER J, WALLON J, LOOZEN S, LEFEVRE A, GEPTS W, HAOT J (1983) The Pancreatic Polypeptide Cells in the Human Pancreas: The Effects of Age and Diabetes*. The Journal of Clinical Endocrinology & Metabolism 56(3):441–444. https://doi.org/10.1210/jcem-56-3-441

14. Stefan Y, Orci L, Malaisse-Lagae F, Perrelet A, Patel Y, Unger RH (1982) Quantitation of Endocrine Cell Content in the Pancreas of Nondiabetic and Diabetic Humans. Diabetes 31(8):694–700. https://doi.org/10.2337/diab.31.8.694

15. Wang X, Misawa R, Zielinski MC, et al (2013) Regional Differences in Islet Distribution in the Human Pancreas - Preferential Beta-Cell Loss in the Head Region in Patients with Type 2 Diabetes. PLoS One 8(6):e67454. https://doi.org/10.1371/journal.pone.0067454

16. Komulainen J, Lounamaa R, Knip M, Kaprio EA, Akerblom HK (1996) Ketoacidosis at the diagnosis of type 1 (insulin dependent) diabetes mellitus is related to poor residual beta cell function. Childhood Diabetes in Finland Study Group. Archives of Disease in Childhood 75(5):410–415. https://doi.org/10.1136/adc.75.5.410

17. Leete P, Willcox A, Krogvold L, et al (2016) Differential Insulitic Profiles Determine the Extent of β-Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes 65(5):1362–1369. https://doi.org/10.2337/db15-1615

18. Leete P, Oram RA, McDonald TJ, et al (2020) Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia 63(6):1258–1267. https://doi.org/10.1007/s00125-020-05115-6

19. In't Veld P (2011) Insulitis in human type 1 diabetes. Islets 3(4):131–138. https://doi.org/10.4161/isl.3.4.15728

20. Vantyghem M-C, Raverdy V, Balavoine A-S, et al (2012) Continuous Glucose Monitoring after Islet Transplantation in Type 1 Diabetes: An Excellent Graft Function (β-Score Greater Than 7) Is Required to Abrogate Hyperglycemia, Whereas a Minimal Function Is Necessary to Suppress Severe Hypoglycemia (β-Score Greater Than 3). J Clin Endocrinol Metab 97(11):E2078–E2083. https://doi.org/10.1210/jc.2012-2115

21. Buckingham B, Cheng P, Beck RW, et al (2015) CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but do poorly in predicting C-peptide levels in the two years following onset of diabetes. Diabetologia 58(6):1167–1174. https://doi.org/10.1007/s00125-015-3559-y

22. Herold KC, Hagopian W, Auger JA, et al (2002) Anti-CD3 Monoclonal Antibody in New-Onset Type 1 Diabetes Mellitus. New England Journal of Medicine 346(22):1692–1698. https://doi.org/10.1056/NEJMoa012864

23. Hagopian W, Ferry RJ, Sherry N, et al (2013) Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomized, placebo-controlled Protégé trial. Diabetes 62(11):3901–3908. https://doi.org/10.2337/db13-0236

24. Herold KC, Bundy BN, Long SA, et al (2019) An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N Engl J Med 381(7):603–613. https://doi.org/10.1056/NEJMoa1902226

25. Sims EK, Bundy BN, Stier K, et al (2021) Teplizumab improves and stabilizes beta cell function in antibody-positive high-risk individuals. Science Translational Medicine 13(583):eabc8980. https://doi.org/10.1126/scitranslmed.abc8980

26. Wilmot EG, Choudhary P, Leelarathna L, Baxter M (2019) Glycaemic variability: The under-recognized therapeutic target in type 1 diabetes care. Diabetes Obes Metab 21(12):2599–2608. https://doi.org/10.1111/dom.13842

27. Reddy M, Godsland IF, Barnard KD, et al (2015) Glycemic Variability and Its Impact on Quality of Life in Adults With Type 1 Diabetes. J Diabetes Sci Technol 10(1):60–66. https://doi.org/10.1177/1932296815601440

28. Chatwin H, Broadley M, Jensen MV, et al (2021) 'Never again will I be carefree': a qualitative study of the impact of hypoglycemia on quality of life among adults with type 1 diabetes. BMJ Open Diabetes Research and Care 9(1):e002322. https://doi.org/10.1136/bmjdrc-2021-002322

29. Wild D, von Maltzahn R, Brohan E, Christensen T, Clauson P, Gonder-Frederick L (2007) A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. Patient Educ Couns 68(1):10–15. https://doi.org/10.1016/j.pec.2007.05.003

30. Cryer PE (2008) Hypoglycemia: still the limiting factor in the glycemic management of diabetes. Endocr Pract 14(6):750–756. https://doi.org/10.4158/EP.14.6.750

31. Martyn-Nemeth P, Quinn L, Penckofer S, Park C, Hofer V, Burke L (2017) Fear of hypoglycemia: Influence on glycemic variability and self-management behavior in young adults with type 1 diabetes. J Diabetes Complications 31(4):735–741. https://doi.org/10.1016/j.jdiacomp.2016.12.015

32. Adolfsson P, Rentoul D, Klinkenbijl B, Parkin CG (2018) Hypoglycaemia Remains the Key Obstacle to Optimal Glycaemic Control – Continuous Glucose Monitoring is the Solution. Eur Endocrinol 14(2):50–56. https://doi.org/10.17925/EE.2018.14.2.50

33. Fox SM, Naughton JP, Haskell WL (1971) Physical activity and the prevention of coronary heart disease. Ann Clin Res 3(6):404–432

34. American College of Sports Medicine (last) (2013) ACSM's Guidelines for Exercise Testing and Prescription. Lippincott Williams & Wilkins

35. Gellish RL, Goslin BR, Olson RE, McDonald A, Russi GD, Moudgil VK (2007) Longitudinal modeling of the relationship between age and maximal heart rate. Med Sci Sports Exerc 39(5):822–829. https://doi.org/10.1097/mss.0b013e31803349c6

36. Tanaka H, Monahan KD, Seals DR (2001) Age-predicted maximal heart rate revisited. J Am Coll Cardiol 37(1):153–156. https://doi.org/10.1016/s0735-1097(00)01054-8

37. Shookster D, Lindsey B, Cortes N, Martin JR (2020) Accuracy of Commonly Used Age-Predicted Maximal Heart Rate Equations. Int J Exerc Sci 13(7):1242–1250

38. Narendran P, Greenfield S, Troughton J, et al (2020) Development of a group structured education programme to support safe exercise in people with Type 1 diabetes: the EXTOD education programme. Diabet Med 37(6):945–952. https://doi.org/10.1111/dme.14064

39. Carr ALJ (2021) CGMProcessing, R 2021 https://github.com/alicelouisejane/CGMprocessing

APPENDIX

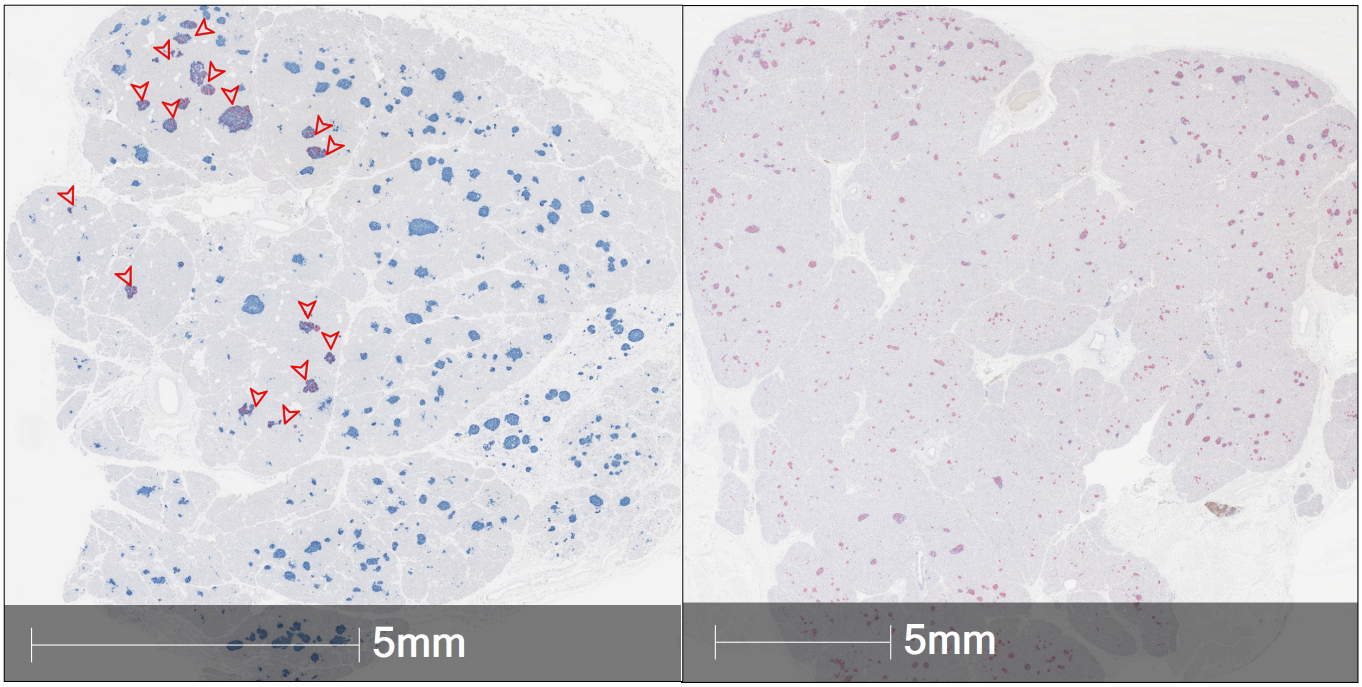
Appendix 1- histological analyses

Appendix 1.1

Example of alignment of serial sections, single-stained for insulin and glucagon. This example is demonstrated using the HALO V3.0 image analysis software (Indica Labs). Using the glucagon stained section islets are identified using the Random Forest Classifier Module as groups of endocrine cells covering an area of ≥ 1000 μm^2. The serial-stained insulin section is linked, using the software, to the glucagon section which enables an overlay of the outline of islets identified in this section and identification of insulin positive islets.

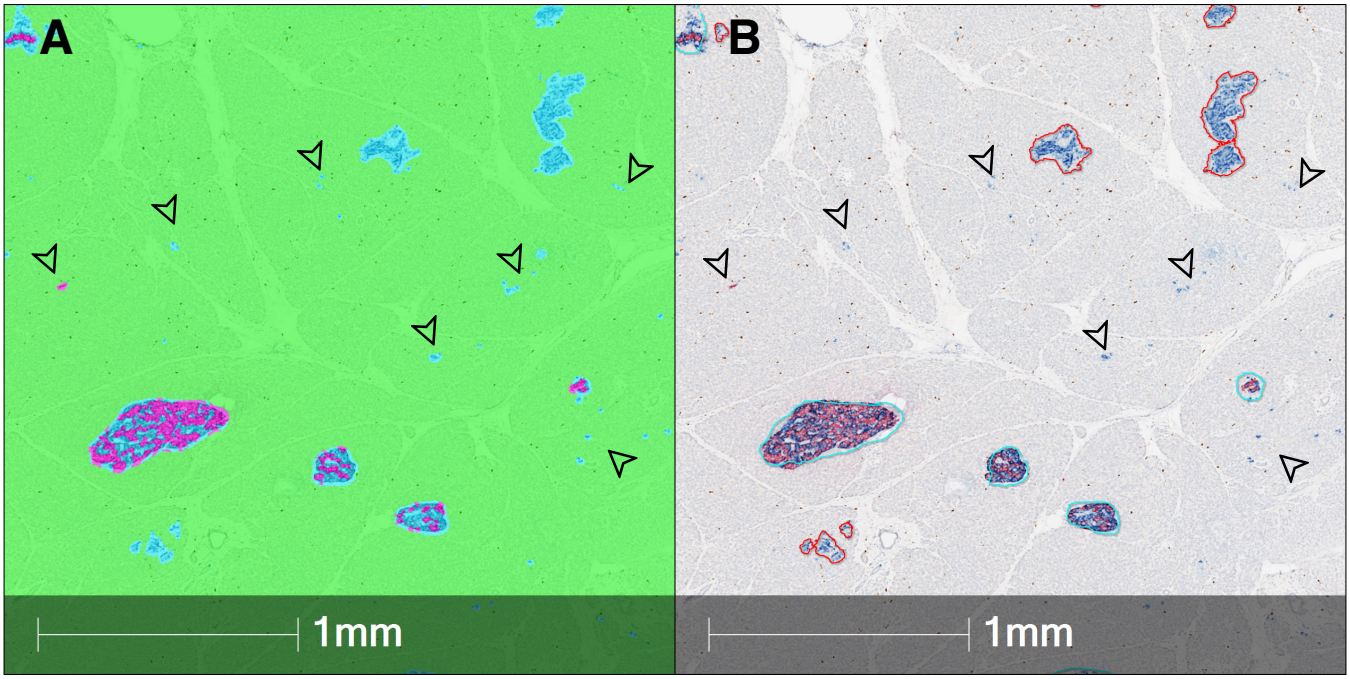
Appendix 1.2

A representative section from nPOD donor 6484 (location: pancreases other) with type 1 diabetes immunostained for insulin (red) and glucagon(blue) demonstrating lobular loss of islets containing insulin positive beta cells (red arrows) as compared to a pancreas from nPOD donor 6508 (location: pancreas other) without type 1 diabetes where all islets contain insulin.



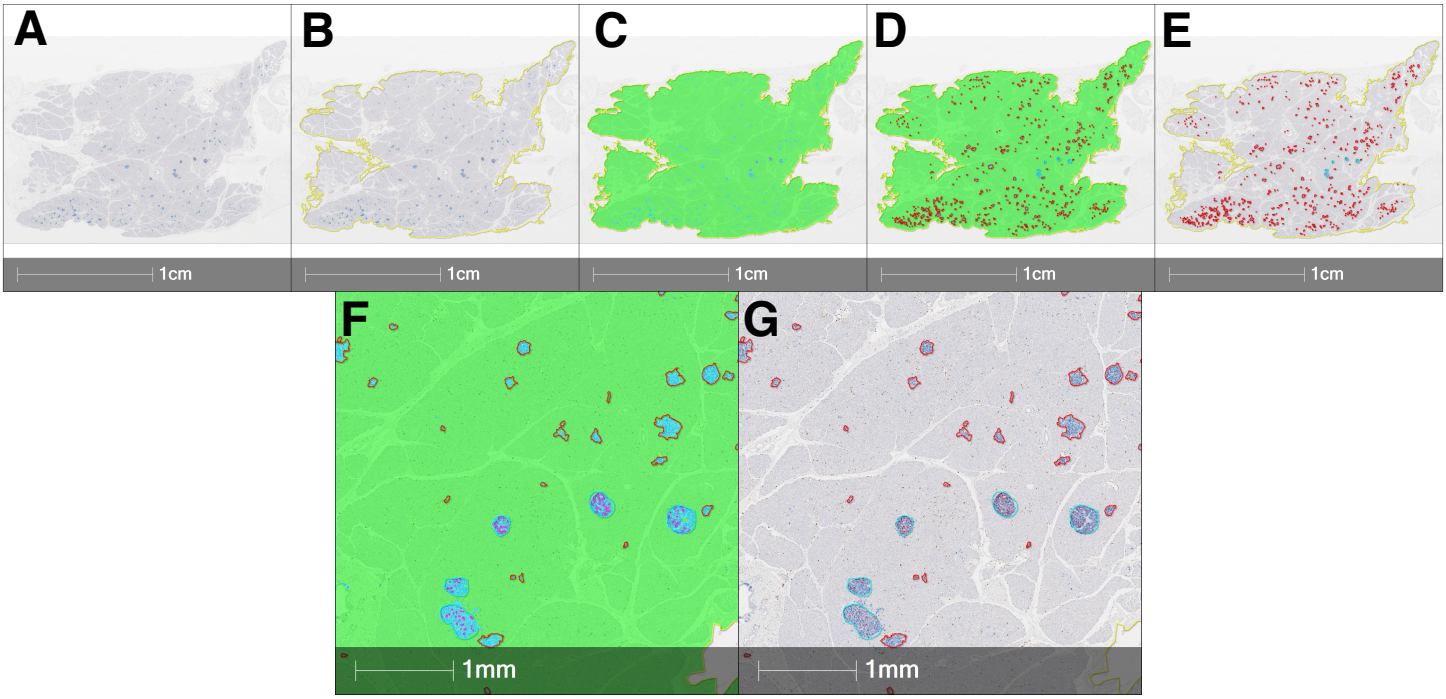
Appendix 1.3

Example of islet classification by the Random Forest Classifier Module from the HALO V3.0 image analysis software (Indica Labs). Islets are defined in the classifier as groups of endocrine cells covering an area of ≥ 1000 μm^2. In **A** there are small numbers of cells staining for insulin (magenta) and glucagon (cyan) (black arrows). However as they do not cover an area of ≥ 1000 μm^2 this is not classified as an islet as outlined in red or cyan in **B**. Occasionally there are small numbers of cells that may stain for insulin or glucagon which, in most cases, is a artifact of sectioning. (nPOD donor 6472, location: pancreas other)



Appendix 1.4

A representative pipeline of analysis using HALO image analysis platform (Indica labs) to identify insulin positive islets and calculate beta cell area completed on a section from nPOD donor 6443 (location: pancreas tail) with type 1 diabetes immunostained for insulin (red) and glucagon (blue) (**A**). (**B**) Following identification of the outer section area (yellow line), (**C**) a Random Forest classifier within the HALO image analysis platform is used to identify insulin positive – pink, glucagon positive –blue and other tissue areas – green (close up in **F**). At this point insulin positive and glucagon positive area is calculated which is used in the beta cell area calculation in Chapter 3. (**D**) Each islet is then classified as either insulin-positive (cyan lines) or insulin-negative (red lines). At this point, islet count and proportion of islets containing insulin positive beta cells can be calculated, as described in Chapter 3. (**E**) Demonstrates the final classification of insulin-positive and insulin-negative (close up in **G)**.



Appendix 2 – Continuous Glucose Monitoring

Appendix 2.1- Coefficient of variation

Coefficient of variation (CV) is one of the 14 key metrics outlined by the International Consensus of Continuous Glucose Monitoring as important to report in the analysis of Continuous Glucose Monitoring data. The simple equation for CV is as follows:

$$CV = \frac{standard\ deviation\ of\ glucose}{average\ glucose} \times 100$$

CV a widely accepted as an index for assessing within-day glycaemic variability independently of average glucose. Monnier et al (Monnier, Diabetes Care 2017) defined a threshold for CV at >36% to distinguish between stable and unstable glucose within a mixed type 1 and type 2 diabetes cohort. This threshold was later adopted by the International Consensus of Continuous Glucose Monitoring. The limit of 36% was chosen as beyond this limit the frequency of hypoglycaemia was increase, particularly on those insulin-treated.

Although a useful measure for outlining glycaemic variability in relation to hypoglycaemia, the metric has its limitations at the extremes of both standard deviation and average glucose. It is important to interpret this metric in the context of average glucose to ensure it is reflective of desired glycaemic targets.

Appendix 2.2- CGMprocessing

CGMprocessing is a work package of sharable R code scripts that provide functions for preparing, cleaning and analysing Continuous Glucose Monitoring (CGM) data aligned with consensus definitions (ref international concensus). The functions in the

package were developed from Dexcom G4 data with 5 minute intervals and was specifically developed to enable processing and analysing of data used in Chapter 4 of this thesis. The scripts have been modified to deal with Abbott Freestlye Libre data, with a parameter within the function set to TRUE or FALSE depending on this.

All code within CGM processing and full instructions on how to use can be found at https://github.com/alicelouisejane/CGMprocessing. Below is a print out from GitHub of the README for *CGMProcessing* containing descriptions of the functions *cleanCGM* and *analyseCGM*.

Additionally, scripts within CGM processing have been built upon for use in analysis of exercise/post-exercise time aligned CGM data, as used in Chapter 5 of this thesis (described briefly in the exerciseanalysis parameter of the *analyseCGM* function*).* Code for this time aligning pipeline is not yet open source and is therefore not shared within this thesis. Additionally, the code for creating average time-matched control data for comparison with exercise data is not shared within this thesis but is described in Chapter 5.

CGMProcessing

The goal of {CGMprocessing} is to provide functions for preparing, cleaning and analysing Continuous Glucose Monitoring (CGM) data. The functions in the package were developed from Dexcom G4 data with 5 minute intervals and was specifically developed to enable processing and analysing of data from the 2 separate EXTOD Randomised Control Trials (RCT) lead by Professor Rob Andrews and Dr Parth Narendran See here for published information about the EXTOD Education program and here for the published EXTOD RCT.

Glycemic variables and definitions are based off the International Concensus on Use of Continuous Gucose Monitoring.

This code was developed based off work by T. Vigers

File Structure

- **/data-raw**: raw data from sensor
- **/data-preprocessed**: Exeter specific preprocessed data from sensor
- **/data-clean**: data ran through cleanCGM() function
- **/CGMupload**: Output csv of glycemic metrics generated from analysedCGM() function

❗ **Important**: As this was developed for analysis of RCT data files preprocessed are outputted with the filename formatted as *ID_timepoint.csv* based on the excel sheet name. If dealing with RCT data please ensure files are named in this way before running through cleanCGM() and analyseCGM() pipeline. Edit scripts if neccesary if not dealing with RCT data ❗

Pre-processing of CGM files (Relevent to Exeter in house data)

Functionality: The script **prepare_spitsheets.R** is a rough script pipeline (non-function) that prepares files from EXTOD and EXTOD education for further processing with functions part of {CGMprocessing}. Chunks of traces with non-consecutive dates are separated with **wrongstart** appended to traces prior to the final consecutive chunk. The final chunk with consecutive dates is outputted to **/data-preprocessed**. Manual inpection is recommended at this point to inspect and remove any **wrongstart** files from this folder. ___

- **cgmvariable_dictionary.xlsx** is used to rename variables of interest. This could be updated for variable names of other sensors and integrated into cleanCGM() function. Final variable names should be **id**, **timestampfp** ,**fingerprickglucose**, **timestamp**, **sensorglucose** defined as below.

Table: Definitions of the final variables

variable	definition
id	Patient ID or sensor ID
timstampfp	Finger stick SMBG timestamp (optional and sensor dependent)
fingerprickglucose	Finger stick SMBG value
timestamp	Sensor glucose timestamp
sensorglucose	Sensor glucose value

Table: Dictionary for renaming old variables in raw CGM data files, edit as required

old_vars	new_vars
id	id
date_and_time_event_4	timestampfp
date_and_time_event_5	timestampfp
finger_prick_glucose	fingerprickglucose
date_and_time_event_7	timestamp
cgms_glucose_reading	sensorglucose
displaytime	timestampfp
value	fingerprickglucose
displaytime3	timestamp
value4	sensorglucose
glucosedisplaytime	timestamp
glucosevalue	sensorglucose
meterdisplaytime	timestampfp
metervalue	fingerprickglucose
serial.number	id
meter.timestamp	timestamp
record.type	recordtype
historic.glucose.mmol.l.	sensorglucose
Scan.Glucose.mmol.L.	scanglucose

- Files from preprocessing are outputted with changed CGM variable names already and reformatted slightly (conversion of HI/LO etc.) compared to raw data as below:

Table: Preprocess script output

id	timestampfp	fingerprickglucose	timestamp	sensorglucose
10013	14/06/2013 13:24	8.88	14/06/2013 13:54	10.88
10013	14/06/2013 13:50	12.49	14/06/2013 13:59	11.27
10013	14/06/2013 18:18	8.32	14/06/2013 14:04	11.32
10013	15/06/2013 17:42	6.22	14/06/2013 14:09	11.43
10013			14/06/2013 14:14	11.54
10013			14/06/2013 14:19	11.6
10013			14/06/2013 14:24	11.76

- Files are named as **ID_timepoint.csv** based on the excel sheet name

❗ **Important**: **Glucose readings must be in mmmol/l. Manually change files in raw excel files. More information on conversion found here ❗

cleanCGM

Functionality: cleanCGM() is a function written to clean CGM data for simpler file outputs and perform (optional) calibration against fingerstick SMBG values developed of Dexcom G4 data. ___ - Function can take raw files from Dexcom, Libre or previosuly preprocessed from an input folder directory. Files can be of any format, csv is preferred.

❗ **Important:** Files should be named as *ID_optional.ext* ❗

- Text Low/High are in filled with the min/max limits depending on the inputted sensor type
- Calibration is performed againsted logged fingerstick SMBG readings and nearest 15 min later sensor reading. Calibration excluded 1) whole traces if 2 blood glucose calibrations were not completed at the start of the sensor wear, 2) a day of wear if the MARD of the sensor glucose and blood glucose calibration on that day is >20% or if <2 blood glucose calibrations were completes on that day. This can be set to false for CGM.

-If calibration check is TRUE then the calibration table of fingerstick SMBG matched to nearest 15 min later sensor glucose with the correlation (checking there were 2 fingersticks per day) and the MARD between the sensor and fingerstick with be output to the specified calibrationoutput directory

👷‍♀️ ***FOR DEVELOPMENT*** 👷 *:Libre sensors store glucose every 15 mins, in order for analyseCGM to work based consensus CGM analysis here we must make the 15 min intervaltimeseries data into 5 min interval data. Currently cleanCGM() handles this with the line below, adding dummy 5 min data by adding 2 rows after every original row that is the same as the original row :*

```
r table<-slice(table,rep(1:n(), each = 3))
```

- Each file outputed should look like the below table:

Table: Cleaned CGM output

id	timestamp	sensorglucose	Date	percent_cgm_wear	percentage_expected_wear	percenageexpectedwear_7daycut
1010	20/09/2018 15:20	8.27	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:25	8.38	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:30	8.1	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:35	8.16	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:40	8.38	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:45	8.44	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:50	7.88	20/09/2018	92.2418107	98.4580026	98.4580026
1010	20/09/2018 15:55	7.94	20/09/2018	92.2418107	98.4580026	98.4580026

analyseCGM

Functionality: analyseCGM() is a function written to create concensus glycemic metrics based off definitions outlined here. Fuction takes files from the **data-clean** folder where the output of cleanCGM() is stored. ___

🖉 **NOTE**: There is an paramater for exercise analysis specified in this function specific to in house Exeter/Liverpool processing of data. This additional option is used to analyse specific files of exercise aligned glucose that were not ran through the cleanCGM() function. Keep parameter as FALSE.🖉

- For calculation of time spent variables data is checked to be consecutive. If timestamps are >20 min apart a missing row is added to the table to prevent events from runnning on if the time gap is >20 min.
- Time spent variables are created for: - Above 10, 13.9, 16 - Below 3, - Range 3-<3.9, 3.9-10 - Hyperglycemia (at levels >10 and >13.9) - Hypoglycemia
- Hyper/hypoglycemia are defined as excursions. The start of an excursion is going above/below the specified value for 15 mins. If this doesn't happpen it is not defined as an excursion and isn't included in the hyper/hypo time spent, but will be included in the general time spent for the defined time spent variables.

🖉 **NOTE**:

In this function Hypoglycemia is defined as:

Beginning of a CGM event: readings below 3 mmol/l for 15 min defines a clinically significant hypoglycemic event. Code checks if 4 consecuative rows are below 3 (4 rows in total: row 1 detected below 3mmol/l the next 3 rows = 15 mins with 1 row being assumed as a 5 min reading). If this is the case then the first isantace of dropping below 3 is marked as the start row of a true hypoglycemia event

End of a CGM event: readings for 15 min at ≥ 3.9 mmol/l. From the start row of the true hypoglycemic event defined above the code checks if the glucose in consecutive rows is ≥ 3.9 mmol/l and marks these values as hypoglycemia. At the row glucose becomes > 3.9 the code checks if the next 3 rows remain > 3.9 mmol/l (4 rows in total: row 1 detected > 3.9 mmol/l the next 3 rows = 15 mins with 1 row being assumed as a 5 min reading). If glucose dips below 3.9 again within these 4 rows then the event does not end. If the glucose stays above 3.9 for these 4 rows the event ends at the end of the last row ie. including the 15 min of remaining above 3.9 mmol/l in the hypoglycemia time.

If a missing row is present (inserted because the consecuative timestamps had a gap >20 min) the hypoglycemic event has to end at this point).
🖉 ___

- All CGM variables generated by analyseCGM() are detailed below:

Table: CGM metrics generated for each individual

Output variable	Description
subject_id	Patient ID
totaltime_mins	Total time in the table, this is caluclted from the number of rows present, assuming each row is 5 min reading NOT max and min as this would not be accurate if there were gaps in the data
start_cgm_analysis	min datetime in table
end_cgm_analysis	max datetime in table
interval	Most common interval in the data, for CGM this is 300 seconds (5min) for libre this is put to 900 seconds (15 min)
num_days_good_data	Total time / (246060))
num_hrs_good_data	Total time / 3600
total_sensor_readings	Total number of rows in the table
percent_cgm_wear	Calculated in cleanCGM() this is the amount of data left post calibration (ie. When dates may have been removed)
percentage_expected_wear	Calculated in cleanCGM() Before cutting data at 7 days this is the amount of data we have vs what we expected based on expected 7 days of wear (before calibration)
percentage_expected_wear_aftersensorlifetimecutoff	Calculated in cleanCGM() When we have cut data off at 7 days. This is the amount of data we have vs what we expected based on expected 7 days of wear (before calibration)
average_sensor	Average sensor glucise
estimated_a1c	Estimated Hba1C based on hba1c equation and mean glucose
gmi	Glucose Management Indicator inndicates the average Hba1C level that would be expected based on mean. gmi and estimated Hba1C should therefore be similar
q1_sensor	Lower quartile sensor glucose
median_sensor	Median sensor glucose
q3_sensor	upper quartile sensor glucose
standard_deviation	Standard deviation of sensor glucose
cv	Coefficient of variation. NOTE: SD is highly influenced by the mean glucose – someone with a higher mean glucose will have a higher SD. The CV divides the SD/mean x100. This division helps "correct" and normalize glucose variability, allowing us to set a single variability goal that applies to people with different mean glucose levels.
min_sensor	Minimum sensor glucose

max_sensor	Maximum sensor glucose
excursions_over_10	Count of number of times glucose went above 10 mmol/l for 15 mins (excursion start defined as above threshold fpr 15 mins)
min_spent_excursion_over_10	Time in mins spent in excursions above 10 mmol/l (excursion start defined as above threshold fpr 15 mins)
percent_time_excursion_over_10	Percentage of time spent in excursions above 10 mmol/l in relation to total time of the sensor calculated previously (excursion defined as above threshold fpr 15 mins)
excursions_over_13	Count of number of times glucose went above 13.9 mmol/l for 15 mins (excursion start defined as above threshold fpr 15 mins)
min_spent_excursion_over_13	Time in mins spent in excursions above 13.9 mmol/l (excursion start defined as above threshold fpr 15 mins)
percent_time_excursion_over_13	Percentage of time spent in excursions above 13.9 mmol/l in relation to total time of the sensor calculated previously (excursion defined as above threshold fpr 15 mins)
excursions_over_16	Count of number of times glucose went above 16 mmol/l for 15 mins (excursion start defined as above threshold fpr 15 mins)
min_spent_excursion_over_16	Time in mins spent in excursions above 16 mmol/l (excursion start defined as above threshold fpr 15 mins)
percent_time_excursion_over_16	Percentage of time spent in excursions above 16 mmol/l in relation to total time of the sensor calculated previously (excursion defined as above threshold fpr 15 mins)
hypo_under_3_prolonged	Count of number of times hypoglycemic episode (as defined see below) was > 2 hours
hypo_under_3	Count of number of hypoglycemic episodes. Clinically significant hypoglycemic event excursion begins as readings below 3 mmol/l for 15 min, ending when readings for 15 min at ≥ 3.9 mmol/l. See below NOTE for more information.
min_spent_under_hypo3	Time in mins spent in hypoglycemia episode
percent_time_under_hypo3	Percentage of time spent in hypoglycemic episodes in relation to total time of the sensor calculated previously
min_spent_<3	Time in mins spent below 3 mmol/l
percent_time_<3	Percentage of time spent below 3 mmol/l in relation in relation to total time of the sensor calculated previously
min_spent_3_3.8	Time in mins spent between 3-3.9 mmol/l
percent_time_3_3.8	Percentage of time spent between 3-3.9 mmol/l in relation in relation to total time of the sensor calculated previously
min_spent_3.9_10	Time in mins spent between 3.9-10 mmol/l
percent_time_3.9_10	Percentage of time spent between 3.9-10 mmol/l in relation in relation to total time of the sensor calculated previously
min_spent_3.9_7.8	Time in mins spent between 3.9-7.8 mmol/l
percent_time_3.9_7.8	Percentage of time spent between 3.9-7.8 mmol/l in relation in relation to total time of the sensor calculated previously
min_spent_over10	Time in mins spent above 10 mmol/l
percent_time_over10	Percentage of time spent above 10 mmol/l in relation in relation to total time of the sensor calculated previously
min_spent_over13	Time in mins spent above 13.9 mmol/l
percent_time_over13	Percentage of time spent above 13.9 mmol/l in relation in relation to total time of the sensor calculated previously
total_auc	Total area under the glucose curve
r_mage	Mean Amplitude Glycemic Excursion. Option to asses average of the differences greater than either entire dataset SD, 2SD, etc
j_index	Combination of information from mean and SD of all glucose values doi: 10.1055/s-2007-979906.
conga_1	Continuous overlapping net glycemic action, with _n appended as the indicated number of hours being assessed (in this case 1 hour)
modd	Mean of daily difference
lbgi	Low Blood Glucose Index, equation is for glucose in mmol/l (dc1386 appendix)
hbgi	High Blood Glucose Index, equation is for glucose in mmol/l (dc1386 appendix)
timepoint	The RCT timepoint (specifc to the filenanes given to Exeter inhouse data, comment this out of code if not necessary)

Appendix 3 – chapter supplementary material

Appendix 3.1- Chapter 2: Histological validation of a type 1 diabetes clinical diagnostic model for classification of diabetes

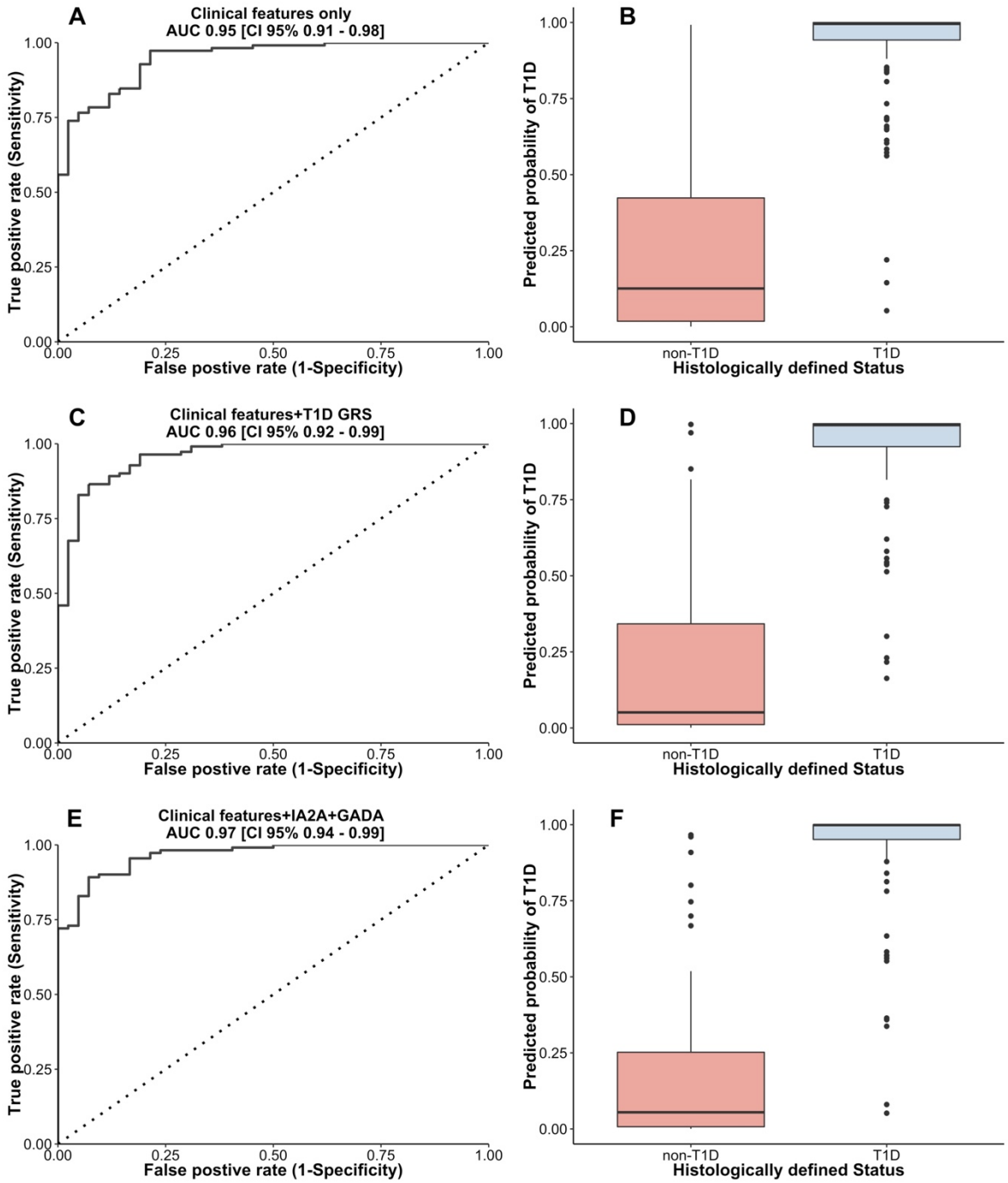


Figure S1. The comparative discriminative ability of diagnostic model variations 1,2 and 3 to identify type 1 diabetes cases. Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) statistics and boxplot of predicted probabilities of type 1 diabetes are shown for model 1 (A-B), model 2 (C-D) and model 3 (E-F).

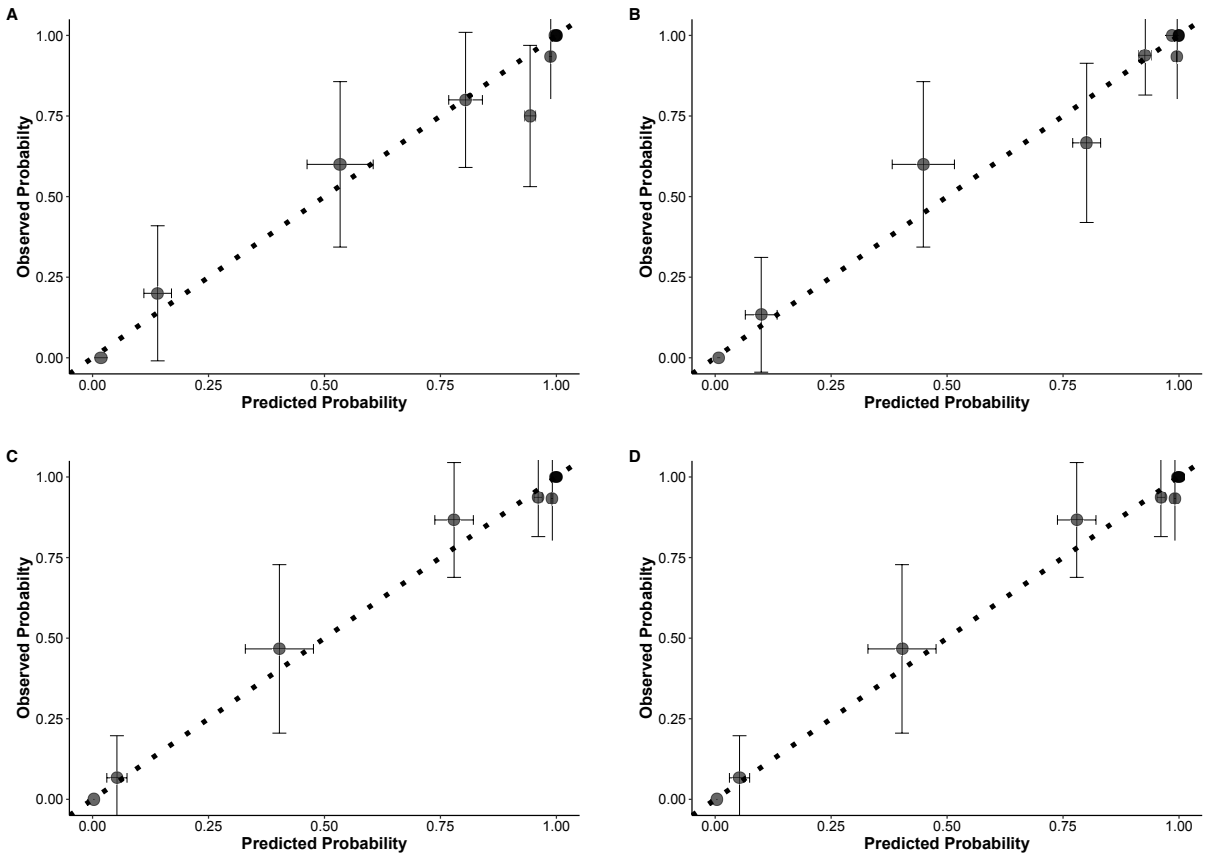


Figure S2: Calibration plots for 4 variations of a clinical diagnostic model 1-4 (A-D respectively). Dashed line represents the line of perfect calibration. Points represent observed proportion against mean predicted probability for deciles. Error bars represent standard error.

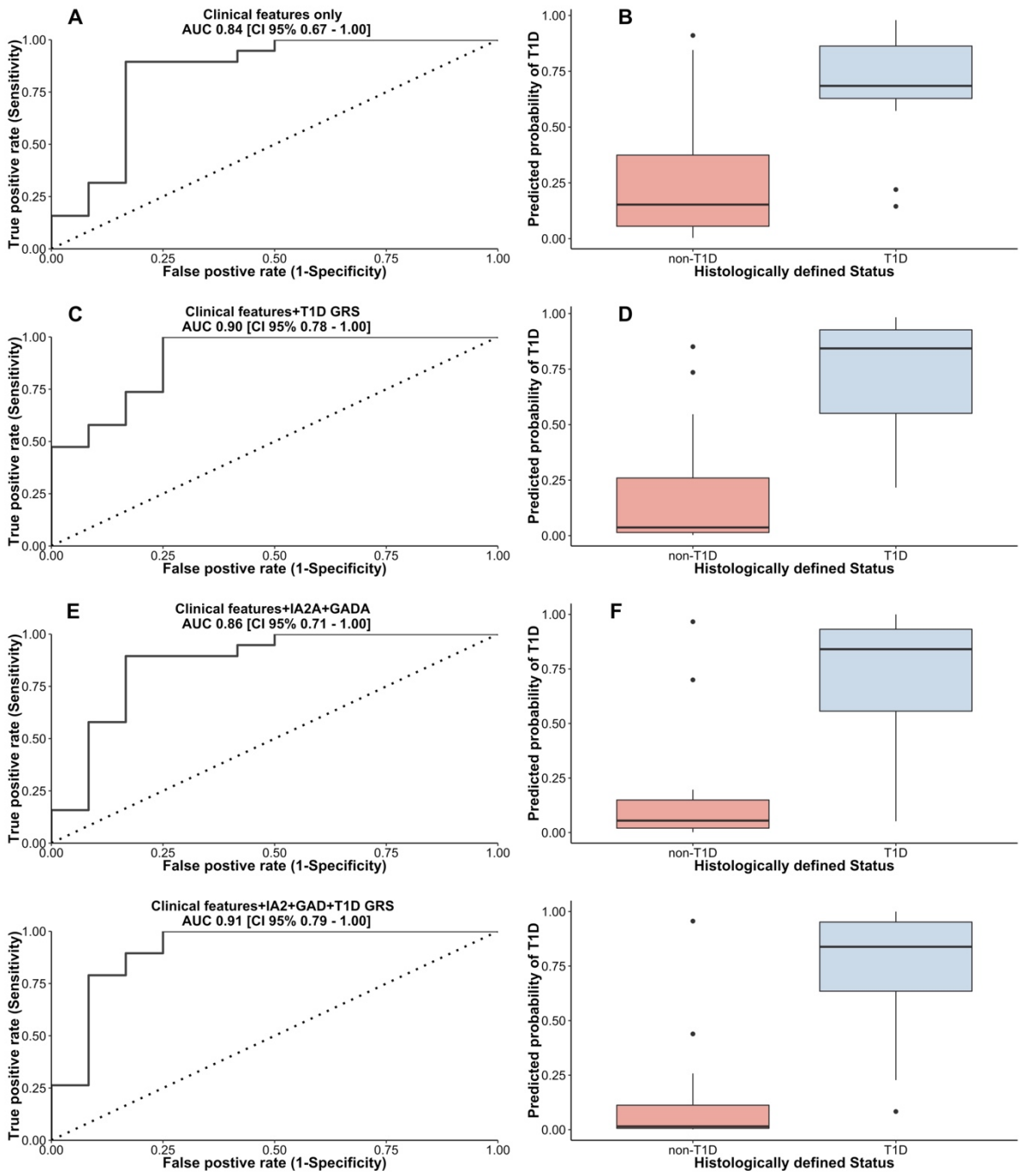


Figure S3: The comparative discriminative ability for all variations of the diagnostic model to identify type 1 diabetes cases in a White European subset diagnosed 18-50 years of age. Receiver operating characteristic (ROC) curve and corresponding area under the curve (AUC) statistics and boxplot of predicted probabilities of type 1 diabetes are shown for model 1 (A-B), model 2 (C-D), model 3 (E-F) and model 4 (G-H).

TABLE S1: Table of regression equations from a combined prediction model developed in a separate clinical cohort as previously described (17). Table adapted from (17). *To convert to probability use exp(lp)/(1+exp(lp)). †Dummy variable: negative = 0, positive = 1 ‡Dummy variables: false = 0, true = 1, AntiStatus1 = GADA positive only, AntiStatus2 = IA-2 positive only, AntiStatus3 = Both GADA and IA-2 positive.

Model	Linear predictor (lp) regression equation*
Clinical features	37.94 + (-5.09 * log(age)) + (-6.34 * log(BMI))
Clinical features + T1D GRS	24.46138054 + (-4.443506884 * Log(Age)) + (-5.534741384 *Log(BMI)) + (33.93968 * T1D GRS)
Clinical features + GADA + IA-2	33.49649577 + (-4.665598345 * Log(Age)) + (-5.81137397 * Log(BMI)) + (3.082366 * AntiStatus1‡) + (3.494462 * AntiStatus2‡) + (4.350717 * AntiStatus3‡)
Clinical features + GADA + IA-2 + T1D GRS	21.57649882 + (-4.086215772 * Log(Age)) + (-5.096252172 * Log(BMI)) + (2.702010666 * AntiStatus1‡) + (3.063255174 * AntiStatus2‡) + (3.813850704 * AntiStatus3‡) + (30.11052 * T1D GRS)

TABLE S2: Characteristics of White European subset diagnosed 18-50 years of age of histologically defined T1D cohort

	Non-T1D N=12	T1D N=19
BMI (kg/m2), Median [25th;75th]	29.0 [25.7;33.2]	25.5 [23.5;27.2]
Age at diagnosis (yrs), Median [25th;75th]	36 [30;42]	26 [21;29]
Diabetes duration (yrs), Median [25th;75th]	11 [5;18]	11 [6;19]
Age of death (yrs), Median [25th;75th]	48 [43;56]	39 [29;48]
Sex, N (%):		
Female	6 (50%)	7 (37%)
Male	6 (50%)	12 (63%)
GRS, Median [25th;75th]	0.22 [0.19;0.24]	0.27 [0.25;0.28]
C-peptide (pmol/l), Median [25th;75th]	432 [169;694]	<17* [<17*;22]
Islet autoantibodies†, N (%):		
0	11 (92%)	9 (47%)
1	1 (8.3%)	9 (47%)
2	0 (0.0%)	0 (0.0%)
3	0 (0.0%)	1 (5.3%)
Race, N (%): White Europeans	12 (100%)	19 (100%)

*Limit of detection

† Islet autoantibodies counted include GADA, IA-2A and ZnT8A. mIAA is not included in this count as it is not a reliable marker of autoimmunity in persons receiving exogenous insulin.

TABLE S3: Comparison of calibration statistics for clinical diagnostic model, model combinations 1-4.

Model	C Statistic (AUC)	Brier score (B)	Spiegelhalter *z*-statistics (Z)	mean P [CI 95%]
1	0.95	0.08	1.76 (p=0.08)	74% [68%, 80]%
2	0.96	0.07	1.03 (p=0.30)	72% [66%, 79%]
3	0.97	0.06	1.71 (p=0.08)	73% [67%, 79%]
4	0.97	0.06	0.95 (p=0.34)	72% [65%, 78%]

TABLE S4: Characteristics of cases with discordant model 4 prediction and histological classification

nPOD Case ID	Probability of T1D from model 4	BMI (kg/m^2)	Age at diagnosis (yrs)	Diabetes duration (yrs)	Age of death (yrs)	Sex	GRS	C-peptide (pmol/l)	Islet autoantibodies †	Race	Histology	nPOD Status
6040	0.083	31.6	30	20	50	Female	0.250	<17*	0	White European	T1D	T1D
6127	0.227	30.4	34	10	44	Female	0.302	27	0	White European	T1D	T2D
6175	0.956	19.8	30	12	42	Male	0.263	1160	1	White European	Non-T1D	T1D
6263	0.985	23.5	13	21	34	Male	0.304	1060	0	Hispanic/ Latino	Non-T1D	T1D
6308	0.874	34.1	12	1	13	Female	0.282	1733	0	White European	Non-T1D	T2D

*Limit of detection

† Islet autoantibodies counted include GADA, IA-2A and ZnT8A. mIAA is not included in this count as it is not a reliable marker of autoimmunity in persons receiving exogenous insulin

Appendix 3.2- Chapter 3: Circulating C-peptide levels in living children and young people and pancreatic beta cell loss in pancreas donors across type 1 diabetes disease duration

ESM Table 1 Breakdown of characteristics for the EADB and nPOD biobanks (N=235) from people with type 1 diabetes diagnosed < 18 years.

	EADB n=124 (53%)	**nPOD** n=111 (47%)
Age (years), Median [25th;75th]	11.0 [6.9;15.0]	22.0 [16.3;32.5]
Diabetes Duration (years), Median [25th;75th]	0.2 [0.03;3.0]	12.0 [6.0;23.0]
Age-at-diagnosis (years), Median [25th;75th]	8.0 [4.0;13.0]	8.0 [5.0;12.8]
Sex, Male, N (%):	46 (37%)	56 (50%)
Donors with islets containing insulin+ beta cells, N (%):		
None	40 (32%)	75 (68%)
Present	84 (68%)	36 (32%)

ESM Table 2 Summary break down of proportions of donors from the UK GRID cohort (N=4079) with detectable C-peptide and pancreas donors from EADB and nPOD cohorts (N=235) with islets containing insulin+ beta cells within age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years) groups.

		UK GRID (N=4079)		**EADB and nPOD (N=235)**	
Duration (years)	**Age-at-diagnosis (years)**	Total number of donors (N)	Number of donors with detectable C-peptide (n (%))	Total number of donors (N)	Number of donors with islets containing insulin+ beta cells (n (%))
<1	**<7**	20	18 (90%)	26	23 (88%)
<1	**7-12**	110	107 (97%)	33	32 (97%)
<1	**≥13**	61	58 (95%)	25	22 (88%)
1-5	**<7**	522	172 (33%)	12	1 (8.3%)
1-5	**7-12**	995	604 (61%)	13	7 (54%)
1-5	**≥13**	289	225 (78%)	8	7 (88%)
5-10	**<7**	635	43 (6.8%)	14	3 (21%)
5-10	**7-12**	533	102 (19%)	17	7 (41%)
5-10	**≥13**	69	30 (43%)	14	9 (64%)
≥10	**<7**	489	21 (4.3%)	34	3(8.8%)
≥10	**7-12**	249	25 (10%)	26	4 (15%)
≥10	**≥13**	107	12 (11%)	13	2 (15%)

ESM Table 3 Summary of pancreas donors from EADB and nPOD cohorts with islets containing insulin+ beta cells (n=120) and the donors of which beta cell area data was available (n=100) within age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years) groups.

Duration (years)	**Age-at-diagnosis (years)**	Total number of donors, N=235 (N)	Number of donors with islets containing insulin+ beta cells, n=120 (N(%))	Number of donors with islets containing insulin+ beta cells and beta cell area data, n=100 (N)	Beta cell area, n=100 (Median % [IQR])
<1	**<7**	26	23 (88%)	22	15 [6.7,27]
<1	**7-12**	33	32 (97%)	27	21 [11,38]
<1	**≥13**	25	22 (88%)	17	31 [15,42]
1-5	**<7**	12	1 (8.3%)	1	2.0 [2.0,2.0]
1-5	**7-12**	13	7 (54%)	6	12 [7.5,25]
1-5	**≥13**	8	7 (88%)	6	14 [5.9,17]
5-10	**<7**	14	3 (21%)	3	5.3 [3.7,15]
5-10	**7-12**	17	7 (41%)	4	20 [14.5,26]
5-10	**≥13**	14	9 (64%)	7	4.6 [2.4,20]
≥10	**<7**	34	3(8.8%)	1	53 [53,53]
≥10	**7-12**	26	4 (15%)	4	5.6 [3.1,9.3]
≥10	**≥13**	13	2 (15%)	2	26 [13,38]

ESM Table 4 C-peptide in entire GRID cohort (N=4079) by age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years).

Age-at-diagnosis, years (N)	**<7 (1666)**				**7-12 (1887)**				**≥13 (526)**			
Duration, years (N)	**<1 (20)**	**1-5 (522)**	**5-10 (635)**	**≥10 (489)**	**<1 (110)**	**1-5 (995)**	**5-10 (533)**	**≥10 (249)**	**<1 (61)**	**1-5 (289)**	**5-10 (69)**	**≥10 (107)**
C-peptide (pmol/l), Median [IQR]	54 [29-111]	<9* [<9*-21]	<9* [<9*-<9*]	<9* [<9*-<9*]	156 [65-233]	24 [<9*-88]	<9* [<9*-<9*]	<9* [<9*-<9*]	189 [102-282]	79 [14-209]	<9* [<9*-41]	<9* [<9*-<9*]

*Limit of detection

ESM Table 5 Two by two table of C-peptide detectability and presence of islets containing insulin+ beta cells, in a subset of nPOD donors diagnosed <18 years without renal disease/failure (n=95), ($p=2.1x10^{-6}$)

	With islets containing insulin+ beta cells	Without islets containing insulin+ beta cells
Detectable C-peptide (≥16.4pmol/L)	17	4
Un-detectable C-peptide (<16.4pmol/L)	17	57

ESM Table 6 Characteristics, including admission course, of nPOD donors identified as having detectable C-peptide and 0% islets containing insulin+ beta cells (n=4). Insulin (Ins) and Glucagon (Gluc) have been abbreviated.

Study Number	Donor Type	Sex	BMI (Kg/m^2)	Age (years)	Duration of diabetes (years)	Age-at-diagnosis (years)	Islets containing insulin+ beta cells (%)	C-peptide (pmol/l)	Transport Duration (Minutes)	nPOD Histopathology Notes
6074	T1D	F	19.5	66	73	7	0	70	NA	Ins-/Gluc+ islets, numerous. Occ. insulin+ cell in acinar regions or within 1 islet. Few CD3+ cells in acinar and parenchyma regions. Moderate arteriosclerosis.
6145	T1D	M	23.1	11	18	7	0	20	849	Ins-/Gluc+ islets, atrophic. No infiltrates.
6244	T1D	M	23.8	28	34	6	0	16.7	981	Ins-/Gluc+ islets. Exocrine atrophy moderate. Low Ki67. IHC- some may be repeated due to background.
6268	T1D	F	26.6	3	12	9	0	16.7	1050	Ins+ (very rare)/Gluc+ islets, possibly reduced islet numbers but increased glucagon+ single cells. Insulitis

	present at insulin+ and insulin- islets. Ki67+ cells moderate numbers in acinar region, also in occasional islet and duct. Moderate acinar atrophy with prominent nerve fibres.

Appendix 3.3- Chapter 5: Glycemic variability and hypoglycemia is increased the day after free-living exercise in people with type 1 diabetes

Supplementary Table 1 Summary of all isCGM derived glycemic metrics based on the order each 14-day intervention periods were completed

Characteristic	1, N = 10[1]	2, N = 10[1]	3, N = 10[1]	p value[2]
Percentage expected wear	99 (98, 100)	100 (99, 100)	100 (100, 100)	0.7
Number of CGM hours per person	317 (305, 322)	320 (302, 327)	304 (291, 320)	0.3
Percentage data attained	94 (91, 96)	95 (90, 97)	90 (86, 95)	0.3
Average glucose (mmol/L)	9.2 (8.0, 10.1)	9.1 (8.6, 9.6)	9.6 (8.0, 10.6)	0.5
SD (mmol/L)	3.4 (3.2, 3.7)	3.5 (3.1, 4.2)	3.7 (3.1, 4.2)	0.3
CV (%)	38 (33, 40)	38 (33, 45)	37 (33, 42)	0.4
MAGE	6.8 (6.1, 7.5)	6.9 (6.0, 7.9)	7.4 (5.5, 8.2)	0.3
Estimated HbA_{1c} (mmol/mol)	57 (49, 64)	57 (53, 60)	60 (49, 67)	0.5
Time spent >10 mmol/L, level 1 elevated, (%)	34 (26, 47)	39 (31, 39)	42 (26, 52)	0.4
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	31 (22, 42)	35 (28, 36)	39 (23, 48)	0.5
Time spent >13.9 mmol/L, level 2 elevated, (%)	10 (3.0, 17)	8.0 (6.0, 19)	14 (4.0, 18)	0.5
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	9 (2.0, 14)	6.0 (6.0, 17)	12 (3.0, 16)	0.3
HBGI	9.8 (8.0, 13.7)	9.7 (9.4, 14.2)	12.5 (7.9, 14.7)	0.06
Time spent 3.9-10 mmol/L, (%)	60 (50, 69)	60 (54, 62)	51 (46, 65)	0.7
Time spent 3-<3.9 mmol/L, level 1 low, (%)	2.4 (1.4, 4.5)	2.1 (0.9, 4.2)	1.8 (1.4, 4.1)	0.7
Time spent <3 mmol/L, level 2 low, (%)	0.6 (0.1, 1.6)	1.2 (0.02, 2.8)	0.2 (0.2, 1.9)	0.7
Time spent in hypoglycemia, (%)	1.3 (0.10, 3.4)	1.7 (0.0, 4.1)	1.0 (0.1, 3.3)	0.8
LBGI	4.7 (2.9, 6.3)	4.3 (2.3, 7.2)	3.2 (2.3, 6.9)	0.7

[1]Median (IQR); [2]Friedman rank sum test

Supplementary Table 2 Summary of all isCGM derived glycemic metrics for participants at each 14-day intervention period

Characteristic	CON, N = 10[1]	HIIE, N = 10[1]	MICE, N = 10[1]	p-value[2]
Expected wear (%)	100 (100, 100)	100 (100, 100)	100 (100, 100)	0.7
Number of CGM hours per person (hours)	313 (299, 322)	317 (302, 324)	318 (292, 322)	0.7
Data attained (%)	93 (89, 96)	94 (90, 96)	95 (87, 96)	0.7
Average glucose (mmol/L)	9.4 (8.7, 9.7)	9.6 (7.9, 9.8)	9.1 (8.0, 10.6)	0.9
SD (mmol/L)	3.5 (3.1, 3.9)	3.3 (3.1, 4.3)	3.6 (3.2, 4.0)	0.4
CV (%)	38 (32, 40)	40 (34, 42)	37 (34, 46)	0.3
MAGE	6.8 (6.0, 7.7)	6.6 (5.5, 8.0)	7.0 (6.3, 8.0)	0.5
Estimated HbA_{1c} (mmol/mol)	58 (54, 61)	60 (49, 61)	56 (50, 67)	0.8
Time spent level 1 elevated, >10 mmol/L, (%)	40 (34, 45)	40 (26, 43)	35 (26, 53)	0.5
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	36 (30, 41)	37 (22, 40)	32 (24, 50)	0.4
Time spent level 2 elevated >13.9 mmol/L, (%)	11 (7.0, 17)	9.0 (3.0, 18)	12 (4.0, 20)	0.2
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	9.0 (6.0, 14)	8.0 (2.0, 16)	11 (3.0, 18)	0.06
HBGI	11.0 (9.4, 14)	11 (8.0, 14)	11 (7.7, 16)	0.5
Time spent 3.9-10 mmol/L, (%)	55 (49, 62)	58 (51, 64)	58 (42, 68)	0.3
Time spent level 1 hypoglycemia 3-<3.9 mmol/L, (%)	2.1 (0.9, 3.6)	2.7 (1.6, 5.0)	1.9 (0.8, 4.7)	0.9
Time spent level 2 hypoglycemia <3 mmol/L, (%)	0.9 (0.1, 2.3)	0.6 (0.1, 2.0)	0.2 (0.02, 2.1)	0.8
Time spent clinically significant hypoglycemia, (%)	1.3 (0.1, 3.9)	1.6 (0.4, 3.3)	0.2 (0.0, 3.7)	0.9
LBGI	4.7 (2.3, 7.1)	4.5 (2.5, 6.5)	3.1 (2.5, 6.6)	0.9

[1]Median (IQR); [2]Friedman rank sum test

Supplementary Table 3 Summary of insulin dose and dietary intake between intervention periods

Characteristic	CON, N = 10[1]	HIIE, N = 10[1]	MICE, N = 10[1]	p-value[2]
Valid diet days	14 (12, 14)	14 (12, 14)	13 (12, 14)	0.6
Mean Daily insulin dose (unit/kg)	0.5 (0.5, 0.6)	0.4 (0.4, 0.5)	0.4 (0.4, 0.5)	0.5
Unknown	*3*	*3*	*4*	
Mean Daily Calories (kcal)	1,484 (1,414, 1,805)	1,567 (1,390, 1,808)	1,472 (1,326, 1,830)	0.6
Unknown	*1*	*1*	*2*	
Mean Daily CHO (g)	178 (176, 203)	176 (165, 198)	181 (153, 205)	0.6
Unknown	*1*	*1*	*2*	
Mean Daily Fat (g)	55 (48, 76)	51 (46, 73)	54 (43, 66)	0.7
Unknown	*1*	*1*	*2*	
Mean Daily Sugar (g	38 (31, 50)	49 (38, 51)	49 (35, 59)	0.6
Unknown	*1*	*1*	*2*	

[1]Median (IQR); [2]Friedman rank sum test

Supplementary Table 4 Summary of all isCGM derived glycemic metrics for participants 0-4 hours home-base hours post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respective matched control (CON) periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
Average glucose (mmol/L)	9.3 (7.2, 10.2)	9.5 (9.1, 10.2)	0.8	9.3 (8.2, 11.2)	9.3 (8.4, 10)	0.4
SD (mmol/L)	3.6 (2.6, 4.0)	2.3 (2.1, 2.8)	0.002	3.2 (2.7, 3.9)	2.4 (2.1, 2.8)	0.01
CV (%)	37 (30, 41)	25 (22, 26)	0.006	34 (31, 37)	26 (24, 28)	0.02
MAGE	6.6 (5.2, 7.9)	5.5 (4.0, 6.7)	0.020	6.4 (4.7, 7.5)	5.4 (4.2, 6.0)	0.2
Estimated HbA_{1c} (mmol/mol)	59 (43, 64)	60 (57, 65)	0.8	59 (51, 71)	59 (52, 64)	0.4
Time spent >10 mmol/L, level 1 elevated, (%)	31 (14, 52)	42 (31, 57)	0.3	41 (18, 50)	33 (27, 61)	0.6
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	26 (10, 45)	38 (26, 51)	0.4	33 (13, 40)	29 (19, 52)	0.6
Time spent >13.9 mmol/L, level 2 elevated, (%)	12 (2.2, 16)	2.2 (0.0, 13)	0.06	13 (2.1, 22)	2.9 (0.0, 6.5)	0.3
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	9.1 (1.6, 14)	1.1 (0.0, 10)	0.05	9.2 (1.2, 18)	1.7 (0.0, 4.2)	0.2
HBGI	11 (5.6, 12)	8.4 (6.6, 11.6)	0.2	11 (6.1, 15)	7.5 (5.2, 12)	>0.9
Time spent 3.9-10 mmol/L, (%)	60 (46, 69)	57 (42, 69)	0.7	55 (49, 74)	64 (39, 73)	0.8
Time spent 3-<3.9 mmol/L, level 1 low, (%)	3.0 (0.5, 3.9)	0.0 (0.0, 0.0)	0.02	1.4 (0.9, 2.6)	0.0 (0.0, 0.0)	0.01
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.2	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4
LBGI	3.1 (1.3, 4.3)	0.5 (0.4, 1.2)	0.002	2.5 (1.7, 3.9)	1.0 (0.4, 1.7)	0.03

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Supplementary Table 5 Summary of all isCGM derived glycemic metrics for participants during overnight sleep periods (00:00-06:00) post home-based hours post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respective matched control (CON) periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
Average glucose (mmol/L)	7.9 (7.2, 10.0)	9.5 (7.9, 10.4)	0.6	9.0 (6.9, 11.5)	9.8 (8.7, 10.7)	>0.9
SD (mmol/L)	2.6 (2.0, 3.6)	2.0 (1.1, 2.9)	0.08	3.1 (1.7, 3.4)	1.6 (1.2, 2.2)	0.2
CV (%)	30 (24, 43)	22 (13, 33)	0.07	31 (21, 36)	16 (14, 23)	0.03
MAGE	7.3 (4.4, 12)	4.2 (2.6, 5.3)	0.02	5.8 (3.1, 7.6)	3.3 (2.5, 5.2)	0.2
Unknown	*1*	*1*		*2*	*2*	
Estimated HbA_{1c} (mmol/mol)	49 (44, 62)	59 (49, 65)	0.6	56 (42, 73)	62 (54, 68)	>0.9
Time spent >10 mmol/L, level 1 elevated, (%)	29 (17, 44)	37 (31, 60)	0.2	37 (7.6, 63)	44 (29, 59)	0.6
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	27 (12, 41)	32 (27, 47)	0.2	30 (6.2, 57)	40 (23, 52)	0.8
Time spent >13.9 mmol/L, level 2 elevated, (%)	1.7 (0.0, 6.3)	0.0 (0.0, 5.0)	0.7	5.8 (0.0, 22.4)	0.0 (0.0, 11.3)	0.4
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	0.0 (0.0, 5.4)	0.0 (0.0, 3.8)	0.7	4.3 (0.0, 20.7)	0.0 (0.0, 9.3)	0.4
HBGI	9.1 (6.1, 10)	8.9 (7.6, 10)	>0.9	10.1 (3.5, 15.0)	8.9 (6.8, 11.0)	0.4
Time spent 3.9-10 mmol/L, (%)	53 (40, 77)	49 (40, 61)	0.6	52 (37, 79)	45 (40, 71)	0.8
Time spent 3-<3.9 mmol/L, level 1 low, (%)	1.2 (0.0, 9.3)	0.0 (0.0, 5.3)	0.7	0.0 (0.0, 1.4)	0.0 (0.0, 0.0)	>0.9
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	>0.9
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.4	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	>0.9
LBGI	2.3 (0.5, 6.2)	0.7 (0.0, 4.7)	0.6	1.1 (0.0, 2.3)	0.0 (0.0, 0.5)	0.04

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Supplementary Table 6 Summary of all isCGM derived glycemic metrics for participants during the next day (06:00-24:00) post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, as compared to the respective matched control (CON) periods at same time of day, on the same day of the week.

Characteristic	HIIE, N = 10[1]	HIIE CON, N = 10[1]	p-value[2]	MICE, N = 10[1]	MICE CON, N = 10[1]	p-value[2]
Average glucose (mmol/L)	8.5 (7.8, 9.1)	8.9 (8.4, 9.7)	0.03	9.1 (8.5, 10.1)	9.1 (8.7, 9.8)	0.8
SD (mmol/L)	3.2 (2.9, 3.6)	2.5 (2.1, 3.0)	0.01	3.8 (2.8, 4.0)	2.5 (2.0, 2.9)	0.002
CV (%)	39 (34, 41)	28 (25, 30)	0.002	36 (33, 44)	28 (22, 31)	0.006
MAGE	6 (5.2, 7.1)	5.2 (3.7, 5.9)	0.02	7.2 (5.2, 8.5)	5.2 (3.8, 5.7)	0.002
Estimated HbA_{1c} (mmol/mol)	53 (48, 57)	55 (51, 61)	0.06	56 (53, 63)	57 (54, 62)	0.6
Time spent >10 mmol/L, level 1 elevated, (%)	28 (24, 33)	30 (25, 40)	0.4	36 (30, 49)	31.7 (28, 40)	0.2
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	25 (20, 28)	25 (20, 36)	0.4	32 (26, 43)	26 (23, 36)	0.06
Time spent >13.9 mmol/L, level 2 elevated, (%)	7.7 (1.3, 10)	2.2 (1.3, 8.3)	0.3	12 (5.2, 23)	2.8 (0.3, 9.8)	0.04
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	6.4 (0.9, 8.1)	1.5 (0.4, 6.8)	0.2	10 (4.4, 20)	2.2 (0.2, 8.0)	0.04
HBGI	8.9 (7.2, 11)	7.3 (6.2, 8.9)	0.11	11.0 (8.5, 17)	7.0 (6.6, 9.2)	0.01
Time spent 3.9-10 mmol/L, (%)	64 (61, 72)	66 (59, 73)	0.8	60 (47, 67)	66 (59, 69)	0.06
Time spent 3-<3.9 mmol/L, level 1 low, (%)	3.3 (1.3, 5.3)	0.0 (0.0, 1.1)	0.002	1.8 (0.7, 5.4)	0.0 (0.0, 0.0)	0.01
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.5)	0.0 (0.0, 0.0)	0.3	0.0 (0.0, 0.4)	0.0 (0.0, 0.0)	0.2
Time spent in hypoglycemia, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.6	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.2
LBGI	3.7 (2.4, 4.7)	1.6 (1.1, 2.1)	0.004	2.7 (1.7, 4.3)	1.4 (0.4, 2.5)	0.05

[1]Median (IQR)

[2]Wilcoxon signed rank exact test; Wilcoxon signed rank test with continuity correction

Supplementary Table 7 The absolute difference between isCGM glycemic derived metrics 0-4h post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, and the respective matched control (CON) periods at same time of day, on the same day of the week.

Absolute difference in characteristic from control	HIIE, N = 10[1]	MICE, N = 10[1]	p-value[2]
Average glucose (mmol/L)	0.01 (-2.0, 0.7)	-0.3 (-0.8, -0.2)	0.9
SD (mmol/L)	1.1 (0.6, 1.5)	0.9 (0.3, 1.2)	0.3
CV (%)	14 (9.0, 18)	8.0 (5.5, 15)	0.4
MAGE	1.9 (0.8, 2.4)	1.5 (-1.1, 3.0)	0.5
Estimated HbA_{1c} (mmol/mol)	0.0 (-14, 5.2)	-2.2 (-5.5, -1.4)	0.9
Time spent level 1 elevated, >10 mmol/L, (%)	-9.2 (-22, 11)	-3.7 (-19, 2.1)	0.7
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	-5.6 (-20.7, 9.5)	-5.9 (-18, 1.2)	>0.9
Time spent level 2 elevated >13.9 mmol/L, (%)	2.9 (0.0, 11.2)	1.7 (0.0, 12.3)	>0.9
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	3.6 (0.0, 10)	2.2 (0.0, 10)	0.8
HBGI	1.6 (-0.6, 4.5)	-0.3 (-2.2, 1.6)	0.4
Time spent 3.9-10 mmol/L, (%)	0.5 (-8.9, 13)	2.0 (-2.5, 14)	0.8
Time spent level 1 hypoglycemia 3-<3.9 mmol/L, (%)	2.6 (0.5, 3.9)	1.4 (0.9, 2.6)	0.4
Time spent level 2 hypoglycemia <3 mmol/L, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)	0.6
Time spent clinically significant hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.9
LBGI	1.8 (0.6, 3.7)	1.7 (0.8, 2.3)	0.5

[1]Median (IQR)

[2]Wilcoxon rank sum exact test; Wilcoxon rank sum test

Supplementary Table 8 The absolute difference between isCGM glycemic derived metrics during overnight sleep periods (00:00-06:00) post high-intensity interval exercise (HIIE) and moderate-intensity interval exercise (MICE) session, and the respective matched control (CON) periods at same time of day, on the same day of the week.

Absolute difference in characteristic from control	Home-HIIT, N = 10[1]	Home-MICT, N = 10[1]	p-value[2]
Average glucose (mmol/L)	-0.3 (-1.7, 0.6)	-0.6 (-1.2, 1.4)	>0.9
SD (mmol/L)	1.0 (0.2, 1.5)	0.5 (-0.08, 1.7)	>0.9
CV (%)	8.0 (1.5, 21)	5.5 (-3.0, 21)	0.8
MAGE	5.4 (1.4, 8.1)	3.4 (-0.2, 5.6)	0.4
Unknown	*1*	*3*	
Estimated HbA_{1c} (mmol/mol)	-2.2 (-11, 4.4)	-4.9 (-7.7, 9.3)	>0.9
Time spent >10 mmol/L, level 1 elevated, (%)	-11 (-24.3, 10)	-1.3 (-24, 14)	0.8
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	-9.6 (-23.3, 11)	1.3 (-23.7, 9.6)	>0.9
Time spent >13.9 mmol/L, level 2 elevated, (%)	0.0 (0.0, 4.0)	0.5 (0.0, 19)	0.7
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	0.0 (0.0, 2.8)	0.0 (0.0, 18)	0.8
HBGI	-0.7 (-2.9, 4.6)	1.5 (-2.9, 6.2)	0.7
Time spent 3.9-10 mmol/L, (%)	3.7 (-7.2, 22)	2.1 (-14, 22)	>0.9
Time spent 3-<3.9 mmol/L, level 1 low, (%)	0.0 (-3.9, 8.8)	0.0 (0.0, 0.0)	>0.9
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.7
Time spent in clinically significant hypoglycemia, (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.6
LBGI	0.0 (-2.2, 3.7)	0.7 (0.0, 1.8)	0.4

[1]Median (IQR)

[2]Wilcoxon rank sum exact test; Wilcoxon rank sum test

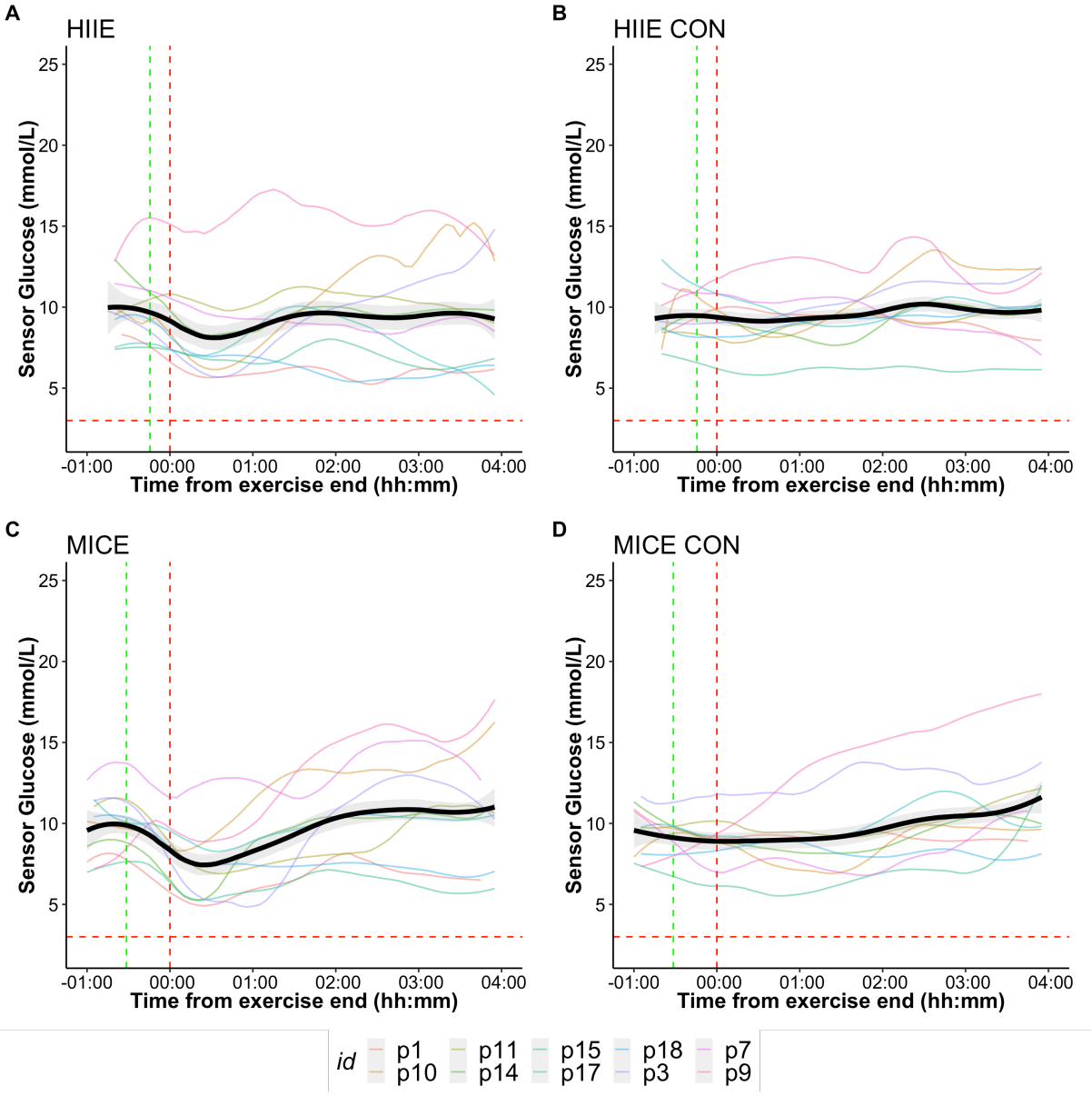
Supplementary Table 9 The absolute difference in isCGM glycemic derived metrics during the next day (06:00-24:00) post home-based HIIE and MICE sessions and their respective matched CON periods at same time of day, on the same day of the week.

Absolute difference in characteristic from control	Home-HIIT, N = 10[1]	Home-MICT, N = 10[1]	p-value[2]
Average glucose (mmol/L)	-0.5 (-0.8, -0.1)	-0.1 (-0.4, 1.1)	0.2
SD (mmol/L)	0.6 (0.3, 1.2)	1.2 (0.5, 1.4)	0.1
CV (%)	9.5 (5.5, 14.3)	10 (6.5, 12)	>0.9
MAGE	1.1 (0.3, 2.0)	1.5 (1.2, 2.8)	0.3
Estimated HbA_{1c} (mmol/mol)	-2.7 (-5.5, -0.3)	-0.6 (-3.0, 7.4)	0.2
Time spent >10 mmol/L, level 1 elevated, (%)	-2.3 (-9.6, 2.9)	8.8 (-1.4, 19)	0.1
Time spent level 1 hyperglycemia, >10 mmol/L ≥15 min, (%)	-1.9 (-9.4, 3.3)	9.3 (0.6, 18)	0.1
Time spent >13.9 mmol/L, level 2 elevated, (%)	3.8 (0.0, 5.6)	5.8 (0.6, 16.6)	0.3
Time spent level 2 hyperglycemia, >13.9 mmol/L ≥15 min, (%)	3.9 (0.2, 4.9)	4.9 (0.5, 15.3)	0.4
HBGI	1.3 (0.03, 3.0)	3.9 (1.7, 6.8)	0.05
Time spent 3.9-10 mmol/L, (%)	0.9 (-4.3, 5.5)	-8.6 (-19, -0.8)	0.1
Time spent 3-<3.9 mmol/L, level 1 low, (%)	1.8 (0.8, 3.9)	1.7 (0.7, 4.7)	0.8
Time spent <3 mmol/L, level 2 low, (%)	0.0 (0.0, 0.5)	0.0 (0.0, 0.4)	0.8
Time spent in clinically significant hypoglycemia, (%)	0.0 (0.0, 0.7)	0.0 (0.0, 0.7)	>0.9
LBGI	1.2 (0.8, 2.4)	1.6 (1.2, 2.0)	>0.9

[1]Median (IQR)

[2]Wilcoxon rank sum exact test; Wilcoxon rank sum test

Supplementary Figure 1 Each individual's loess regression estimation of ambulatory glucose profile (colour) and overall loess regression estimate (black) of ambulatory glucose profile in the time 0-4 hours post-exercise session for high-intensity interval exercise (HIIE) (A) and moderate-intensity continuous exercise (MICE) (C), compared respectively matched control (CON) (B,D). Horizonal dashed red line represents 3 mmol/L. Vertical dashed lines represents the average start (green) and end (red) of an exercise session. Shaded ribbon represents standard error.



Appendix 4 – other published papers

Appendix 4.1- Time to death and risk factors associated with mortality among COVID-19 cases in countries within the WHO African region in the early stages of the COVID-19 pandemic

Published in Epidemiology and Infection 2022

This work was completed during my time in an emergency consulting role for the WHO African Regional Office as a junior data sciences expert (October 2020 – May 2021). This was undertaken independently and alongside my PhD. This work has also led to me working with AppliedEpi, the organisation behind Epidemiologist R Handbook, developing functional R packages for outfield epidemiological analysis for Médecins Sans Frontières.

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Original Paper

*These authors contributed equally to this work.

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Time to death and risk factors associated with mortality among COVID-19 cases in countries within the WHO African region in the early stages of the COVID-19 pandemic

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Abstract

This study describes risk factors associated with mortality among COVID-19 cases reported in the WHO African region between 21 March and 31 October 2020. Average hazard ratios of death were calculated using weighted Cox regression as well as median time to death for key risk factors. We included 46 870 confirmed cases reported by eight Member States in the region. The overall incidence was 20.06 per 100 000, with a total of 803 deaths and a total observation time of 3 959 874 person-days. Male sex (aHR 1.54 (95% CI 1.31–1.81); $P < 0.001$), older age (aHR 1.08 (95% CI 1.07–1.08); $P < 0.001$), persons who lived in a capital city (aHR 1.42 (95% CI 1.22–1.65); $P < 0.001$) and those with one or more comorbidity (aHR 36.37 (95% CI 20.26–65.27); $P < 0.001$) had a higher hazard of death. Being a healthcare worker reduced the average hazard of death by 40% (aHR 0.59 (95% CI 0.37–0.93); $P = 0.024$). Time to death was significantly less for persons ≥60 years ($P = 0.038$) and persons residing in capital cities ($P < 0.001$). The African region has COVID-19-related mortality similar to that of other regions, and is likely underestimated. Similar risk factors contribute to COVID-19-associated mortality as identified in other regions.

Introduction

In late December 2019, a novel coronavirus identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was detected in several cases of pneumonia in Wuhan City, Hubei Province, China [1]. Within a month several countries were reporting cases of the disease, named by the World Health Organisation (WHO) as coronavirus disease 2019 (COVID-19), with the deployment of testing resources demonstrating the rapid spread across international borders.

Although mortality from SARS-CoV-2 is lower, transmission is higher when compared to other emerging coronaviruses causing severe acute respiratory syndrome (SARS) epidemics over the last two decades [2–4]. As of February 2021, the global case fatality ratio (CFR) for SARS-CoV-2 was estimated at 2.3% [5], compared to 9.7% for SARS-CoV which emerged in late 2002 [3], and 34% for Middle East respiratory syndrome coronavirus (MERS-CoV) which emerged in 2012 [4]. The basic reproductive rate (R_0) for SARS-CoV-2 is 2.5 compared to 2.4 for SARS-CoV and 0.69 for MERS-CoV [2, 6]. Although of similar R_0 to SARS-CoV based on available data, SARS-CoV-2 has spread rapidly to all continents.

Initial cases of COVID-19 were detected in Africa in February 2020, introduced by travellers from Europe into Egypt and Algeria [7]. The outbreak in the WHO African region evolved rapidly, and by 13 May 2020, all 47 countries had been affected [8]. Confirmed case numbers began to increase from April 2020, reaching a peak by the end of July 2020, which then declined through August and September 2020, before increasing again during November and December 2020 [5].

As of 24 February 2021, 12 months after the notification of the first laboratory-confirmed COVID-19 case, a cumulative total of 2 811 106 confirmed cases and 71 159 deaths have been reported in the African region, with the CFR estimated at 2.5% [5]. This represents 2.5% of cases globally and 2.8% of deaths [5]. This proportion of cases is low when compared to the Americas (45%) and Europe (34%) [5]. The reason for this disparity may be due to low testing performance within the African region. As of 24 February 2021, 25904273 SARS-CoV-2 tests (molecular and antigen) across 43 African region countries were performed, representing 241.5 tests per 10 000 population. Only 41.8% (18 out of 43) of the countries assessed surpassed the effective testing rate (10 tests per 10 000 population per week) between 28 January and 24 February 2021 (data unpublished). Other factors contributing to this disparity have been suggested including early implementation of travel restrictions, border closures, lockdown measures including curfews and school closures, a younger population, genetics, lower comorbidities rates, possible trained immunity or immunomodulation, suboptimal testing, and favourable climate [9–11].

While several studies documented the occurrence of deaths in COVID-19-confirmed patients, there are limited studies in the WHO African region addressing mortality burden and the risk factors associated with COVID-19 [10, 12]. The purpose of this study is to describe the risk factors associated with mortality among COVID-19 cases reported in the WHO African region in the early stages of the COVID-19 pandemic between 21 March and 31 October 2020, to understand if these differ from other regions, and to inform future measures that should be taken by public health authorities to address and mitigate the impact in the WHO African region.

Methods

Study design

We conducted a retrospective cohort study of deaths associated with confirmed COVID-19 cases reported by Member States in the WHO African region between 21 March and 31 October 2020. The time period was chosen to maximise the number of countries with complete reports.

Case definitions

A confirmed case of COVID-19 was defined as a person with a positive Nucleic Acid Amplification Test (NAAT) or a person with a positive SARS-CoV-2 Ag-RDT *and* meeting either the probable case definition or suspected criteria as per the WHO guideline, or an asymptomatic person with a positive SARS-CoV-2 Ag-RDT *and* who is a contact of a probable or confirmed case [13]. A COVID-19 death is defined as a death resulting from a clinically compatible illness in a probable or confirmed COVID-19 case, unless there is a clear alternative cause of death that cannot be related to COVID-19 disease (e.g. trauma) [13].

Data source

The primary data source was the regional linelist of confirmed COVID-19 cases, a database containing key information about each confirmed case reported to the WHO Regional Office for Africa (WHO AFRO) by its Member States per the reporting requirements of the International Health Regulations (2005) [14]. Variables captured include unique identification, date of reporting, age, sex, location (administrative levels 1 and 2), case classification, occupation, health worker status, date of symptom onset, presence of symptoms, laboratory test result, date of sample collection, date of laboratory result, date of death, date of discharge, patient outcome, current inpatient status, and presence and description of comorbidity.

Exclusion criteria

All countries' cases in the WHO AFRO regional linelist were eligible for inclusion. We excluded cases reported before 21 March or after 31 October 2020 as well as cases missing information on patient outcome, key dates (e.g. outcome date if died or laboratory result date), age or sex. Cases with laboratory result dates after outcome dates were also excluded.

Data cleaning

We identified confirmed cases using the case classification variable. Where this was unavailable, we used the laboratory test result. Start dates were defined for individuals as the date of confirmed laboratory result; where this was unavailable the sample collection date was used. This was deemed to be more complete and reliable than either symptom onset or sample collection date in isolation. End dates for individuals were defined as the earliest occurring date where the individual was reported dead or recovered; in the absence of recovery date, the maximum date of observation was used (31 October 2020). Observation time was calculated as the difference between start and end dates in days. Patients were identified as recovered if the patient outcome variable contained prespecified words related to recovery. Where patient outcome was unavailable, we used current inpatient status. Patient outcome was dichotomised to 'alive' or 'dead'.

We coded the following exposure variables: healthcare worker status, residence in capital city status and comorbidity status and types. Healthcare worker status was identified if the free-text occupation variable contained prespecified key words related to healthcare. Healthcare worker status was defined as 'Not Reported' in the absence of the occupation variable. Capital city residence was identified if the free-text location variable (administrative level 2) contained the country's capital city. Capital city residence was defined as 'Not Reported' in the absence of the free-text location variable. Comorbidity types were identified if the comorbidity free-text variables contained prespecified key words for each comorbidity type of interest (diabetes, asthma, hypertension, cancer, renal disease, cardiovascular disease, obesity, tuberculosis, sickle cell disease, chronic pulmonary or other). Comorbidity type was defined as 'Not Specified' if presence was indicated with 'Yes' but no further description was provided. Comorbidity presence was categorised as 'Yes' if a defined comorbidity type was detected in the previous step, 'No' if indicated so in free-text description or if the free-text description contained non-comorbidities and 'Not reported' in the absence of the comorbidity free-text variables. These exposure variables were dichotomised as 'Yes' and 'No/Not Reported'.

Based on age at reporting, we created a dichotomous age variable (⩾60 years) and an age group variable in years as follows: ⩽10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70 and 70+.

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Data analysis

Incidence and case fatality ratios

We calculated the incidence per 100 000 population as the number of confirmed cases divided by the population multiplied by 100 000. We sourced population data for each country in 2020 from the United Nations World Population Prospects [15] and summed these to calculate the overall.

We calculated the CFR, the proportion of confirmed cases that died due to the consequences of COVID-19 [16], by dividing the cumulative number of deaths by the cumulative number of confirmed cases. We stratified results by country, age group, sex, comorbidity (and number of comorbidities), residence and healthcare worker status to ascertain the most affected categories.

Age and sex distributions

To determine the distribution of cases and deaths stratified by age group and sex, we calculated the proportion in each group of the total number, and plotted this in an age-sex pyramid. We additionally plotted the CFR by age group and sex.

Comorbidities

In addition to describing CFR by individual comorbidities, we conducted a combination analysis for the comorbidities/condition of interest (as defined in data cleaning). This investigated the most frequently occurring combinations of comorbidities among all confirmed cases and among those who died. We also investigated the exposure–response relationship between increasing number of comorbidities and mortality compared to those without comorbidity using weighted Cox regression (see regression details in risk factors for death section).

Risk factors for death

We used Cox regression to compute hazard ratios and corresponding 95% confidence intervals to investigate associations between mortality and several dichotomous exposure variables (healthcare worker status, residence in capital city status, comorbidity status) and age as a continuous variable. These variables were chosen based on associations with increased mortality identified in previous literature.

Given the low number of variables included in the univariate analysis, we decided *a priori* to include all variables in the multivariable analysis regardless of significance level in univariate analyses. We excluded pregnancy status from multivariable analysis, given that this is only relevant to females, leading to data separation.

We investigated all variables for confounding and effect modification using Mantel–Haenszel statistics and associated Woolf's tests, in order to identify necessary interaction terms.

The model proportional hazards assumption was tested using scaled Schoenfeld residuals, with non-linearity assumptions assessed visually. As the hazards were found to be non-proportional, we present the average hazard ratio and corresponding 95% CI calculated using weighted Cox regression [17].

Time to death

At a population level, the case fatality was not above 50%, thus it was not possible to calculate median survival times using Cox regression. Instead, we calculated the medians and interquartile ranges of observation time in days among those who died, stratified by exposure variables and compared the distribution of these times using Kruskal–Wallis tests.

All analyses were two-tailed, with a significance level of 0.05, and carried out using R statistical software version 3.6.1 (Foundation for Statistical Computing, Vienna, Austria).

Results

Inclusion

Of the 194 777 COVID-19 cases reported in 20 countries of the WHO African region from 21 March to 31 October 2020, we selected 46 870 cases (24%) for the study with a total observation time of 3 959 874 person-days. The cases meeting our selection criteria were reported from eight WHO Member States in the WHO African region. Cases from WHO Member States who stopped reporting before 31 October 2020 ($n = 47\,416$ from nine countries) and who did not report outcome dates ($n = 31\,678$ from two countries) were excluded. Reported cases with negative results ($n = 13\,173$), missing outcomes and incomplete dates ($n = 54\,596$) and missing age and sex information ($n = 1044$) were also excluded (Fig. 1). Characteristics and missingness for variables of interest among confirmed cases are outlined in Supplementary Table S1.

A comparative summary for 116 304 COVID-19 cases reported between 21 March and 31 October 2020, for 11 WHO Member States reporting up until and including 31 October, prior to exclusion due to covariate data availability, is outlined in Supplementary Table S2.

Incidence and case fatality ratio

The overall incidence among the eight WHO Member States included in this analysis was 20.06 per 100 000. Namibia had the highest incidence (49.43 per 100 000), followed by Sao Tome and Principe (42.02 per 100 000) and Eswatini (11.21 per 100 000). Uganda ($n = 12\,126$) and the Democratic Republic of Congo ($n = 10\,274$) reported high numbers of cases, but had a relatively low incidence (9.25 and 1.15 per 100 000, respectively) (Table 1).

A total of 803 deaths of confirmed COVID-19 cases included in our analysis were reported from the eight WHO Member States. Kenya reported the largest proportion of these (70%, $n = 554$) and also had the highest CFR (7.47%). Niger was the only other member state to have CFR >1% (4.36%), with all others having CFR <1% (Table 1). The overall CFR among the eight WHO Member States was 1.71%. Among the 116 304 COVID-19 cases from 11 WHO Member States, prior to exclusion due to covariate data availability, the incidence was 43.25 per 100 000. The number of deaths totalled 2166 with the overall CFR at 1.86% (Supplementary Table S2).

Healthcare workers made up 2.9% ($n = 1381$) of included cases and had a lower CFR at 1.38%. Namibia reported the highest absolute number among healthcare workers ($n = 532$), but Niger had the highest proportion at 15.2% ($n = 178$). CFR among healthcare workers varied by country (min: 0%, max: 4.09%) with the highest CFR reported by Kenya (Table 1).

Age and sex distribution

Among confirmed COVID-19 cases included in our study, 52% occurred in the ages between 21 and 40 years, with the majority of deaths occurring in persons aged over 40 years (84%). There were a higher proportion of male cases compared to females

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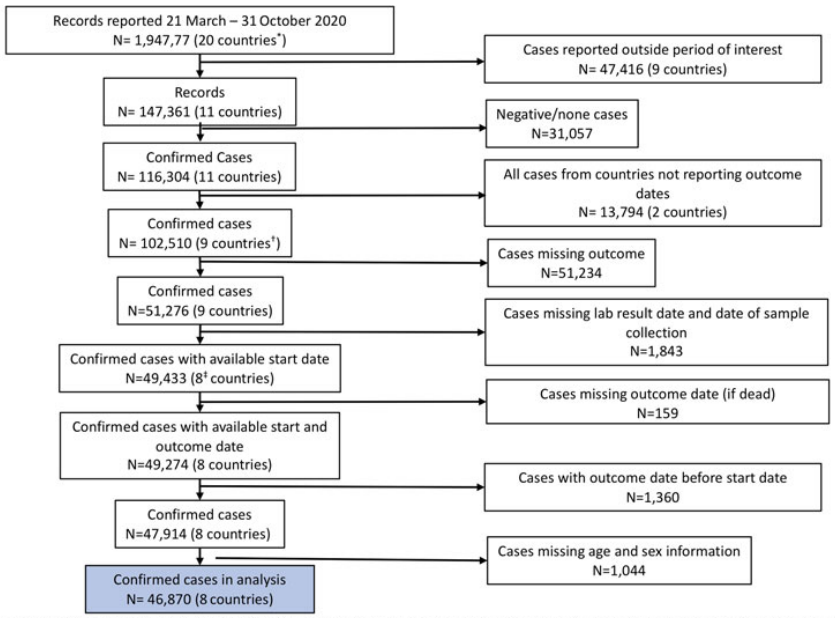

***Countries reporting:** Burkina Faso, Kenya, Sao Tome and Principe, Eswatini, Seychelles, Chad, Uganda, Zimbabwe, Cote d'Ivoire, Democratic Republic of Congo, Republic of Congo, Guinea, Liberia, Mozambique, Mauritius, Namibia, Niger, Rwanda, Senegal, Sierra Leone
†**Countries reporting up until 31 October and reporting outcome dates:** Kenya, Sao Tome and Principe, Eswatini, Chad, Uganda, Democratic Republic of Congo, Mauritius, Namibia, Niger
‡ All cases from Mauritius excluded at this step due to not reporting lab result date or date of sample collection

Fig. 1. Flowchart of inclusion pathway for cases.

overall (1.7:1) and also within all age groups over 20 years (Fig. 2a). Similarly, deaths were reported twice as often in males, with a notably increased proportion of deaths in all age groups over 30 years of age (Fig. 2b). In the age groups under 50 years, CFR was approximately equal for males and females and remained stable. In the age groups older than 50 years however, the CFR was approximately 0.7 times higher with every 10 years of age in both males and females, and was higher in males in each of the age groups (Fig. 3). For both males and females, the lowest CFR occurred between 11 and 20 years (males: 0.3%, females: 0.2%) with the highest occurring in persons aged 70 years and over (males: 17.7%, females: 13.7%).

Among 1381 healthcare worker cases, 56.6% were female ($N = 783$), with a higher proportion occurring in the age groups below 30 years (33% *vs.* 25% for males). Male healthcare workers had a higher overall CFR (2.17% *vs.* 0.77%) (Supplementary Table S3).

Comorbidities

Cardiovascular disease was the most commonly reported comorbidity among confirmed COVID-19 cases included in our analysis, both overall ($n = 951$) and as a single comorbidity ($n = 694$) (Fig. 4a). This means, among those reporting cardiovascular disease, 73% had no other comorbidities. Diabetes ($n = 585$) and hypertension ($n = 535$) were also commonly reported among cases (Fig. 4a); 52% ($n = 312$) and 64% ($n = 343$), respectively, were reported as single comorbidities. While 39 deaths (CFR 4.1%) occurred among those with cardiovascular disease, only nine deaths (CFR 1.3%) occurred in those with isolated cardiovascular disease (Supplementary Table S4).

The highest number of deaths were reported in cases with only hypertension ($n = 112$, CFR 33%) (Fig. 4b). The overall CFR among all cases with hypertension (singularly or in combination) was 34.4% (Supplementary Table S4). Cases with only diabetes reported had the second highest number of deaths ($n = 50$, CFR = 16%) (Fig. 4b) with an overall CFR 20.2% for all cases with diabetes (Supplementary Table S4).

Cases with renal disease had the overall highest CFR (45%), with 27 dead out of a total of 60 (Supplementary Table S4). However, 70% of these deaths occurred in cases with other comorbidities, including renal disease with hypertension ($n = 6$, 20%) and renal disease with diabetes ($n = 3$, 10%) (Fig. 4b). The overall CFR for a further six common comorbidities is outlined in Supplementary Table S4, as well as comorbidities categorised as other, non-specified comorbidities and pregnancy.

The hazard of death significantly increased with increasing number of comorbidities. Cases with one comorbidity from the specified list (excluding those only listed as 'other' or non-specified) had 12 times greater hazard of death than those without comorbidities (aHR 11.6 (95% CI 9.87–13.73); $P < 0.001$) (Table 2). The maximum number of comorbidities specified was four, and cases in this group had a 66 times higher hazard of death compared to those without comorbidities (aHR 66.01 (95% CI 28.50–152.42); $P < 0.001$), although this is likely a problem of low numbers, which will give an unrealistically high ratio (Table 2).

Risk factors for death

In univariate regression, males (aHR 1.46 (95% CI 1.27–1.74); $P < 0.001$), increasing age (aHR 1.08 (95% CI 1.07–1.08); $P < 0.001$), persons who lived in a capital city (aHR 1.88 (95% CI 1.63–2.16); $P < 0.001$) and those with one or more comorbidity (aHR 11.89 (95% CI 10.33–13.69); $P < 0.001$) had a higher hazard of

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Table 1. Summary measures for COVID-19 cases reported between 21 March and 31 October 2020 by Member States of the WHO African region included in analysis ($N = 46\,870$)

Country	Cases[a] (*N*)	Recovered[b] (*n*)	Dead (*n*)	Alive[c] (*n*)	CFR (%)	Healthcare worker cases (*N*)	Healthcare worker deaths (*n*)	Healthcare worker cases (%)	CFR among healthcare workers (%)	Incidence per 100 000
Democratic Republic of Congo	10 274	532	100	10 174	0.97	208	4	2.02	1.92	1.15
Kenya	7421	6865	554	6867	7.47	269	11	3.62	4.09	1.38
Namibia	12 560	605	34	12 526	0.27	532	2	4.24	0.38	49.43
Niger	1169	1026	51	1118	4.36	178	0	15.23	0	0.48
Sao Tome and Principe	921	0	4	917	0.43	0	0	0		42.02
Eswatini	1301	1289	12	1289	0.92	117	2	8.99	1.71	11.21
Chad	1098	16	8	1090	0.73	1	0	0.09	0	0.67
Uganda	12 126	0	40	12 086	0.33	76	0	0.63	0	2.65
Total	46 870	10 333	803	46 067	1.71	1381	19	2.94	1.38	20.06

[a]Confirmed cases meeting selection criteria reported 21st March–31st October 2020.
[b]Recovery status missing for Sao Tome and Principe and Uganda.
[c]Recovered cases are included in alive count. Alive and dead counts sum to total cases.

death. Healthcare worker status in women (aHR 0.76 (95% CI 0.46–1.21); $P = 0.248$) and pregnancy (aHR 1.06 (95% CI 0.26–4.28); $P > 0.9$) was not significantly associated with hazard of death (Table 3).

While controlling for other covariates and their interaction in multivariable analysis, males (aHR 1.54 (95% CI 1.31–1.81); $P < 0.001$), increasing age (aHR 1.08 (95% CI 1.07–1.08); $P < 0.001$), persons who lived in a capital city (aHR 1.42 (95% CI 1.22–1.65); $P < 0.001$) and those with one or more comorbidity (aHR 36.37 (95% CI 20.26–65.27); $P < 0.001$) had a higher hazard of death. Being a healthcare worker reduced the average hazard of death by 40% (aHR 0.59 (95% CI 0.37–0.93); $P = 0.024$) (Table 3). For comparison, unweighted hazard ratios for univariate and multivariable Cox regression are outlined in Supplementary Table S5, but should not be interpreted due to violation of the proportional hazards assumption.

Time to death

The time to death in those that died ($N = 803$) was significantly less for persons aged 60 and over (<60 years, median (IQR): 4 (2, 9) days *vs.* ≥60 years, median (IQR): 5 (2, 10) days; $P = 0.038$) and for those not reporting residing in a capital city (residence in capital, median (IQR): 4 (2, 9) days *vs.* residence outside of capital/not reported, median (IQR): 6 (2, 11) days; $P < 0.001$) (Table 4). Sex, healthcare worker status and comorbidity status did not significantly impact time to death for confirmed COVID-19 cases (Table 4). Median time to death varied by country with a range between 1 and 10.5 days (data not shown).

Discussion

Our study reports a total of 46 870 confirmed COVID-19 and 803 deaths from eight countries in the WHO African region during the period 21 March to 31 October 2020. The key risk factors identified for mortality were male sex, older age, presence of one or more comorbidities and residence in capital cities. Of all reported confirmed cases, 2.9% were among healthcare workers, with a higher proportion of these cases being female (56.6%), with, however, an increased CFR in male healthcare workers. Being a healthcare worker was not attributed as a risk factor for mortality or time to death.

Monitoring the occurrence of deaths during a pandemic, and factors influencing this mortality, not only helps track the evolution of the pandemic but also helps decision makers target, prioritise and monitor the effectiveness of prevention and response strategies [18]. Although the African region accounts for <2.5% of COVID-19-associated deaths reported globally, it is crucial to understand the patterns of these deaths, the areas and populations affected, and identify the risk factors for death, in order to guide decision makers at national and regional levels.

Our observed overall incidence and CFR were on the lower end of the scale compared to several regions reporting hundreds of cases per 100 000 population and CFRs several times higher [11, 19]. The highest incidence observed in Sao Tome and Principe (42.02 per 100 000) and Eswatini (11.21 per 100 000) could be explained by the small size and high density population [20, 21]. The overall low case fatality observed in our study makes the region among the least affected. Studies in the early stages of the pandemic suggest that lockdown may have delayed epidemics by about 3 months [22]. It is important to note that the crude (total cases prior to inclusion criteria) estimate of incidence

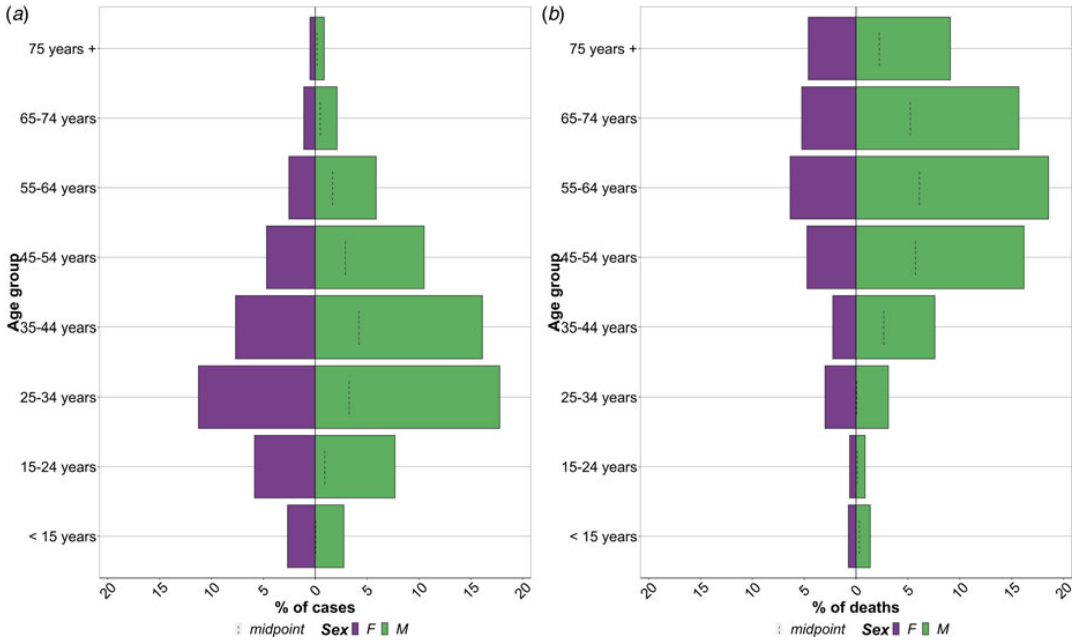

Fig. 2. (a) Age-sex pyramid of confirmed cases (a) and of deaths among confirmed cases (b) reported in eight Member States of the WHO African region included in analysis between 21 March and 31 October 2020 (*N* = 46 870, *N* = 803 respectively).

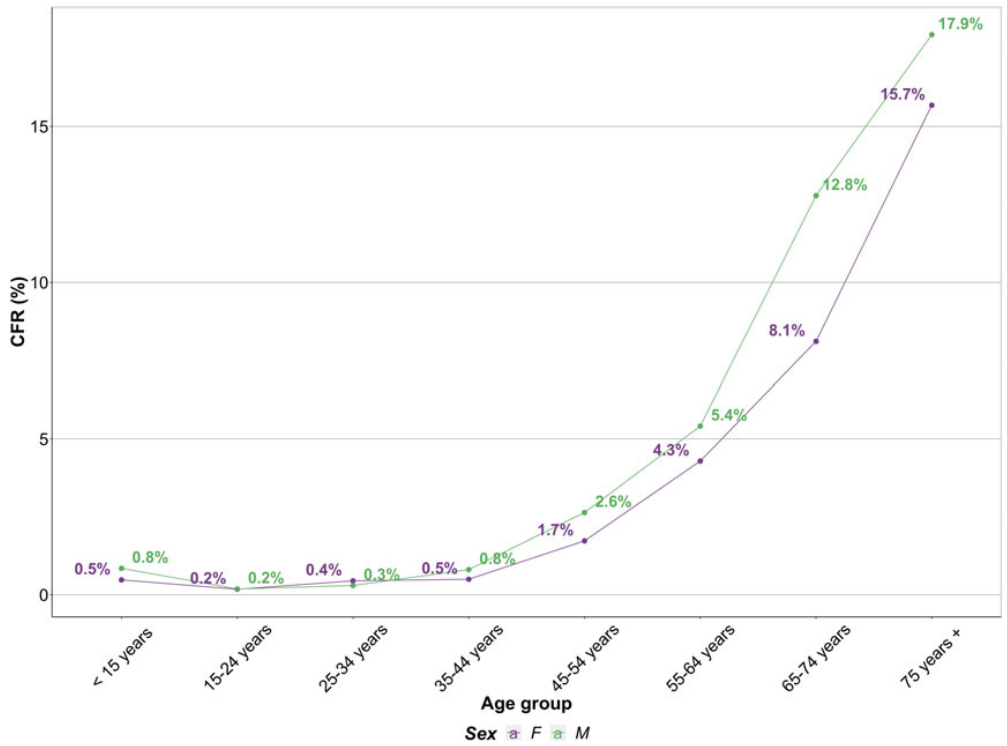

Fig. 3. Age and sex-specific case fatality ratio among confirmed cases reported in eight Member States of the WHO African region included in analysis between 21 March and 31 October 2020 (*N* = 46 870).

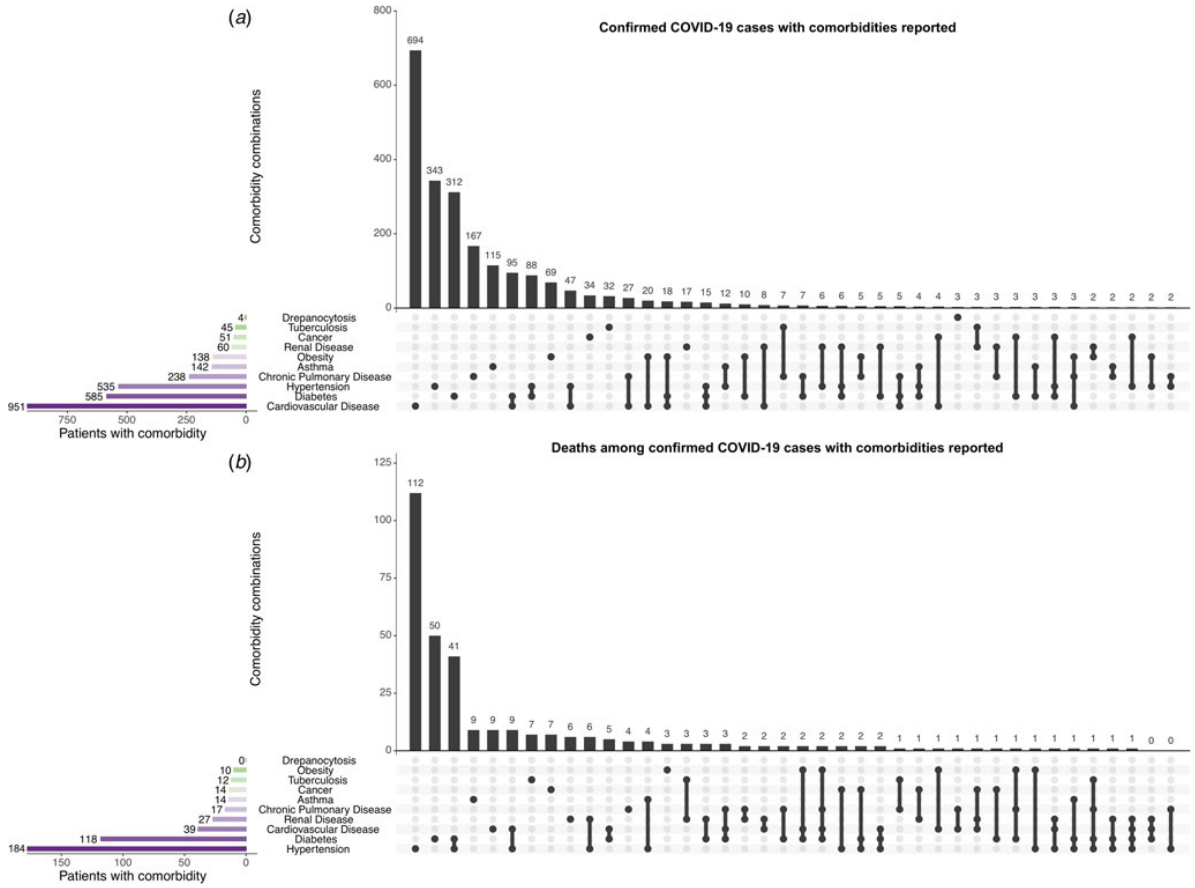

Fig. 4. (a) Combination analysis of comorbidities of interest – demonstrating the 40 most common combinations among confirmed cases (a) and confirmed cases that died (b) with comorbidities of interest in eight Member States of the WHO African region included in analysis between 21 March and 31 October 2020 ($N = 2227$, $N = 310$ respectively).

Table 2. Univariate weighted cox regression for exposure–response relationship comparing increasing number of comorbidities of interest to those without, among confirmed cases with comorbidities of interest in eight Member States of the WHO African region between 21 March and 31 October 2020 ($N = 46\,236$)

Number of comorbidities[a]	CFR	*N*	Dead (*N*)	Time (days)	aHR	95% CI	*P*-value
No/not reported	1.05	44 009	463	3 732 259			
1	11.59	1786	207	137 250	11.64	9.87–13.73	<0.001
2	23.16	367	85	28 719	24.14	19.22–30.33	<0.001
3	20.90	67	14	5543	22.30	13.11–37.91	<0.001
4	57.14	7	4	308	66.01	28.59–152.42	<0.001

aHR, average hazard ratio; CI, confidence interval.
[a]Specific comorbidities identified included diabetes, asthma, hypertension, obesity, cardiovascular disease, tuberculosis, renal disease, drepanocytosis, chronic pulmonary disease and cancer. Other/not specified comorbidity was not included in this analysis.

presented here for the same time period is double what we report, with the overall CFR also being slightly higher. This suggests that our observations may be underestimating the true state of the COVID-19 situation in the region. It should also be considered that CFRs can be influenced by the bias from possible discrepancies in per capita testing rates between the African countries included in this study.

Three key risk factors have stood out to date with relation to COVID-19-associated deaths, namely age, sex and the presence of comorbidities. Our observed incidence and CFR by age and sex aligned with other studies, with older age and being male being widely documented as key risk factors, with higher mortality among older males [23, 24]. Persons over the age of 65 years have been shown to have a 62 times higher mortality rate than

Table 3. Weighted cox regression for mortality by various characteristics among confirmed cases reported in eight Member States of the WHO African region between 21 March and 31 October 2020 ($N = 46\,870$)

				Univariate			Multivariate		
Characteristic	*N*	Dead (N^a)	Time (days[a])	aHR	95% CI	*P*-value	aaHR	95% CI	*P*-value
Sex (male)	46 870	582	2 565 154	1.49	1.27–1.74	<0.001	1.54	1.31–1.81	<0.001
Age (continuous)	46 870			1.08	1.07–1.08	<0.001	1.08	1.07–1.08	<0.001
Health care worker status	46 870	19	149 076	0.76	0.49–1.21	0.248	0.59	0.37–0.93	0.024
Residence in capital city status	46 870	347	1 662 749	1.88	1.63–2.16	<0.001	1.42	1.22–1.65	<0.001
Comorbidity status	46 870	340	227 615	11.89	10.33–13.69	<0.001	36.37	20.26–65.27	<0.001
Pregnancy	17 012[b]	584	10 910	1.06	0.26–4.28	0.935			
Comorbidity status×age (continuous)							0.97	0.96–0.98	<0.001

aHR, average hazard ratio; CI, confidence interval; aaHR, average adjusted hazard ratio.
[a]Unweighted counts.
[b]Females only.

Table 4. Time to death by various characteristics among confirmed cases reported in eight countries between 21 March and 31 October 2020 ($N = 803$)

Characteristic	Dead (N = 803)	Time to death (days)[a]	*P*-value[b]
Sex			0.565
Female	221	4 (2, 10)	
Male	582	5 (2, 9)	
Age			0.038
<60	428	5 (2, 10)	
≥60	375	4 (2, 9)	
Health care worker			0.200
No/not reported	784	5 (2, 9.25)	
Yes	19	7 (4, 11.5)	
Residence in capital city			<0.001
No/not reported	456	4 (2, 9)	
Yes	347	6 (2, 11)	
Presence of comorbidity			0.085
No/not reported	463	4 (2, 9)	
Yes	340	5 (2, 11)	

[a]Statistics presented: median (IQR).
[b]Statistical test: Kruskal–Wallis rank-sum test.

those under 55 years [25]. In our data, we find that those over 60 have a CFR 10 times greater than those 60 and under and 15 times greater than those under 50, demonstrating that the same dynamics hold true despite the different population age structure of countries in the WHO African region. However, it is possible that new variants, arising in later stages of the pandemic, could result in the shifting of this age demographic, and should be investigated in further studies

Persons with non-communicable diseases, such as cardiovascular disease and diabetes, have also been identified as having greater risk of COVID-19-associated mortality, with the risk of death increasing with the number of comorbidities [26, 27]. In a study of causes of deaths in South Africa during the first 99 days of the pandemic, individuals with two or more comorbidities accounted for 58.6% of these deaths, with hypertension and diabetes the most commonly reported diseases [28].

Our study found that cardiovascular disease, diabetes and hypertension were the most commonly reported comorbidities among cases in the countries studied in the WHO African region. Having any comorbidity increased the hazard of death 12-fold, reaching a maximum of 66-fold for cases with four comorbidities (the maximum reported). This is again in keeping with the globally identified mortality risk COVID-19 poses to those with comorbidities [29]. With the absolute burden of non-communicable diseases in Africa being comparable to other regions [30], our findings suggest that we should not disregard the impact of COVID-19 on people with non-communicable diseases, and *vice versa*, in the WHO African region. It is key for public health authorities in the WHO African region to address the growing burden of non-communicable diseases as part of the COVID-19 response, as suggested previously by the WHO [31]. Affordable and proven cost-effective interventions should be made available to countries to prevent and manage non-communicable diseases in the context of COVID-19. Critically, interruptions in non-communicable disease services that have occurred as a result of diversion of resources to COVID-19 responses must be addressed. Some countries have already started, with alternative strategies such as triaging and telemedicine, while continuity of non-communicable diseases services has been ensured by others in their list of essential health services [31].

Several studies have reported higher COVID-19 incidence among healthcare workers, however few have reported on differences in mortality [32, 33]. Healthcare workers have a lower hazard in our study, which may be explained by the fact that there were more young female healthcare workers captured in our data. It should be noted however, that our measures involving healthcare workers may be subject to potential bias, possibly due to ease of testing access within this setting.

Spatial disparities have been reported as an independent risk factor for infection-related mortality when comparing metropolitan and non-metropolitan areas [20]. The reasoning behind this is linked to high-population density and opportunities for increased transmission through more socio-economic

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interactions. Some studies have found that COVID-19 deaths are concentrated in large cities and surrounding metropolitan areas. However, small cities or rural communities were also found to have equivalently high rates when opportunities for large gatherings, such as funerals, presented themselves and there was an infected individual(s) in attendance [21]. Our findings reflect the latter, and in addition our data suggest that living outside a capital city significantly decreases time to death.

The time to death for COVID-19 has been shown to vary widely across studies and has been linked to age and presence of comorbidities [34, 35]. To our knowledge, no other studies report on time from having a positive test to death. Several studies report on the (more clinically useful) time from hospital admission of COVID-19 patients to death [36, 37], which could have impacted the lack of association found with time to death and comorbidity presence. Though not directly comparable, our median times being lower than the studies mentioned may be suggestive of limited critical resources and access to early supportive care in some countries which may lead to accelerated deterioration [38].

Study limitations

Several important limitations to this analysis should be noted, most importantly the generalisability of these results. The low number of countries included (8 of 47 countries in the WHO African region) means that our results are not representative of the whole African region. In addition, it is not possible to make valid comparisons between countries due to likely differences in testing and reporting of cases and associated deaths. Most countries, despite reporting up until the 31 October, did not report confirmed cases right up to that date. This means that for those countries we may be underestimating CFR for the period, and thereby also for the overall CFR estimate. We must also note the weaknesses of the civil registration system, with death reporting universally adopted in only eight countries of the African continent [39].

Cases dropped due to missing or incorrect date variables, as well as those missing information on age and sex, may have further contributed to CFR inaccuracy (under or overdepending on whether/how systematically missing). Beyond the issues in data collection, the choice to interpret 'not reported' as 'no' for exposure variables may have led to inaccurate stratified CFRs and may also have resulted in inaccurate aHR estimates again depending on the pattern of missingness.

Our regressions only include information on a few variables, meaning that we are likely missing a multitude of important confounders and effect modifiers; thus the results of multivariable regressions should be interpreted with caution.

We acknowledge the association with comorbidities in our study may be due to reporting bias, because those who are the most ill will be more likely to be in hospital and thereby more likely to be captured, tested and reported. However, when considering that we included specific comorbidities of interest, it is possible that the association with non-communicable disease comorbidities would be even higher had this information been systematically collected. It should also be considered that differences in disease burden between countries within the African region may influence our findings. It is also possible that our observations on those residing in capital cities are due to reporting bias, as there will be higher clinical, testing and public health capacity in capital cities. However, this could also mean our observation for time to death is valid due to the disparities in healthcare availability and capacity in rural and urban areas. In addition, selection bias may also influence measures surrounding healthcare workers, due to ease of testing access within this setting. This may result in an over-representation of healthcare workers in our sample.

Finally, our analysis has only captured people who have a confirmed positive test, and so are only informative at the population level hazard of death associated with a positive diagnosis of COVID-19. This is an important limitation for both clinical and public health decisions making. Future studies that either have more complete data (in terms of both countries included, cases captured and information collected), or are designed to investigate specific risk factors in clinical settings and within the specific African region countries, may provide more comprehensive information for the region.

Conclusion

This study is, to our knowledge, the only study of its size that investigates the mortality burden and risk factors for COVID-19 in the WHO African region. The utility of analysing observational data for decision making, as opposed to relying solely on assumption-based mathematical models, cannot be understated. Our study found that the overall incidence and CFR were on the lower end of the scale compared to several regions reporting hundreds of cases per 100 000 population and CFRs several times higher, but this may be due to under-reporting. Four key risk factors were associated with mortality, namely male sex, older age, presence of one or more comorbidities and residence in capital cities.

Mortality from COVID-19 in Africa is likely to be comparable with that elsewhere, although under-reported, with many of the same risk factors for this present in these populations. The incidence of non-communicable diseases across the region is also comparable to other, perhaps better studied, regions. This makes it important to consider these diseases in future studies and in health system and future pandemic planning.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S095026882100251X.

Data availability statement. The data that support the findings of this study are available on request from the corresponding author (BI). Some of the data are publicly available through situation reports produced by Ministries of Health and WHO/AFRO on their respective websites.

References

1. **World Health Organisation (WHO)**. *Novel Coronavirus – China. WHO*. World Health Organization. Available at http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/ (Accessed 26 January 2021).
2. **Petersen E *et al.*** (2020) Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. *The Lancet Infectious Diseases* **20**, e238–e244.
3. **World Health Organization** (2003) *Consensus Document on the Epidemiology of Severe Acute Respiratory Syndrome (SARS)*. Geneva: World Health Organization.
4. **World Health Organisation EMRO**. *MERS situation update, January 2020*. Available at http://www.emro.who.int/pandemic-epidemic-diseases/mers-cov/mers-situation-update-january-2020.html (Accessed 22 January 2021).
5. **World Health Organisation (WHO)**. *WHO Coronavirus (COVID-19) Dashboard*. Available at https://covid19.who.int (Accessed 30 September 2021).

6. **Breban R, Riou J and Fontanet A** (2013) Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* **382**, 694–699.
7. **WHO Regional Office for Africa** (2020) *A second COVID-19 case is confirmed in Africa*. *WHO|Regional Office for Africa*. Available at https://www.afro.who.int/news/second-covid-19-case-confirmed-africa (Accessed 21 January 2021).
8. **WHO Regional Office for Africa**. *Situation reports on COVID-19 outbreak – Sitrep 11, 13 May 2020*. *WHO|Regional Office for Africa*. Available at https://www.afro.who.int/publications/situation-reports-covid-19-outbreak-sitrep-11-13-may-2020 (Accessed 26 January 2021).
9. **Ghosh D, Bernstein JA and Mersha TB** (2020) COVID-19 pandemic: the African paradox. *Journal of Global Health* **10**, 020348.
10. **Mougeni F, Mangaboula A and Lell B** (2020) The potential effect of the African population age structure on COVID-19 mortality. medRxiv. doi: 2020.05.19.20106914.
11. **Sajadi MM *et al.*** (2020) Temperature, humidity, and latitude analysis to estimate potential spread and seasonality of coronavirus disease 2019 (COVID-19). *JAMA Network Open* **3**. Published online: 11 June 2020. doi: 10.1001/jamanetworkopen.2020.11834.
12. **Achoki T *et al.*** (2020) COVID-19 pandemic in the African continent: forecasts of cumulative cases, new infections, and mortality. medRxiv. doi: 2020.04.09.20059154.
13. **World Health Organisation (WHO)** (2019) *Public health surveillance for COVID-19: interim guidance*. Available at https://www.who.int/publications-detail-redirect/who-2019-nCoV-surveillanceguidance-2020.8 (Accessed 28 January 2021).
14. **World Health Organisation (WHO)** (2016) *International Health Regulations (2005)*, 3rd Edn. Geneva: World Health Organisation.
15. **United Nations Department of Economic and Social Affairs**. *World population prospects – population division – United Nations*. Available at https://population.un.org/wpp/ (Accessed 21 January 2021).
16. **World Health Organisation (WHO)** *Estimating mortality from COVID-19*. Available at https://www.who.int/news-room/commentaries/detail/estimating-mortality-from-covid-19 (Accessed 21 January 2021).
17. **Dunkler D *et al.*** (2018) Weighted cox regression using the R package coxphw. *Journal of Statistical Software* **84**, 1–26.
18. **Setel P *et al.*** (2020) Mortality surveillance during the COVID-19 pandemic. *Bulletin of the World Health Organization* **98**, 374–374.
19. ***WHO Coronavirus Disease (COVID-19)*** *Dashboard*. Available at https://covid19.who.int (Accessed 26 January 2021).
20. **Bhadra A, Mukherjee A and Sarkar K** (2020) Impact of population density on Covid-19 infected and mortality rate in India. *Modeling Earth Systems and Environment*. Published online: 14 October 2020. doi: 10.1007/s40808-020-00984-7.
21. **Stier A, Berman MG and Bettencourt L** (2020) COVID-19 attack rate increases with city size. Published online: 30 March 2020.
22. **van Zandvoort K *et al.*** (2020) Response strategies for COVID-19 epidemics in African settings: a mathematical modelling study. *BMC Medicine* **18**, 324.
23. **Peckham H *et al.*** (2020) Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nature Communications* **11**, 6317.
24. **O'Driscoll M *et al.*** (2020) Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* **590**, 1–6.
25. **Yanez ND *et al.*** (2020) COVID-19 mortality risk for older men and women. *BMC Public Health* **20**, 1742.
26. **Ssentongo P *et al.*** (2020) Association of cardiovascular disease and 10 other pre-existing comorbidities with COVID-19 mortality: a systematic review and meta-analysis. *PLoS ONE* **15**, e0238215.
27. **Harrison SL *et al.*** (2020) Comorbidities associated with mortality in 31,461 adults with COVID-19 in the United States: a federated electronic medical record analysis. *PLoS Medicine* **17**, e1003321.
28. **Wyk VP *et al.*** (2020) COVID deaths in South Africa: 99 days since South Africa's first death. *South African Medical Journal* **110**, 1093–1099.
29. **Horton R** (2020) Offline: COVID-19 is not a pandemic. *The Lancet* **396**, 874.
30. **GBD 2019 Diseases and Injuries Collaborators** (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* **396**, 1204–1222.
31. **World Health Organisation (WHO)** (2020) *The Impact of the COVID-19 Pandemic on Noncommunicable Disease Resources and Services: Results of a Rapid Assessment*. Geneva: World Health Organisation.
32. **Office for National Statistics** (2020) *Which occupations have the highest potential exposure to the coronavirus (COVID-19)?* Available at https://www.ons.gov.uk/employmentandlabourmarket/peopleinwork/employmentandemployeetypes/articles/whichoccupationshavethehighestpotentialexposuretothecoronaviruscovid19/2020–05–11 (Accessed 22 January 2021).
33. **Bandyopadhyay S *et al.*** (2020) Infection and mortality of healthcare workers worldwide from COVID-19: a systematic review. *BMJ Global Health* **5**, e003097.
34. **Verity R *et al.*** (2020) Estimates of the severity of coronavirus disease 2019: a model-based analysis. *The Lancet Infectious Diseases* **20**, 669–677.
35. **Alaa A *et al.*** (2020) Retrospective cohort study of admission timing and mortality following COVID-19 infection in England. *BMJ Open* **10**, e042712.
36. **Faes C *et al.*** (2020) Time between symptom onset, hospitalisation and recovery or death: statistical analysis of Belgian COVID-19 patients. *International Journal of Environmental Research and Public Health* **17**. Published online: October 2020. doi: 10.3390/ijerph17207560.
37. **Azoulay E *et al.*** (2020) Increased mortality in patients with severe SARS-CoV-2 infection admitted within seven days of disease onset. *Intensive Care Medicine* **46**, 1714–1722.
38. **Asiimwe E and Saraswati K** (2020) Covid-19 and sub-Saharan Africa's critical care infrastructure. *BMJ*. Available at https://blogs.bmj.com/bmj/2020/04/23/covid-19-and-sub-saharan-africas-critical-care-infrastructure/ (Accessed 28 January 2021).
39. **United Nations** (2017) Economic Commission for Africa. Report on the status of civil registration and vital statistics in Africa: outcome of the Africa programme on accelerated improvement of civil registration and vital statistics systems monitoring framework. *Addis Ababa. © UN. ECA*. Published online: November 2017.

Appendix 4.2- 100 years of insulin: celebrating the past, present and future of diabetes therapy 100 years of insulin: celebrating the past, present and future of diabetes therapy

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100 years of insulin: celebrating the past, present and future of diabetes therapy

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The year 2021 marks the centennial of Banting and Best's landmark description of the discovery of insulin. This discovery and insulin's rapid clinical deployment effectively transformed type 1 diabetes from a fatal diagnosis into a medically manageable chronic condition. In this Review, we describe key accomplishments leading to and building on this momentous occasion in medical history, including advancements in our understanding of the role of insulin in diabetes pathophysiology, the molecular characterization of insulin and the clinical use of insulin. Achievements are also viewed through the lens of patients impacted by insulin therapy and the evolution of insulin pharmacokinetics and delivery over the past 100 years. Finally, we reflect on the future of insulin therapy and diabetes treatment, as well as challenges to be addressed moving forward, so that the full potential of this transformative discovery may be realized.

In 2021, the world celebrates the 100th anniversary of the discovery of insulin, a treatment that transformed type 1 diabetes from a once-fatal diagnosis into a chronic, medically manageable condition. Beyond its immediate therapeutic impact, insulin has served as the centerpiece for incredible advances in the fields of crystallography, molecular biology, prohormone processing, autoimmunity, physiology, and precision health and genetics, while forming the basis for four Nobel Prizes. In honor of this centennial, we commemorate the unlikely scientific journey that led to insulin's discovery, chronicle the subsequent molecular characterization of the insulin molecule, which has permitted new insulin-based therapeutics, and describe the parallel clinical discoveries that have forged our contemporary understanding of diabetes classification and etiology.

The discovery of insulin

The events surrounding the discovery of insulin are well chronicled. Michael Bliss summarized it perfectly in his 1982 history describing them as "richly dramatic", both for the "medical miracle" of resurrecting people near death by a "magical elixir of life" and for the incredible scientific journey that ended with the successful extraction of pancreatic insulin and its rapid clinical use[1,2]. The story's dramatic arc is one woven together by stubborn determination, numerous experimental failures, recurrent serendipity and, ultimately, disputed academic credit. At its center is a pair of unlikely protagonists, Frederick Banting, a surgeon with no apparent formal research experience, and Charles Best, a medical student who won a coin toss for the assignment to work with Banting on a summer research project. After reading an article on the pancreas, Banting appealed to and ultimately received support and advice from J. J. R. Macleod, a Professor of Physiology at the University of Toronto, to begin a project with a simple premise. He proposed to perform surgical ligation of the canine pancreatic duct to isolate the organ's internal secretions[3–8]. He aimed to use these secretions for the treatment of diabetes.

At the time Banting and Best began their experiments in May 1921, diabetes was understood to be a disease of the pancreas. The name 'diabetes' was coined by Demetrius of Apamea around the first century BC based on the Greek term *diabainein* meaning 'siphon' due to the symptoms of polyuria and polydipsia[9]. In the 1600s, 'mellitus' was added to indicate that urine sweetness differentiated this condition from other causes of polyuria, with the idea that this sweetness might be linked to a similar finding in the blood[10]. However, it took nearly another century to link the polyuria and polydipsia of diabetes mellitus with excessive glucose in both the blood and urine[11]. The first working evidence that the pancreas controlled carbohydrate metabolism would not come until 1889, when German scientists Oskar Minkowski and Joseph von Mering performed pancreatectomies on dogs who then developed hyperglycemia and diabetes[12]. Almost 20 years before Minkowski and von Mering's seminal work, the first detailed histologic studies of the pancreas were published by Paul Langerhans, as a medical student. His meticulous work described nine different cell types that formed numerous "cell heaps" scattered throughout the gland[13]. The French scientist G. E. Laguesse would revisit pancreas histology in 1893 and name these collections the "îlots de Langerhans"[14,15]. The term 'insulin' was subsequently coined in 1909 by the Belgian scientist J. de Meyer to describe the still-speculative internal secretion of the pancreas thought to be capable of regulating blood glucose[16]. At the time experiments were beginning in Toronto in the summer of 1921, a handful of other scientists throughout the world were already pursuing the goal of harnessing this mysterious substance for therapeutic use[4].

Whereas others failed or, in the case of the Romanian scientist N. C. Paulesco, would have their work interrupted by World War I (ref. [4]), the Toronto group in a mere 9 months successfully isolated

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insulin from the pancreas. They would go on to prove that the pancreatic extract regulated blood glucose levels and urinary glucose excretion by reinjecting it into pancreatectomized dogs, while keeping the longest living of these dogs, Marjorie, alive for more than 70 days. James Collip, a biochemist from the University of Alberta, on sabbatical at the University of Toronto, joined the team late in the fall of 1921 and played a critical role in developing methods to reliably isolate insulin from the pancreas using alcohol extraction. The first documented patient to receive insulin was 14-year-old Leonard Thompson. He received his first injection in January 1922, at a time when he, by all accounts, was near death. As reports of his treatment spread throughout North America, the team in Toronto received a growing number of desperate appeals from patients and their physicians for the new therapy. They struggled to scale up the production to reach this growing demand. Ultimately this problem was solved through a partnership with the pharmaceutical corporation Eli Lilly and Company in Indianapolis, Indiana. Scientists at Lilly optimized methods of isoelectric precipitation enabling the extraction of large quantities of insulin from porcine pancreata, allowing it to be purified for commercial distribution.

The capability for insulin purification quickly spread to physicians and scientists beyond North America. At a private dinner in 1922, Elliot Joslin shared the news with the Nobel Prize-winning Danish scientist August Krogh and his wife Marie Krogh, who had recently been diagnosed with adult-onset diabetes. The Kroghs extended their trip by several days to visit Macleod in Toronto, obtained a license to bring the team's insulin purification protocol to Europe, and immediately began production of insulin following their return to Copenhagen (serving as the starting foundation for what eventually became Novo Nordisk)[17].

By the end of this incredible journey, the team in Toronto would be deeply fractured by conflict over who deserved scientific credit for the discovery of insulin. However, to ensure access of this life-saving drug to patients with diabetes, the team agreed to sell their patents back to the University of Toronto for the price of CAN$1. Ultimately Banting and Macleod were awarded the 1923 Nobel Prize in Physiology or Medicine, with Banting sharing his portion of the award with Best, and Macleod doing the same with Collip[4,18].

Advances in the understanding of diabetes pathophysiology. The transformative discovery of insulin, in part, represented an inevitable culmination of a body of work performed by many investigators over many years (Fig. 1). The evolution of our understanding of diabetes pathophysiology has similarly occurred due to the collective observations of numerous clinicians and researchers. Before the clinical availability of insulin, astute observers delineated subgroups of affected individuals based on age of presentation, body habitus and survival on low-carbohydrate diets[19]. Once insulin therapy was available, clinicians related these differences to insulin requirements, with insulin-insensitive patients usually presenting with symptoms later in life, in association with obesity and a more insidious presentation[20,21]. Those who were more sensitive to insulin often presented at younger ages and required smaller doses of insulin to suppress urine glucose and become hypoglycemic[22]. Direct comparison of forearm arteriovenous glucose gradients after simultaneous glucose and insulin administration showed differences in lean, young patients with diabetes compared to older, overweight patients[23]. These findings suggested that differences in glucose gradients may be related to forearm muscle resistance to insulin action and that those with diabetes could be separated into distinct subgroups—the first with disease resulting from insulin insufficiency and the second with disease occurring due to insulin insensitivity[23].

In the 1950s, the ability to quantify circulating insulin allowed for confirmation of insulin deficiency in certain groups of patients. Initial work used bioassays demonstrating that compared to human plasma from older obese females with nonketotic hyperglycemia, human plasma from young, ketotic patients with diabetes was unable to lower blood glucose values when injected into diabetic rats[24]. Similarly, the extractable insulin content of pancreata was tested for its ability to induce mouse seizures. These experiments showed that pancreatic insulin was almost undetectable in young people with diabetes. This was in contrast to the nearly ~50% reduction observed in older people with diabetes relative to nondiabetic controls[25]. Rosalyn Yalow and Solomon Berson's development of a reliable radioimmunoassay allowed for direct measurement of insulin levels, allowing for the separation of insulin-deficient versus insulin-insensitive diabetes based on measurement of circulating insulin[26]. Yalow was awarded the 1977 Nobel Prize in Physiology or Medicine for this seminal work, becoming only the second woman to earn this award.

In search of a simple binary classification system, multiple naming iterations would be trialed, including groups I and II; types I and II; insulin sensitive and insensitive; insulin dependent and noninsulin dependent; and diabetes gras (fat) and diabetes maigre (thin)[3,27,28]. Still the actual etiologic basis for these different disease types remained unclear. Not until the 1950s, following the discovery of an autoimmune basis for other endocrine diseases, did researchers begin to consider autoimmunity as an etiology of insulin-deficient diabetes[29–31]. Patients with diabetes and an insulin-deficient phenotype were noted to frequently have detectable autoantibodies associated with other autoimmune diseases, including thyroid and gastric antibodies[32,33]. In animals, injections of anti-insulin serum, or homogenized pancreatic or islet tissues, resulted in development of islet immune lesions, supporting the idea that islets could generate an immune response[34–36]. Early reports examining small numbers of pancreatic sections from individuals with diabetes had only rarely identified examples of immune cell infiltration into the islet (that is, insulitis)[37]. However, in 1965, Willy Gepts analyzed a larger number of pancreatic samples obtained from children who died near the time of clinical diagnosis and showed islets with lymphocytic infiltrates in the majority of autopsy specimens, suggesting a clearer link to an immunologic origin of disease[38]. These findings in postmortem tissue were ultimately validated by key studies showing autoimmunity using blood samples from living donors. Leukocyte migration assays demonstrated that individuals with type 1 diabetes exhibited evidence of anti-pancreatic cell-mediated immunity[2]. In a now famous 'eureka' moment, in 1974, Franco Bottazzo, a research fellow in Deborah Doniach's laboratory in London, was the first to successfully visualize islet cell antibodies using indirect immunofluorescence, thereby confirming the presence of antibodies reactive to the islet. During experiments originally designed to support his thesis work on Addison's disease, he observed that pancreatic islets "lit up" after incubation with sera from some patients with polyendocrine autoimmunity, most of whom had or would go on to develop diabetes[3,5]. These findings would quickly be confirmed by multiple groups around the world[3].

By the end of the 1970s, this work led to the recognition that immune-mediated loss of insulin-secreting cells was the cause of insulin-dependent diabetes[3]. In parallel, development of techniques to measure insulin-mediated glucose disposal allowed for direct confirmation of insulin resistance in individuals matching a noninsulin-dependent diabetes phenotype[39–41]. Based on these findings, in 1979 the National Diabetes Data Group proposed classifying diabetes using the terms employed today: type 1 (insulin dependent), type 2 (noninsulin dependent), and 'other' denoting forms of disease not fitting into either of these two categories[42].

Human cohorts provide a contemporary understanding of diabetes pathophysiology. New animal models of spontaneous disease[43,44] and improvements in immunologic, metabolic and genetic phenotyping in human cohorts have continued to shape our understanding of type 1 and type 2 diabetes over the past half-century. The widely

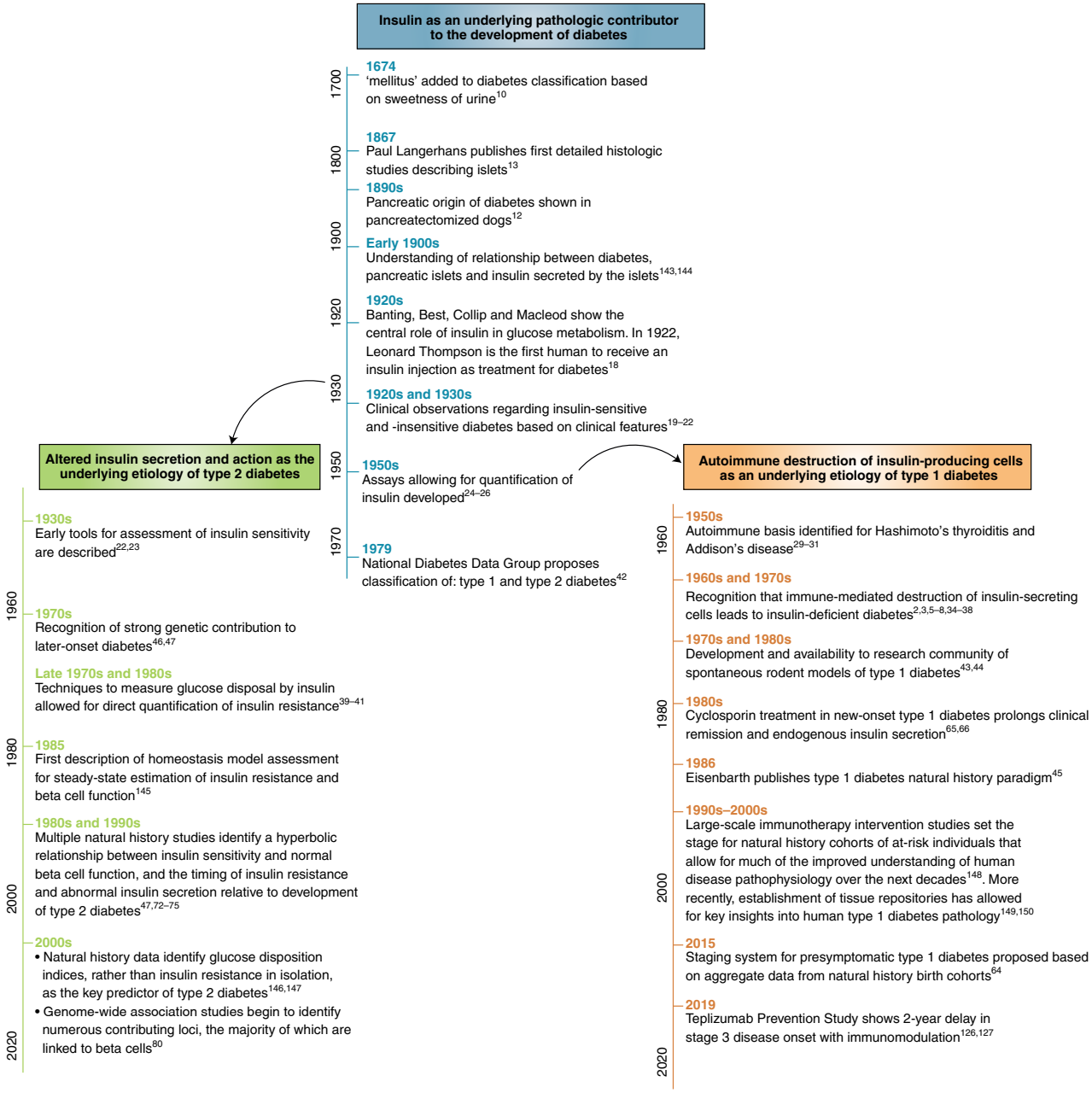

Fig. 1 | A timeline of key discoveries in our understanding of insulin and diabetes pathophysiology. Shown in the main branch of the timeline are key discoveries in our understanding of insulin as a central contributor to diabetes pathophysiology[10,12,13,18–22,24–26,42,143,144]. Included in the left branch of the timeline are important milestones that have enabled the understanding of type 2 diabetes as a disease of impaired insulin secretion and action[22,23,39–41,46,47,67,72–75,80,145–147]. The right branch highlights notable discoveries that have led to the understanding of type 1 diabetes as an autoimmune disease[2,3,5–8,29–31,34–38,43–45,64–66,126,127,148–150].

adopted 1986 Eisenbarth model suggested that type 1 diabetes was a chronic autoimmune disease, with genetically predisposed individuals encountering a hypothetical triggering event that activated islet autoimmunity, yielding progressive beta cell destruction and insulin deficiency[45]. Although a genetic contribution to diabetes was clear based on increased prevalence among family members, analyses of kindreds were limited by lack of a reliable biomarker for 'pre-diabetes', as well as a confusing picture based on different inheritance patterns, disease presentations and phenotypes, which also pointed to environmental exposures as contributors[6,43–45]. A theme of early twin studies indicated >90% concordance of diabetes in those diagnosed at older ages (that is, type 2 diabetes) and approximately 50% concordance of diabetes occurring in children and young adults (that is, type 1 diabetes)[8,46,47]. The description of the critical role of human leukocyte antigen (HLA) antigen-presentation genes in the transplantation setting[48] was followed with the association of these

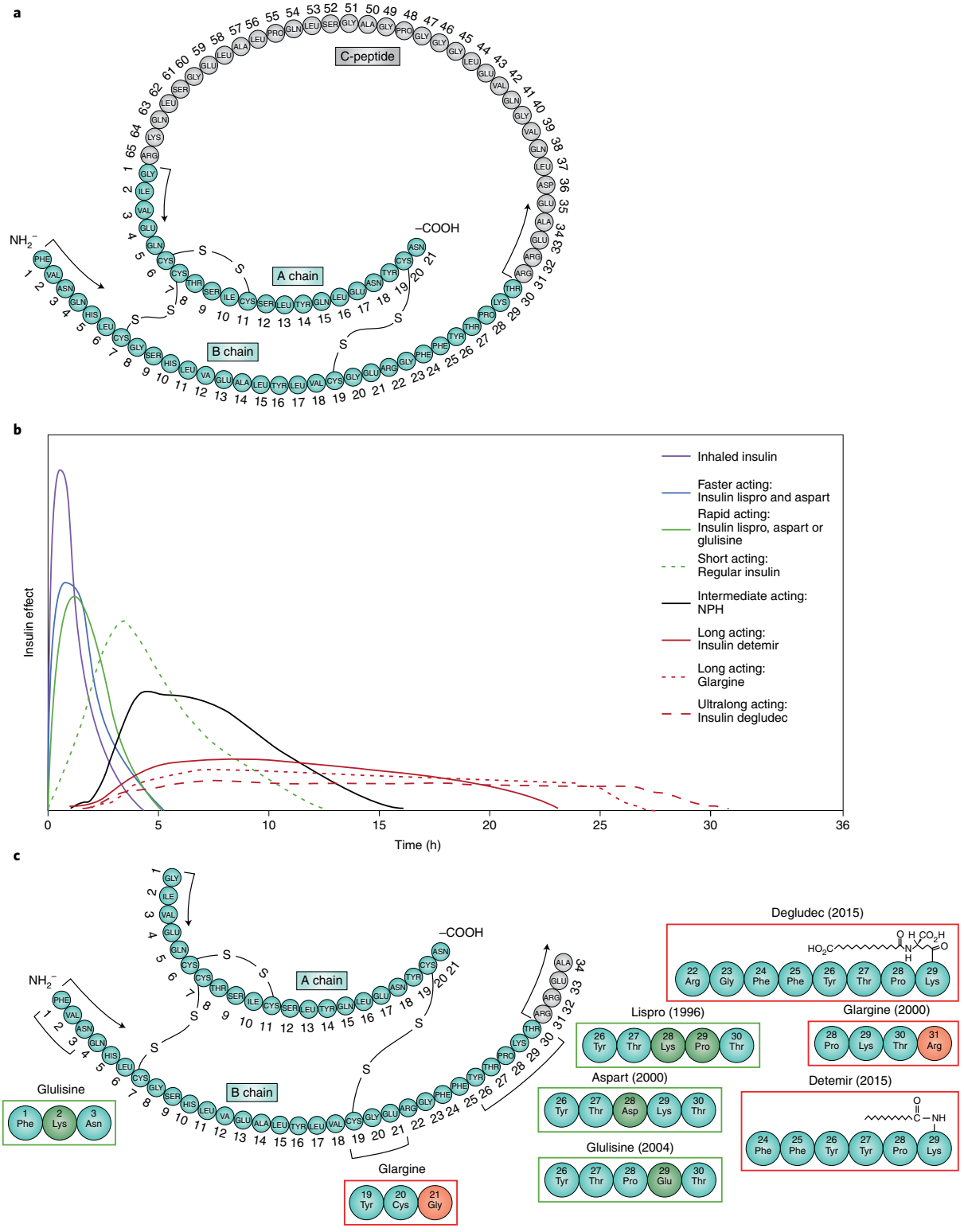

Fig. 2 | The evolution of improvements in insulin pharmacokinetics. a, The native structure of human proinsulin. **b**, Representative pharmacokinetic profiles of available insulins administered subcutaneously. **c**, Structural changes of insulin analogs and years of introduction in the USA including rapid-acting insulin analogs (green boxes) and long-acting insulin analogs (red boxes)[96,106,151–154].

genes with autoimmune diseases including ankylosing spondylitis, multiple sclerosis and type 1 diabetes in the early 1970s (ref. [6]). To this day, a standout feature of many autoimmune diseases including type 1 diabetes is that a small number of HLA class 2 alleles, critical for antigen presentation by the immune system, explain a large proportion of disease heritability.

In the 1990s, linkage analysis of sibling pairs affected by type 1 diabetes identified an area on chromosome 11p15 (ref. [49]) that was subsequently mapped to a region upstream of the insulin gene[50–52] as associated with type 1 diabetes. Subsequent genome-wide linkage and then genome-wide association studies of cases and controls have described more than 60 loci outside the HLA region that also contribute to type 1 diabetes genetic risk[53]. The majority of variants point towards the role of inflammation and the immune system in type 1 diabetes pathogenesis. More recently, there has been increased focus on whether many of these variants may influence beta cell interactions with the immune system, with over 40% of genes associated with type 1 diabetes being expressed in the islet or beta cell. Coupled with molecular studies in human islets and mouse models of disease, these genetic associations highlight an ongoing dialogue as to whether beta cell or immune system abnormalities are the key driving factor in the pathogenesis of type 1 diabetes[54–59], a juxtaposition first described by Bottazzo as the notion of "beta cell homicide or suicide"[60].

Contemporary and large natural history studies assembled based on HLA genotypes and family history have shown that type 1 diabetes is a heterogenous disorder and that features beyond autoimmunity, including metabolic factors, exocrine function and environmental exposures, impact progression to clinical disease[61]. In addition, birth cohort studies have shed light on the timing of autoantibody development, describing a wide range but a surprising peak incidence of islet autoantibody development at 9 months of age[62], particularly focused on insulin autoimmunity. A seminal analysis of four different birth cohorts from the USA, Finland and Germany demonstrated that the presence of a single islet autoantibody is associated with a 13% risk of developing type 1 diabetes over 15 years. In contrast, having two or more antibodies is associated with a 70% risk over 10 years of observation and an 84% risk over 15 years[63]. These and other data led to a proposed modified staging system in 2015. Here, stage 1 diabetes is defined by two or more autoantibodies, while stage 2 diabetes is defined as the presence of multiple autoantibodies and dysglycemia. Stage 3 type 1 diabetes is defined by the progression to overt diabetes based on the American Diabetes Association standards, which include a fasting blood glucose of greater than 7.0 mmol l^{-1} (1.26 g l^{-1}), a random glucose of >11.1 mmol l^{-1} (2 g l^{-1}) with symptoms, an abnormal oral glucose tolerance test or a hemoglobin A1C level of >48 mmol mol^{-1} (6.5%)[64]. This staging paradigm has provided a regulatory and conceptual framework for efforts focused on disease prevention[65,66] and for mechanistic studies focused on developing stage-specific metabolic and immune signatures.

In parallel, beautifully detailed physiologic studies using intravenous and oral glucose tolerance tests and hyperglycemic clamps have provided further insight into the metabolic underpinnings of type 2 diabetes. These studies have demonstrated that to maintain glucose homeostasis, a feedback loop exists in which decreased insulin sensitivity is tightly associated with increased insulin secretion from the beta cell, with this hyperbolic relationship between beta cell responsivity and insulin sensitivity termed the disposition index[67,68]. Natural history studies of cohorts progressing to type 2 diabetes have demonstrated early impairments in insulin sensitivity, which are evident more than 10 years in advance of diabetes development. Initially, beta cell function is increased, maintaining glucose levels at higher but still normal levels and below the diagnostic threshold for diabetes. However, the ability of the beta cell to maintain this response is finite in some individuals. As the beta cells undergo a process of failure that has been linked with a number of molecular processes, including oxidative and endoplasmic reticulum stress, lipotoxicity and dedifferentiation[69–71], beginning around 3 years before the onset of diabetes, decreasing insulin secretion and an accelerated rise in blood glucose levels are observed[72–75]. However, the temporal relationship between changes in insulin secretion and insulin sensitivity continue to be elucidated, as insulin hypersecretion may also contribute to or exacerbate insulin resistance, and has even been documented before insulin resistance in some individuals[76].

Despite the high heritability observed in twin studies[46,47], it took longer to begin to identify the genetic loci responsible for the high concordance observed in twin studies of type 2 diabetes. The first associated loci, in genes including *TCF7L2*, *INSR*, *IRS1*, *GCK* and *KCNJ11* (refs. [77,78]), were originally identified by linkage or candidate gene studies, and since the turn of the century, increasing size and depth of genome-wide association studies have rapidly expanded the list of associated loci in type 2 diabetes to more than 250 with 400 independent signals[79]. The majority of associated loci are linked to beta cells, supporting the idea that impaired beta cell function is critical to type 2 diabetes pathogenesis[78,80]. Despite the large number of associated loci, their individual contributions to overall risk are moderate, explaining just under 20% of heritability and highlighting the proportion of 'missing heritability' that is still to be fully elucidated. Whereas type 1 diabetes seems to be a discrete entity defined by islet autoimmunity, beta cell destruction and a relatively small group of genes, an outstanding question is whether type 2 diabetes will be resolved into multiple subtypes/clusters defined by genetic associations, mechanisms and phenotype[81–83] and whether this approach will improve precision intervention and treatment.

Advances in the molecular characterization of insulin. Soon after the discovery of insulin and in parallel to its application in clinical medicine, there was a steady march to shed light on the molecular characteristics of the insulin molecule. In 1935, a research fellow, Dorothy Crowfoot Hodgkins, took the first diffraction images of insulin crystals[84]. She would continue her work on the insulin molecule on and off throughout her career, ultimately solving the crystal structure in 1969 and showing that insulin was a hexamer composed of three heterodimers[85]. Hodgkins earned the Nobel Prize in Chemistry in 1964 for her pioneering work in crystallography, all while battling her own autoimmune condition, rheumatoid arthritis[86]. In the early 1950s, Frederick Sanger determined the amino acid sequences of the A and B chains of insulin[87–90]. By 1955, he would demonstrate the position of the two disulfide bonds linking the A and B chains and the intrachain disulfide bond within the A chain, and in 1958, he was awarded the Nobel Prize in Chemistry[91–93]. In addition to being the first protein that was successfully sequenced, insulin was the first molecule to be characterized as a prohormone. In another moment of serendipity, Donald Steiner had the opportunity to study an insulinoma tumor removed from a patient at the University of Chicago in 1965. While analyzing extracts of the tumor and in subsequent experiments, Steiner identified proinsulin as the larger single-chain precursor of insulin, established proinsulin as the origin of C-peptide, and showed that insulin and C-peptide were secreted from the beta cell in equimolar ratios[94,95]. In 1968, Ronald Chance at Lilly Research Laboratories in Indianapolis published the porcine sequence of the proinsulin molecule[96].

These structural accomplishments would pave the way for studies describing the interaction of insulin with the insulin receptor[97], and would serve as a precursor to our understanding of monogenic forms of diabetes resulting from mutations in the insulin gene, which yield distinct phenotypes based on structural impacts. Altered interaction of structurally abnormal insulin with the insulin receptor leads to altered insulin action, hyperinsulinemia and adult-onset diabetes with autosomal dominant inheritance[98]. In

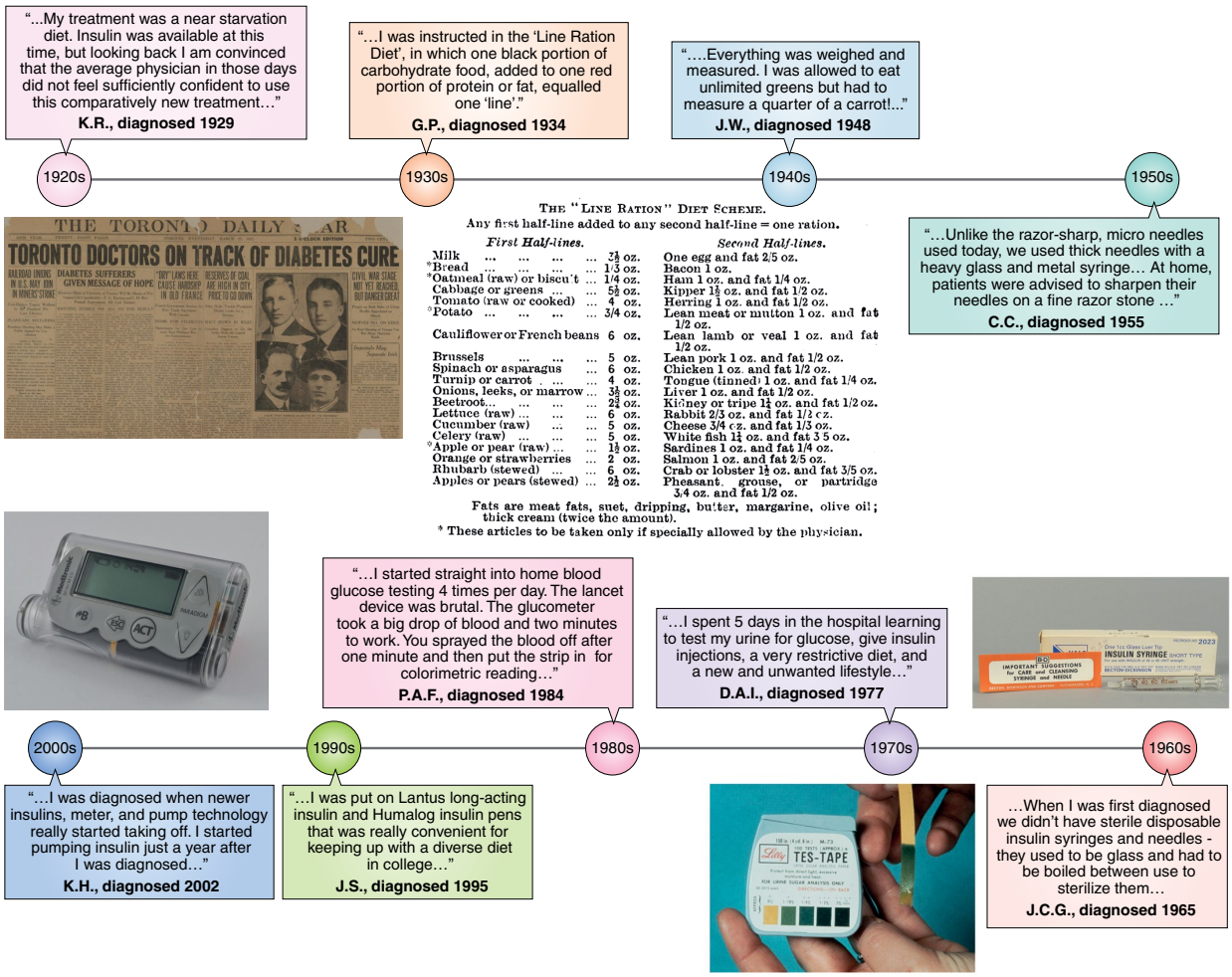

Fig. 3 | Advances in diabetes management viewed through the lens of individuals with type 1 diabetes. The full names of contributing individuals for the years 1965–2006 are included in the Acknowledgements. The quotes from K.R. (diagnosed 1929) and G.P. (diagnosed 1934) are from ref. [155]. The quote from J.W. (diagnosed 1948) is from ref. [156]. The quote from C.C. (diagnosed 1955) is from ref. [157].

contrast, recessive mutations impacting insulin biosynthesis result in neonatal diabetes[99]. Heterozygous mutations can also impair the normal folding of insulin precursors, yielding abnormal molecules that act in a dominant-negative fashion to impair the exit of all proinsulin from the endoplasmic reticulum[100]. This initially causes insulin deficiency, followed by severe beta cell endoplasmic reticulum stress and apoptosis[100,101]. Molecular studies defining the biologic impact of mutant *INS* gene-induced diabetes of youth (MIDY) mutations have also yielded valuable insights into the normal molecular pathways of insulin biosynthesis, precursor processing and transit through the secretory pathway[101], recently highlighting how certain conserved residues are critical for normal insulin folding[102]. Finally, decades after Steiner's original identification of proinsulin as insulin's precursor, increased proinsulin secretion relative to insulin or C-peptide is accepted as a serum proxy for beta cell stress and dysfunction and a predictive biomarker for both type 1 and type 2 diabetes[103,104].

Advances in the clinical use of insulin. The molecular characterization of insulin would also dramatically shape diabetes therapy. After the first clinical use of 'regular' insulin for patients, the pancreatic extract was further purified, the source of insulin moved to pork and later beef pancreas, and the concentration was increased from the original commercially available U-5 insulin (for example, 5 units ml^{-1}) to U-10, U-20, U-40 and U-80 preparations[105]. Later, in the early 1970s, the most common insulin preparation became U-100. More concentrated insulins also became available, and were employed for people with severe insulin resistance (U-200, U-300 and U-500); the first was U-500 beef regular insulin, which was developed in 1952.

Although exogenously administered regular insulin was lifesaving, its pharmacokinetics did not mirror that of endogenously produced human insulin. Administered insulin molecules self-associate into hexamers, which must dissociate into dimers and then monomers before entering the circulation, with typical delays of 60–90 min from injection to peak action. This contrasts with the circulating endogenous insulin peak action of approximately 15–30 min after the start of food ingestion. In addition to the delay in action, these first insulins were all short acting (Fig. 2) and required multiple injections per day.

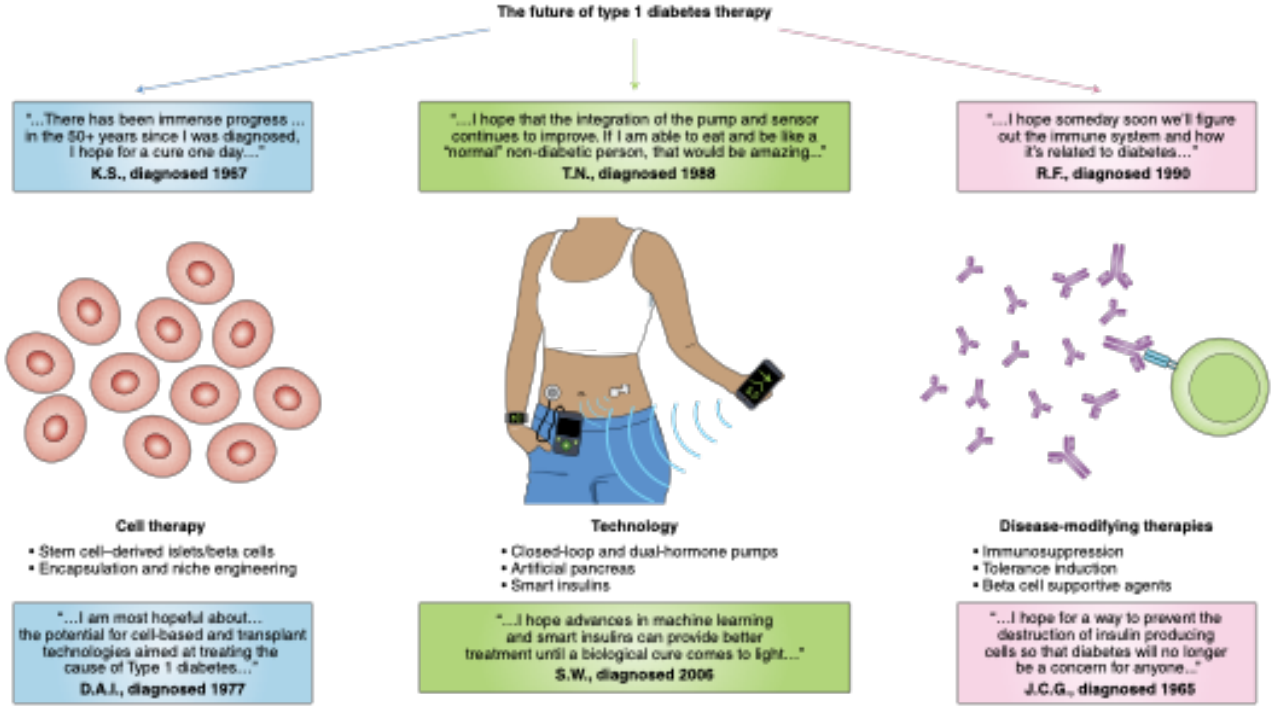

Fig. 4 | The future of insulin and diabetes therapy. The future of diabetes therapy and prevention includes efforts focused on: the development of a renewable, cellular source of insulin; improvements in technology, including better insulins and novel insulin delivery platforms; and disease-modifying therapies, including immune-modulating therapies and beta cell supportive agents. Quotes are included from individuals who depend on insulin, expressing their hopes surrounding the future of diabetes therapy. Image originally created using biorender.com and AutoDesk SketchBook.

Towards a goal of reducing the need for multiple daily injections, the first long-acting insulin was developed in the 1930s by H. C. Hagedorn. It was a suspension protamine zinc insulin that was based on the discovery that insulin action could be prolonged by adding protamine obtained from river trout semen[106]. The action of protamine zinc insulin lasted 24–36 h. In 1946, Nordisk developed an intermediate-acting neutral protamine Hagedorn insulin that formed microcrystals, could be mixed with regular insulin and lasted 18–24 h. The first 'peakless' basal insulin, known as ultralente (belonging to the lente family of insulins), was developed during the 1950s by employing an extended zinc suspension without protamine[107]. Ultralente was mixed with a semilente (an insulin with a different proportion of zinc and a time–action profile slightly slower than that of regular insulin) to make the intermediate-acting lente. However, because these insulins were suspension based, they had variable day-to-day action, complicating their clinical use, and they still required more than one injection a day to provide a basal coverage.

Before the 1980s, all insulin preparations were derived from animal sources. However, with increased clinical demand and tedious extraction processes (for example, more than 23,500 pancreata were needed to make 1 lb of insulin), available supplies were being outstripped[108]. Additionally, even with the advent of highly purified monocomponent animal insulin in the 1970s[109], many people with diabetes continued to have allergic reactions to the formulations. A pure, scalable insulin source was needed. Just as there had been a race to isolate insulin 50 years earlier, now teams were using what had been learned in the molecular biology renaissance of the previous decade to produce human insulin using recombinant DNA technology.

Insulin cloning in bacteria was a complicated process[110]. First, the A and B chains needed to be synthesized (the B chain synthesis required cleaving the peptide into two sections). Then the A and B chains needed to be linked together. Finally, the insulin needed to be harvestable for commercial use, which required breaking off the beta-galactosidase required to insert the insulin into *Escherichia coli* bacteria. In 1978, David Goeddel, Arthur Riggs and their Genentech colleagues working at City of Hope produced the first recombinant DNA human insulin[111]. Subsequently, Genentech and Lilly agreed to commercialize this new insulin and Humulin R and N insulins came to market in 1983. Novolin R (Novo Nordisk) followed in 1991 and Insuman R (Hoechst) in 1997. Although this represented an improvement in source, these insulins were still zinc-based formulations with slower pharmacokinetic profiles than natively secreted insulin.

The 1993 publication of the Diabetes Control and Complications Trial[112] and 1998 United Kingdom Prospective Diabetes Study[113–115] demonstrated definitive relationships between glycemic control and microvascular complications and showed that lower A1cs were associated with higher rates of severe hypoglycemia. These observations spurred efforts focused on improving exogenously administered insulin's pharmacokinetic and pharmacodynamic properties (absorption rate, time to peak and duration of action). This has been accomplished over time (Fig. 2) using recombinant DNA technology and genetic engineering, and adding excipients. Tweaking amino acid sites/composition in the native insulin molecule changed the pharmacokinetics and permitted faster absorption, earlier peak action and faster offset. In 1988 a synthetically designed insulin was produced by replacing the B28 proline with aspartic acid, which favored a molecular conformation leading to rapid dissociation of dimerized insulin chains. The first rapid-acting insulin, insulin lispro (produced by inverting the B29 lysine and B28 proline), came to market in 1996. Next was aspart in 2000, and then glulisine in 2004.

An ultrarapid-acting version of insulin aspart was subsequently developed by adding nicotinamide and L-arginine as excipients that improve the insulin's stability and rate of absorption[116]. An ultrarapid insulin lispro has also been developed by using a prostacyclin analog to enhance vasodilation and absorption and citrate to enhance local vascular permeability.

The first long-acting once-daily basal insulin, glargine, was approved in 2000 (ref. [117]). It was designed to have an extended duration of action through amino acid modifications in both chains (A chain A21 asparagine substituted by glycine and B chain elongated by adding two arginines). These changes achieved a prolonged duration by shifting the isoelectric point to make the insulin soluble at an acidic pH but precipitate at the injection site at a pH of 7.4, allowing for slow dissociation. The next long-acting basal insulin, detemir, was approved in 2005 (ref. [118]). Detemir has a fatty-acid (myristic acid) side chain bound to position B29 that facilities self-association and an affinity for albumin allowing for prolonged duration of action without peaks. Insulin degludec followed a decade later; degludec forms a depot of soluble multihexamers at the injection site giving it an ultralong (>42 h) glucose-lowering effect[119].

Improvements in insulin therapy have also been realized by changes in the method of delivery. Initially, insulin was available only through administration via vials and syringes. In 1985 the first insulin pen was launched by Novo Nordisk[120]. More recently developed 'smart' insulin pens allow for tracking of insulin dosing, and integration with smartphone applications to provide reminders, integrate with blood glucose data and provide dosing recommendations[120]. Advances in insulin delivery have also included the development of inhaled insulin with a faster onset of action and offset of effect than any of the injected insulins[121]. The first, Exubera, came to market in 2006, but was rapidly withdrawn due to poor market uptake. Inhaled technosphere insulin, Afrezza, was launched commercially in the next decade by Mannkind, although cost, limited dosing flexibility and continued concern about pulmonary effects have limited its clinical uptake and use.

Arguably, the most impactful technology-driven advances in insulin delivery have revolved around the technology of continuous subcutaneous insulin infusion using insulin pumps[122]. The first closed-loop insulin pump that incorporated automatic blood glucose sensing was designed by Arnold Kadish in 1963 (ref. [123]). It was large (like an "army backpack") and impractical for daily use. The first bedside computer-controlled closed-loop system, the Biostator, was invented by Miles Laboratory (Elkhart IN) in 1974 (ref. [124]). During the late 1970s, rigorous testing of insulin pumps began in earnest—leading to the first wearable systems, including the 'big blue brick.' By the 1980s, continuous subcutaneous insulin infusion had become a viable alternative means of delivering insulin[122]. In 1983, MiniMed brought the first commercial pump to market. Improvements over the next several decades have included the emergence of new pump models by multiple manufacturers, including tubeless patch pump models, the ability to modify the timing/duration of insulin bolus delivery and improvements in device usability. The development of reliable and accurate continuous glucose monitors allowed for the possibility of integration of glucose data with pump insulin delivery and sparked a flurry of interest to develop safe and effective algorithms for closed-loop systems, notably championed by do-it-yourself movements from the diabetes community itself[122,125]. Now, increasingly, many pumps employ hybrid closed-loop technologies with automatic insulin dosing by the pump based on continuous glucose readings and trends.

What does the future hold? In the 100 years since the discovery of insulin, there has been remarkable progress in our ability to treat type 1 and type 2 diabetes, facilitated by an improved understanding of the pathophysiology of the disease and improvements in insulin formulation and delivery. This progress is captured in an impressive series of scientific accomplishments summarized in this Review and shown in Fig. 1, several of them recognized by the most prestigious awards in Medicine, Physiology and Chemistry. However, the true impact of these achievements is best illustrated by the voices of patients who have seen dramatic changes in the management of their type 1 diabetes (Fig. 3).

What do the next 100 years hold for insulin and those who depend on it (Fig. 4)? Furthermore and importantly, will treatment with exogenous insulin therapy become another note in the history books? For type 1 diabetes, this goal is a centerpiece of clinical trials testing disease-modifying interventions, including work that is ongoing in several large networks such as Type 1 Diabetes TrialNet, the Immune Tolerance Network and INNODIA. In 2019, following a nearly three-decade search for successful disease prevention, the Type 1 Diabetes TrialNet study of the anti-CD3 antibody teplizumab showed that a single 14-day course of drug could delay the onset of clinical diabetes (that is, stage 3 type 1 diabetes) by a median of 32.5 months in high-risk multiple-autoantibody-positive individuals with dysglycemia (that is, stage 2 type 1 diabetes)[126,127]. Results from this seminal study have underscored the importance of identifying the correct therapeutic window for intervention, but have also raised the practical question of how to identify at-risk individuals outside a research setting. In this regard, population-based screening is now being increasingly performed in several countries and regions, and is based on autoantibody measurement and, in some cases, assessment of genetic risk. Genetic risk stratification has focused on assessment of HLA risk or more recently calculation of polygenic genetic risk scores that sum the effects of a large number of variants[128]. The education and anticipatory guidance provided as part of these programs have been shown to significantly reduce the risk of ketoacidosis at the onset of stage 3 type 1 diabetes[129]. However, additional research will be needed to identify the ideal timing and frequency of screening and how to prioritize at-risk individuals for interventions. For type 2 diabetes, complementary disease-modifying therapies that may reduce or eliminate the need for insulin administration have also represented a rapidly expanding field of interest[130–132].

In addition to efforts focused on disease modification, there are continuing efforts to improve insulin therapies and there is still much to be refined in our approach to exogenous insulin delivery. There is a hope for development of better insulins including: insulins with even faster pharmacokinetics, once-weekly insulin, oral insulin and, ultimately, glucose-responsive 'smart' insulins that increase circulating concentrations under conditions of hyperglycemia. Additional technological advancements on the horizon include improved algorithms for automated insulin delivery devices, implantable devices and dual-hormonal systems that combine automated delivery of insulin and glucagon[133,134]. Finally, there is also considerable interest in developing renewable, cellular sources of insulin through the generation of beta-like cells from either induced pluripotent stem cells or embryonic stem cells. While a beta-like cell with behavior that fully recapitulates the physiology of a normal beta cell is yet to be realized, there has been a steady series of improvements to directed differentiation strategies over the past 20 years[135–138]. In parallel, a large body of work has focused on developing the ideal cellular niche and encapsulation strategies to support normal patterns of hormone secretion while also protecting these cellular implants from autoimmune destruction[139,140]. Improvements in insulin delivery and monitoring and alternative cell-based sources of insulin have the potential to broadly impact diabetes management and will benefit individuals with type 1 and type 2 diabetes, as well as rarer forms of the disease.

Closing. In the 1920s, having developed a transformative and life-saving therapy, the Toronto team faced an almost impossible challenge, and they struggled at the outset to produce enough insulin

to meet a rapidly growing demand and to distribute insulin in a fashion that was equitable[1]. As we celebrate this remarkable centennial anniversary and the subsequent discoveries that have improved life expectancy and quality of life for those with diabetes (Fig. 3), there are continued challenges with accessibility and equity, which have only been exacerbated by advances in diabetes care technology. In a recent analysis of children and adults with type 1 diabetes in the USA, the average cost associated with diabetes totaled nearly US$800 per month, with nearly 50% driven by pharmacy costs[141]. Even the most basic component of diabetes management, insulin itself, has become unaffordable for many[142]. From 2012 to 2016, the average list price of insulins increased by 14–17% per year in the USA. These increases are often driven by gaps between the list price and the net price ultimately received by manufacturers, which have been largely attributed to rebates and discounts negotiated between stakeholders in a supply chain with poor transparency[143]. As members of the Toronto team arranged to sell their patents for insulin back to the University of Toronto for CAN$1, Banting is reported to have remarked, "Insulin belongs to the world, not to me." Thus, while we envision a future of possibilities for those who require insulin to survive, it is important that we not forget Banting's altruism and become complacent to this most basic and fundamental challenge of the present. Only once equal access for patients around the globe is established will the remarkable achievements surrounding insulin over the past century truly realize their greatest impact[144–150].

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References

1. Bliss, M. *The Discovery of Insulin* (Univ. Chicago Press, 1982).
2. Nerup, J. et al. Cell-mediated immunity in diabetes mellitus. *Proc. R. Soc. Med.* **67**, 506–513 (1974).
3. Gale, E. A. The discovery of type 1 diabetes. *Diabetes* **50**, 217–226 (2001).
4. Bliss, M. The history of insulin. *Diabetes Care* **16**, 4–7 (1993).
5. Bottazzo, G., Florin-Christensen, A. & Doniach, D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* **304**, 1279–1283 (1974).
6. Singal, D. P. & Blajchman, M. A. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* **22**, 429–432 (1973).
7. Nerup, J. et al. HL-A antigens and diabetes mellitus. *Lancet* **2**, 864–866 (1974).
8. Cudworth, A. G. & Woodrow, J. C. HL-A antigens and diabetes mellitus. *Lancet* **2**, 1153 (1974).
9. Gemmill, C. L. The Greek concept of diabetes. *Bull. N. Y. Acad. Med.* **48**, 1033–1036 (1972).
10. Allan, F. N. The writings of Thomas Willis, M.D.; diabetes three hundred years ago. *Diabetes* **2**, 74–77 (1953).
11. Dobson, M. Nature of the urine in diabetes. *Med. Obs. Inq.* **5**, 298–310 (1776).
12. v. Mering, J. & Minkowski, O. Diabetes mellitus nach Pankreasexstirpation. *Naunyn Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **26**, 371–387 (1890).
13. Sakula, A. Paul Langerhans (1847–1888): a centenary tribute. *J. R. Soc. Med.* **81**, 414–415 (1988).
14. Laguesse, G. E. Sur la formation des îlots de Langerhans dans le pancréas. *C. R. Seances Soc. Biol. Fil.* **5**, 819–820 (1893).
15. Goet, J. P. Gustave Edouard Laguesse; his demonstration of the significance of the Islands of Langerhans. *Diabetes* **2**, 322–324 (1953).
16. De Meyer, J. Action de la sécrétion interne du pancréas sur différents organes et en particulier sur la sécrétion rénale. *Arch. Fisiol.* **7**, 96–99 (1909).
17. Lindsten, J. August Krogh and the Nobel Prize to Banting and Macleod. *The Nobel Prize* https://www.nobelprize.org/prizes/themes/august-krogh-and-the-nobel-prize-to-banting-and-macleod/ (2 April 2001).
18. Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R. & Fletcher, A. A. Pancreatic extracts in the treatment of diabetes mellitus. *Can. Med. Assoc. J.* **12**, 141 (1922).
19. Lancereaux, E. Le diabète maigre, ses symptômes, son évolution, son pronostic et son traitement. *Union Med.* **29**, 161 (1880).
20. Pincus, G., Joslin, E. & White, P. The age-incidence relations in diabetes mellitus. *Am. J. Med. Sci.* **188**, 116–121 (1934).
21. MacLean, H. Some observations on diabetes and insulin in general practice. *Postgrad. Med. J.* **1**, 73–77 (1926).
22. Falta, W. & Boller, R. Insulärer und insulinresistenter Diabetes. *Klin. Wochenschr.* **10**, 438–443 (1931).
23. Himsworth, H. P. Diabetes mellitus: its differentiation into insulin-sensitive and insulin insensitive types. *Lancet* **227**, 127–130 (1936).
24. Bornstein, J. & Lawrence, R. Two types of diabetes mellitus, with and without available plasma insulin. *Br. Med. J.* **1**, 732 (1951).
25. Wrenshall, G. A., Bogoch, A. & Ritchie, R. Extractable insulin of pancreas: correlation with pathological and clinical findings in diabetic and nondiabetic cases. *Diabetes* **1**, 87–107 (1952).
26. Yalow, R. S. & Berson, S. A. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**, 1157–1175 (1960).
27. Himsworth, H. P. Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types. *Int. J. Epidemiol.* **42**, 1594–1598 (2013).
28. Wild, S. H. & Byrne, C. D. Commentary: sub-types of diabetes–what's new and what's not. *Int. J. Epidemiol.* **42**, 1600–1602 (2013).
29. Anderson, J., Goudie, R., Gray, K. & Timbury, G. Auto-antibodies in Addison's disease. *Lancet* **269**, 1123–1124 (1957).
30. Roitt, I., Doniach, D., Campbell, P. & Hudson, R. V. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* **268**, 820–821 (1956).
31. Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R. & Egan, R. W. Chronic thyroiditis and autoimmunization. *J. Am. Med. Assoc.* **164**, 1439–1447 (1957).
32. Irvine, W., Clarke, B., Scarth, L., Cullen, D. & Duncan, L. Thyroid and gastric autoimmunity in patients with diabetes mellitus. *Lancet* **296**, 163–168 (1970).
33. Ungar, B., Stocks, A., Martin, F., Whittingham, S. & Mackay, I. Intrinsic-factor antibody in diabetes mellitus. *Lancet* **290**, 77–78 (1967).
34. Renold, A. E., Soeldner, S. & Steinke, J. in *Ciba Foundation Symposium - Aetiology of Diabetes Mellitus and its Complications (Colloquia on Endocrinology)* Vol. 15 (eds. M.P. Cameron, M. P. & O'Connor, M.) 122–139 (Wiley, 1964).
35. Lacy, P. E. & Wright, P. H. Allergic interstitial pancreatitis in rats injected with guinea pig anti-insulin serum. *Diabetes* **14**, 634–642 (1965).
36. Heydinger, D. K. & Lacy, P. E. Islet cell changes in the rat following injection of homogenized islets. *Diabetes* **23**, 579–582 (1974).
37. Lecompte, P. M. Insulitis in early juvenile diabetes. *AMA Arch. Pathol.* **66**, 450–457 (1958).
38. Gepts, W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* **14**, 619–633 (1965).
39. DeFronzo, R. A., Tobin, J. D. & Andres, R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237**, E214–E223 (1979).
40. Reaven, G. M. Insulin resistance in noninsulin-dependent diabetes mellitus. Does it exist and can it be measured?. *Am. J. Med.* **74**, 3–17 (1983).
41. Shen, S. W., Reaven, G. M. & Farquhar, J. W. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J. Clin. Invest.* **49**, 2151–2160 (1970).
42. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* **28**, 1039–1057 (1979).
43. Makino, S., Muraoka, Y., Kishimoto, Y. & Hayashi, Y. Genetic analysis for insulitis in NOD mice. *Jikken Dobutsu* **34**, 425–431 (1985).
44. Nakhooda, A. F., Like, A. A., Chappel, C. I., Wei, C. N. & Marliss, E. B. The spontaneously diabetic Wistar rat (the "BB" rat). Studies prior to and during development of the overt syndrome. *Diabetologia* **14**, 199–207 (1978).
45. Eisenbarth, G. S. Type I diabetes mellitus. A chronic autoimmune disease. *N. Engl. J. Med.* **314**, 1360–1368 (1986).
46. Gottlieb, M. S. & Root, H. F. Diabetes mellitus in twins. *Diabetes* **17**, 693–704 (1968).
47. Tattersall, R. B. & Pyke, D. A. Diabetes in identical twins. *Lancet* **2**, 1120–1125 (1972).
48. Thorsby, E. A short history of HLA. *Tissue Antigens* **74**, 101–116 (2009).
49. Davies, J. L. et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* **371**, 130–136 (1994).
50. Bennett, S. T. et al. Susceptibility to human type 1 diabetes at *IDDM2* is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* **9**, 284–292 (1995).
51. Vafiadis, P. et al. Insulin expression in human thymus is modulated by *INS* VNTR alleles at the *IDDM2* locus. *Nat. Genet.* **15**, 289–292 (1997).
52. Pugliese, A. et al. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the *INS* VNTR-*IDDM2* susceptibility locus for type 1 diabetes. *Nat. Genet.* **15**, 293–297 (1997).
53. Buniello, A. et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics. *Nucleic Acids Res.* **47**, D1005–D1012 (2019).
54. Coomans de Brachène, A. et al. IFN-α induces a preferential long-lasting expression of MHC class I in human pancreatic beta cells. *Diabetologia* **61**, 636–640 (2018).

55. Marroqui, L. et al. Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia* **60**, 656–667 (2017).
56. Fløyel, T. et al. *CTSH* regulates β-cell function and disease progression in newly diagnosed type 1 diabetes patients. *Proc. Natl Acad. Sci. USA* **111**, 10305–10310 (2014).
57. Eizirik, D. L. et al. The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. *PLoS Genet.* **8**, e1002552 (2012).
58. Eizirik, D. L., Colli, M. L. & Ortis, F. The role of inflammation in insulitis and β-cell loss in type 1 diabetes. *Nat. Rev. Endocrinol.* **5**, 219–226 (2009).
59. Lee, H. et al. Beta cell dedifferentiation induced by IRE1α deletion prevents type 1 diabetes. *Cell Metab.* **31**, 822–836 (2020).
60. Bottazzo, G. F. Lawrence lecture. Death of a beta cell: homicide or suicide? *Diabet. Med.* **3**, 119–130 (1986).
61. Battaglia, M. et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. *Diabetes Care* **43**, 5–12 (2020).
62. Krischer, J. P. et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia* **58**, 980–987 (2015).
63. Ziegler, A. G. et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *J. Am. Med. Assoc.* **309**, 2473–2479 (2013).
64. Insel, R. A. et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* **38**, 1964–1974 (2015).
65. Feutren, G. et al. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* **2**, 119–124 (1986).
66. Stiller, C. R. et al. Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* **223**, 1362–1367 (1984).
67. Kahn, S. E. et al. Quantification of the relationship between insulin sensitivity and β-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* **42**, 1663–1672 (1993).
68. Bergman, R. N., Phillips, L. S. & Cobelli, C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J. Clin. Invest.* **68**, 1456–1467 (1981).
69. Evans-Molina, C., Hatanaka, M. & Mirmira, R. G. Lost in translation: endoplasmic reticulum stress and the decline of β-cell health in diabetes mellitus. *Diabetes Obes. Metab.* **15**, 159–169 (2013).
70. Butler, A. E. et al. β-cell deficit in obese type 2 diabetes, a minor role of β-cell dedifferentiation and degranulation. *J. Clin. Endocrinol. Metab.* **101**, 523–532 (2016).
71. Guo, S. et al. Inactivation of specific β cell transcription factors in type 2 diabetes. *J. Clin. Invest.* **123**, 3305–3316 (2013).
72. Lillioja, S. et al. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *N. Engl. J. Med.* **318**, 1217–1225 (1988).
73. Martin, B. C. et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* **340**, 925–929 (1992).
74. Saad, M. F. et al. Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. *Lancet* **1**, 1356–1359 (1989).
75. Tabak, A. G. et al. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* **373**, 2215–2221 (2009).
76. Trico, D., Natali, A., Arslanian, S., Mari, A. & Ferrannini, E. Identification, pathophysiology, and clinical implications of primary insulin hypersecretion in nondiabetic adults and adolescents. *JCI Insight* **3**, e124912 (2018).
77. Duggirala, R. et al. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am. J. Hum. Genet* **64**, 1127–1140 (1999).
78. Grant, S. F. et al. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat. Genet.* **38**, 320–323 (2006).
79. Mahajan, A. et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat. Genet.* **50**, 1505–1513 (2018).
80. McCarthy, M. I. Genomics, type 2 diabetes, and obesity. *N. Engl. J. Med.* **363**, 2339–2350 (2010).
81. Udler, M. S. et al. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: a soft clustering analysis. *PLoS Med.* **15**, e1002654 (2018).
82. Ahlqvist, E. et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol.* **6**, 361–369 (2018).
83. Dennis, J. M., Shields, B. M., Henley, W. E., Jones, A. G. & Hattersley, A. T. Disease progression and treatment response in data-driven subgroups of type 2 diabetes compared with models based on simple clinical features: an analysis using clinical trial data. *Lancet Diabetes Endocrinol.* **7**, 442–451 (2019).
84. Crowfoot, D. X-ray single crystal photographs of insulin. *Nature* **135**, 591–592 (1935).
85. Adams, M. J. et al. Structure of rhombohedral 2 zinc insulin crystals. *Nature* **224**, 491–495 (1969).
86. Howard, J. A. Dorothy Hodgkin and her contributions to biochemistry. *Nat. Rev. Mol. Cell Biol.* **4**, 891–896 (2003).
87. Sanger, F. & Tuppy, H. The amino-acid sequence in the phenylalanyl chain of insulin. 1. The identification of lower peptides from partial hydrolysates. *Biochem. J.* **49**, 463–481 (1951).
88. Sanger, F. & Tuppy, H. The amino-acid sequence in the phenylalanyl chain of insulin. 2. The investigation of peptides from enzymic hydrolysates. *Biochem. J.* **49**, 481–490 (1951).
89. Sanger, F. & Thompson, E. The amino-acid sequence in the glycyl chain of insulin. 1. The identification of lower peptides from partial hydrolysates. *Biochem. J.* **53**, 353–366 (1953).
90. Sanger, F. & Thompson, E. The amino-acid sequence in the glycyl chain of insulin. 2. The investigation of peptides from enzymic hydrolysates. *Biochem. J.* **53**, 366–374 (1953).
91. Berg, P. Fred Sanger: a memorial tribute. *Proc. Natl Acad. Sci. USA* **111**, 883–884 (2014).
92. Ryle, A. P., Sanger, F., Smith, L. F. & Kitai, R. The disulphide bonds of insulin. *Biochem. J.* **60**, 541–556 (1955).
93. Sanger, F. Chemistry of insulin; determination of the structure of insulin opens the way to greater understanding of life processes. *Science* **129**, 1340–1344 (1959).
94. Steiner, D. F. Adventures with insulin in the islets of Langerhans. *J. Biol. Chem.* **286**, 17399–17421 (2011).
95. Steiner, D. F. & Oyer, P. E. The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. *Proc. Natl Acad. Sci. USA* **57**, 473–480 (1967).
96. Chance, R. E., Ellis, R. M. & Bromer, W. W. Porcine proinsulin: characterization and amino acid sequence. *Science* **161**, 165–167 (1968).
97. De Meyts, P. Insulin and its receptor: structure, function and evolution. *Bioessays* **26**, 1351–1362 (2004).
98. Shoelson, S. et al. Three mutant insulins in man. *Nature* **302**, 540–543 (1983).
99. Garin, I. et al. Recessive mutations in the *INS* gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc. Natl Acad. Sci. USA* **107**, 3105–3110 (2010).
100. Stoy, J. et al. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc. Natl Acad. Sci. USA* **104**, 15040–15044 (2007).
101. Liu, M. et al. Impaired cleavage of preproinsulin signal peptide linked to autosomal-dominant diabetes. *Diabetes* **61**, 828–837 (2012).
102. Rege, N. K. et al. Evolution of insulin at the edge of foldability and its medical implications. *Proc. Natl Acad. Sci. USA* **117**, 29618–29628 (2020).
103. Sims, E. K. et al. Elevations in the fasting serum proinsulin-to-C-peptide ratio precede the onset of type 1 diabetes. *Diabetes Care* **39**, 1519–1526 (2016).
104. Loopstra-Masters, R. C., Haffner, S. M., Lorenzo, C., Wagenknecht, L. E. & Hanley, A. J. Proinsulin-to-C-peptide ratio versus proinsulin-to-insulin ratio in the prediction of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetologia* **54**, 3047–3054 (2011).
105. Ovalle, F. et al. Understanding concentrated insulins: a systematic review of randomized controlled trials. *Curr. Med. Res. Opin.* **34**, 1029–1043 (2018).
106. Hirsch, I. B., Juneja, R., Beals, J. M., Antalis, C. J. & Wright, E. E. The evolution of insulin and how it informs therapy and treatment choices. *Endocr. Rev.* **41**, 733–755 (2020).
107. Murray, I. & Wilson, R. B. The new insulins—lente, ultralente, and semilente. *Br. Med. J.* **2**, 1023–1026 (1953).
108. Fraser, L. Cloning insulin. *Genentech* https://www.gene.com/stories/cloning-insulin (7 April 2016).
109. Lavaux, J. P., Ooms, H. A. & Christiansen, A. H. Insulin antibodies in insulin-treated patients; a clinical trial with highly purified insulins. *Int. Congr. Ser.* **316**, 40–46 (1973).
110. Institute of Medicine. *Sources of Medical Technology: Universities and Industry* (National Academies Press, 1995).
111. Goeddel, D. V. et al. Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc. Natl Acad. Sci. USA* **76**, 106–110 (1979).
112. The Diabetes Control and Complications Trial (DCCT) Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**, 977–986 (1993).
113. United Kingdom Prospective Diabetes Study (UKPDS). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* **352**, 854–865 (1998).

114. United Kingdom Prospective Diabetes Study (UKPDS). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* **352**, 837–853 (1998).
115. United Kingdom Prospective Diabetes Study (UKPDS). 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *Br. Med. J.* **310**, 83–88 (1995).
116. Biester, T., Kordonouri, O. & Danne, T. Pharmacological properties of faster-acting insulin aspart. *Curr. Diabetes Rep.* **17**, 101 (2017).
117. Pieber, T. R., Eugene-Jolchine, I. & Derobert, E. Efficacy and safety of HOE 901 versus NPH insulin in patients with type 1 diabetes. The European Study Group of HOE 901 in type 1 diabetes. *Diabetes Care* **23**, 157–162 (2000).
118. US Food and Drug Administration. Levemir insulin detemir[rDNA origin] injection drug approval package. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021-536_LevemirTOC.cfm (2005).
119. Kurtzhals, P. et al. Multi-hexamer formation is the underlying basis for the ultra-long glucose-lowering effect of insulin degludec. *Diabetologia* **54**, S426 (2011).
120. Kesavadev, J., Saboo, B., Krishna, M. B. & Krishnan, G. Evolution of insulin delivery devices: from syringes, pens, and pumps to DIY artificial pancreas. *Diabetes Ther.* **11**, 1251–1269 (2020).
121. Oleck, J., Kassam, S. & Goldman, J. D. Commentary: why was inhaled insulin a failure in the market? *Diabetes Spectr.* **29**, 180–184 (2016).
122. Kovatchev, B. A century of diabetes technology: signals, models, and artificial pancreas control. *Trends Endocrinol. Metab.* **30**, 432–444 (2019).
123. Kadish, A. H. Automation control of blood sugar. I. A servomechanism for glucose monitoring and control. *Am. J. Med. Electron.* **3**, 82–86 (1964).
124. Albisser, A. M. et al. An artificial endocrine pancreas. *Diabetes* **23**, 389–396 (1974).
125. Kesavadev, J., Srinivasan, S., Saboo, B., Krishna, B. M. & Krishnan, G. The do-it-yourself artificial pancreas: a comprehensive review. *Diabetes Ther.* **11**, 1217–1235 (2020).
126. Herold, K. C. et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* **346**, 1692–1698 (2002).
127. Sims, E. K. et al. Teplizumab improves and stabilizes beta cell function in antibody-positive high-risk individuals. *Sci. Transl. Med.* **13**, eabc8980 (2021).
128. Ferrat, L. A. et al. A combined risk score enhances prediction of type 1 diabetes among susceptible children. *Nat. Med.* **26**, 1247–1255 (2020).
129. Ziegler, A. G. et al. Yield of a public health screening of children for islet autoantibodies in Bavaria, Germany. *J. Am. Med. Assoc.* **323**, 339–351 (2020).
130. Deacon, C. F. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **16**, 642–653 (2020).
131. Rieg, T. & Vallon, V. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia* **61**, 2079–2086 (2018).
132. Wilding, J. P. H. et al. Once-weekly semaglutide in adults with overweight or obesity. *N. Engl. J. Med.* **384**, 989–1002 (2021).
133. Russell, S. J. et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N. Engl. J. Med.* **371**, 313–325 (2014).
134. Haidar, A. et al. Comparison of dual-hormone artificial pancreas, single-hormone artificial pancreas, and conventional insulin pump therapy for glycaemic control in patients with type 1 diabetes: an open-label randomised controlled crossover trial. *Lancet Diabetes Endocrinol.* **3**, 17–26 (2015).
135. D'Amour, K. A. et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol.* **23**, 1534–1541 (2005).
136. Hogrebe, N. J., Augsornworawat, P., Maxwell, K. G., Velazco-Cruz, L. & Millman, J. R. Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells. *Nat. Biotechnol.* **38**, 460–470 (2020).
137. Rezania, A. et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat. Biotechnol.* **32**, 1121–1133 (2014).
138. Evans-Molina, C., Vestermark, G. L. & Mirmira, R. G. Development of insulin-producing cells from primitive biologic precursors. *Curr. Opin. Organ Transplant.* **14**, 56–63 (2009).
139. Agulnick, A. D. et al. Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in vivo. *Stem Cells Transl. Med.* **4**, 1214–1222 (2015).
140. Nair, G. G. et al. Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nat. Cell Biol.* **21**, 263–274 (2019).
141. Simeone, J. C. et al. Healthcare resource utilization and cost among patients with type 1 diabetes in the United States. *J. Manag. Care Spec. Pharm.* **26**, 1399–1410 (2020).
142. Beran, D., Lazo-Porras, M., Mba, C. M. & Mbanya, J. C. A global perspective on the issue of access to insulin. *Diabetologia* **64**, 954–962 (2021).
143. Cefalu, W. T. et al. Insulin Access and Affordability Working Group: conclusions and recommendations. *Diabetes Care* **41**, 1299–1311 (2018).
144. Sharpey-Schäfer, E. A. *An Introduction to the Study of the Endocrine Glands and Internal Secretions: Lane Medical Lectures, 1913* (Stanford Univ. Press, 1914).
145. Matthews, D. R. et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419 (1985).
146. Goldfine, A. B. et al. Insulin resistance is a poor predictor of type 2 diabetes in individuals with no family history of disease. *Proc. Natl Acad. Sci. USA* **100**, 2724–2729 (2003).
147. Utzschneider, K. M. et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care* **32**, 335–341 (2009).
148. Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med.* **346**, 1685–1691 (2002).
149. Morgan, N. G. & Richardson, S. J. Fifty years of pancreatic islet pathology in human type 1 diabetes: insights gained and progress made. *Diabetologia* **61**, 2499–2506 (2018).
150. Campbell-Thompson, M. et al. Network for pancreatic organ donors with diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab. Res. Rev.* **28**, 608–617 (2012).
151. Al-Tabakha, M. M. Future prospect of insulin inhalation for diabetic patients: the case of Afrezza versus Exubera. *J. Control. Release* **215**, 25–38 (2015).
152. Katzung, B. G. M., Susan B. & Trevor, A. J. *Basic & Clinical Pharmacology* 12th edn (McGraw Hill, 2012).
153. Tibaldi, J. M. Evolution of insulin: from human to analog. *Am. J. Med.* **127**, S25–S38 (2014).
154. Polonsky, K. S. The past 200 years in diabetes. *N. Engl. J. Med.* **367**, 1332–1340 (2012).
155. Gill, G., Jones, K., Smyth, C., Bain, S. & Barnett, A. Memories of the early years of insulin treatment in the UK. *Pract. Diabetes Int.* **20**, 103–107.
156. Padmore, E. Reflections on six decades of living with insulin-treated diabetes. *J. Diabetes Nurs.* **10**, 366–370 (2009).
157. Clothier, C. Living with diabetes in the 1950s. *J. Diabetes Nurs.* **23**, 1–8 (2019).

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Competing interests

The authors declare no competing interests.

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Appendix 4.3- Continuous glucose monitoring demonstrates low risk of clinically significant hypoglycemia associated with sulphonylurea treatment in an African type 2 diabetes population: results from the OPTIMAL observational multicenter study

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Continuous glucose monitoring demonstrates low risk of clinically significant hypoglycemia associated with sulphonylurea treatment in an African type 2 diabetes population: results from the OPTIMAL observational multicenter study

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ABSTRACT

Introduction People living with diabetes in low-resource settings may be at increased hypoglycemia risk due to food insecurity and limited access to glucose monitoring. We aimed to assess hypoglycemia risk associated with sulphonylurea (SU) and insulin therapy in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

Research design and methods This study was conducted in the outpatients' diabetes clinics of two hospitals (one rural and one urban) in Uganda. We used blinded continuous glucose monitoring (CGM) and self-report to compare hypoglycemia rates and duration in 179 type 2 diabetes patients treated with sulphonylureas (n=100) and insulin (n=51) in comparison with those treated with metformin only (n=28). CGM-assessed hypoglycemia was defined as minutes per week below 3mmol/L (54mg/dL) and number of hypoglycemic events below 3.0 mmol/L (54 mg/dL) for at least 15 minutes.

Results CGM recorded hypoglycemia was infrequent in SU-treated participants and did not differ from metformin: median minutes/week of glucose <3 mmol/L were 39.2, 17.0 and 127.5 for metformin, sulphonylurea and insulin, respectively (metformin vs sulphonylurea, p=0.6). Hypoglycemia risk was strongly related to glycated haemoglobin (HbA1c) and fasting glucose, with most episodes occurring in those with tight glycemic control. After adjusting for HbA1c, time <3 mmol/L was 2.1 (95% CI 0.9 to 4.7) and 5.5 (95% CI 2.4 to 12.6) times greater with sulphonylurea and insulin, respectively, than metformin alone.

Conclusions In a low-resource sub-Saharan African setting, hypoglycemia is infrequent among people with type 2 diabetes receiving sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycemic control.

Significance of this study

What is already known about this subject?

- Evidence from high-income countries suggest that severe hypoglycemia is rare in patients taking sulphonylureas, but in those with well-controlled diabetes, non-severe hypoglycemia may be common.
- People treated with sulphonylureas in low-income countries may be at increased of hypoglycemia because of food insecurity, lack of access to glucose monitoring, and use of older sulphonylurea agents that have higher hypoglycemia risk; however, the risk of hypoglycemia with these agents in low-income populations is unclear.

What are the new findings?

- Both continuous glucose monitoring assessed and self-reported hypoglycemia were infrequent in participants with sulphonylurea-treated diabetes and did not differ from metformin.
- Hypoglycemia risk was strongly associated with glycemic control, with most episodes occurring in those with tight glycemic control.
- After adjusting for glycemic control (HbA1c), participants receiving sulphonylurea or insulin treatment experienced two and five times more continuous glucose monitoring assessed hypoglycemia, respectively, than those receiving metformin.

How might these results change the focus of research or clinical practice?

- The high rates of poor glycemic control in type 2 diabetes patients and relatively low hypoglycemic events among patients taking sulphonylureas suggest that there is room for optimizing glycemic control using these cheap, readily available and effective agents in low-resource settings.

INTRODUCTION

The prevalence of type 2 diabetes is rapidly increasing especially in low-income and middle-income countries (LMICs) where the majority of people living with type 2 diabetes reside.[1] While complications of type 2 diabetes can be reduced by maintaining glucose control,[2 3] glycemic control for people living with type 2 diabetes in LMICs is often poor.[4] A key barrier to intensifying glucose-lowering therapy in low-resource healthcare settings is fear of hypoglycemia.[5 6] Sulphonylureas (SUs) and insulin remain the most available treatments after metformin for people living with diabetes in LMICs.[7 8] Because of limited resources, treatments with lower risk of hypoglycemia, such as the newer classes of SUs (eg, gliclazide and glimepiride) and analog insulins, are not readily available in LMICs,[8] and robust glucose monitoring is often unaffordable, even for those treated with insulin.[9] Concerns about hypoglycemia mean that SUs may be started at far higher glycemic thresholds than recommended in international guidance.[10 11]

It is not clear whether this fear of hypoglycemia among type 2 diabetes patients in low-resource settings is justified. Previous studies investigating the burden of hypoglycemia among type 2 diabetes patients in low-resource settings are limited, with available data predominantly from high-income countries.[12] Observational and trial data from high-income countries suggest that severe hypoglycemia is rare in patients taking SUs, but in those with well-controlled diabetes, non-severe hypoglycemia may be common.[13 14] Studies in high-income countries suggest substantially higher rates of hypoglycemia with insulin than SUs.[15 16] However, these data may not apply in resource poor settings where use of older SUs, with higher hypoglycemia risk compared with newer generation SUs (eg, gliclazide and glimepiride) and food insecurity (and therefore missed meals) are common. In addition, due to resource constraints, the majority of those receiving treatment associated with hypoglycemia will not be able to access capillary glucose monitoring.

We therefore aimed to assess hypoglycemia risk with SUs and insulin therapy (in comparison with metformin) in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

METHODS

We compared continuous glucose monitoring (CGM) and self-reported hypoglycemia in people treated with metformin, sulfonylureas or insulin attending diabetes clinics in Uganda. CGM was used to obtain an objective assessment of hypoglycemia.

Study population

People living with type 2 diabetes attending a routinely scheduled diabetes clinic in a rural-based hospital (Masaka regional referral hospital) and urban-based hospital (St. Francis Hospital Nsambya) were invited consecutively. Eligible individuals were aged 18 years and above and treated with metformin, SU or insulin. All participants provided written informed consent before entering the study.

Patient and public involvement (PPI)

Patients were involved in prioritization of the research question. Patients were not involved in the design and conduct of the study. However, they were central to dissemination of the results by choosing to have some of the results sent to their respective clinicians and will continue to be involved in ongoing study dissemination.

Study procedures

We used questionnaires to record baseline patient characteristics including sociodemographic, diabetes medical history, current treatment information, and history of severe hypoglycemia in the previous 12 months.

We assessed glucose levels over a 14-day period from the baseline visit using the blinded Freestyle Libre Pro Glucose Monitoring System (Abbott Laboratories, Illinois, USA) as previously described.[17]

Hypoglycemia assessment

CGM-assessed hypoglycemia was defined according to the international consensus on use of CGM guidelines as the number of hypoglycemic events that occur over the given CGM reporting period.[18] Clinically significant hypoglycemic events were defined as readings below the 3.0 mmol/L (54 mg/dL) threshold for at least 15 minutes. The end of a CGM hypoglycemic event was defined at the point where glucose was at least 3.9 mmol/L (70 mg/dL) for 15 min. Hypoglycemia rate and duration below 3 mmol/mol were standardized to events/week and minutes/week per week, respectively, to account for variation in duration of CGM measurement. Self-reported hypoglycemia data were collected using a questionnaire that captured the history of hypoglycemia requiring assistance of another person, history and number of times the participant was hospitalized due to hypoglycemia in the previous 12 months.

Statistical analysis

Statistical analysis was performed using Stata V.16.1 (StataCorp LLC).

Medians and IQrs are reported for descriptive data due to skewed nature of most variables. We compared median hypoglycemia event rate per week and the median minutes below 3 mmol/L per week across treatment classes using the non-parametric Wilcoxon rank-sum test. Frequency of self-reported hypoglycemia and hospital admission due to hypoglycemia was assessed, and proportions were compared across the three treatment groups using χ^2 or Fischer's exact tests.

Hypoglycemia rate and minutes below 3 mmol/L per week results were positively skewed following a Poisson distribution. We therefore assessed whether the differences in hypoglycemia rates between the three treatment groups were due to confounding by differences in clinical features associated with hypoglycemia using Poisson

regression models. To ensure model assumptions of variance, we fitted Poisson regression with robust SEs.[19] The differences in minutes below 3 mmol/L were also assessed using Poisson regression; the Poisson regression with robust SEs (Huber-White-Sandwich linearized estimator of variance) was preferred to log-linear regressions for easy interpretation of results and due to the presence of numerous natural zeros in the outcome of interest (minutes below 3 mmol/L) and overdispersion.[20] We assessed the rates and the minutes below 3 mmol/L, with and without adjustment for glycemic control (glycated haemoglobin (HbA1c) or fasting plasma glucose (FPG)), age, sex, diabetes duration and body mass index (BMI). We then visually assessed the relationship between FPG and HbA1c using scatter plots and compared rate and duration at different HbA1c and FPG values.

The adjusted means of hypoglycemia rates and minutes below 3 mmol/L per week were then estimated using the margins command for each treatment class (ie, metformin only, SUs and insulin) holding HbA1c or FPG (or other adjusted covariates) at the sample population mean. We also estimated adjusted mean rates of hypoglycemia and minutes per week below glucose levels of 3 mmol/L at clinically relevant HbA1c and FPG thresholds.

RESULTS

Baseline characteristics

One hundred and seventy-nine participants met analysis inclusion criteria (online supplemental figure 1). Twenty-eight participants were treated with metformin only, 100 were treated with SUs (with or without metformin) and 51 were treated with insulin (with or without metformin and/or SU) (online supplemental figure 1). Of the 100 participants treated with SUs, 67 patients (67%) were prescribed glibenclamide, 26 (26%) were prescribed glimepiride and 7 (7%) were prescribed gliclazide. Forty-two of 51 (78.8%) of the patients taking insulin were on mixtard insulin. The median duration of CGM was 14 (IQR: 13–14) days. Baseline characteristics are shown in table 1. Participants treated with SU and insulin had substantially higher glycemia than those treaded with metformin: median HbA1c (mmol/mol) of 66 (IQR: 2–83), 84 (IQR: 67–102) and 46 (IQR: 39.5–63.5) respectively.

Metformin group includes patients being treated with metformin only, SU group includes patients on SUs and metformin, and insulin group includes patients being treated with insulin with metformin and/or SUs. Renal impairment was defined as an estimated glomerular filtration rate (eGFR)<60 mL/min/1.73 m^2. Per cent time spent in optimal range was defined as the percentage of readings and time spent between 3.9–10.0 mmol/L (70–180 mg/dL).

Hypoglycemia was infrequent in participants with SU-treated diabetes and did not differ from metformin

Median minutes and rate below 3 mmol/L per week of CGM defined hypoglycemia were low in those treated with SUs and similar to rates observed in those treated with metformin (figure 1 and table 1). Median (IQR) minutes below 3 mmol/L per week were 39.2 (0–174.8), 17.0 (0–229.3) and 127.5 (0–637.5) with metformin, SU, and insulin, respectively. Median hypoglycemic events/week were 1 (IQR: 0–2.3), 0.5 (0–3.0) and 2 (0–6.0) with metformin, SU, and insulin, respectively. Self-reported hypoglycemia results were broadly consistent with CGM findings, with numerically similar proportions of reported hypoglycemia-related hospitalization with SU (3.0% (95% CI 0.6 to 8.5) and metformin (3.6% (95% CI 0.1 to 18.3)) and higher rates in those treated with insulin (11.8% (95% CI 4.4 to 23.9) (table 1).

Hypoglycemia risk was strongly associated with glycemic control, with most episodes occurring in tightly controlled diabetes

In those treated with SU and insulin, time spent in hypoglycemia and hypoglycemic event rate was strongly associated with glycemic control, with differences in HbA1c explaining 33.1% (p=<0.001) and 20.7% (p=0.005) of variation in time below 3 mmol/L for SU and insulin, respectively (figure 2). The majority of hypoglycemia occurred in those with lower HbA1c or fasting glucose (figure 2 (time <3 mmol/L) and online supplemental figure 2) (hypoglycemia rate). Participants with HbA1c below 53 mmol/mol (7%) spent 2.34% (IQR: 0.60–4.49) and 5.61% (0.34–13.80) of their total time per week in hypoglycemia (<3 mmol/L), for SU and insulin, respectively. In comparison, those who had an HbA1c ≥53 mmol/mol on SU spent 0.0% (IQR: 0.00–0.92) and those on insulin spent 1.27% (0.00–5.75) of their total time per week in hypoglycemia (<3 mmol/L). Participants with fasting glucose <7 mmol/L spent 2.40% (IQR: 0.60–4.98) and 6.52% (IQR: 1.24– 13.50) of their total time per week in hypoglycemia, for SU and insulin, respectively, in comparison with only 0.0% (IQR: 0.00–0.46) and 0.67% (IQR: 0.00–3.44) for those who had fasting glucose ≥7 mmol/L (online supplemental table 1).

In analysis adjusted for HbA1c participants receiving SU or insulin treatment experienced two and five times more hypoglycemia, respectively, than those receiving metformin

Table 2 shows mean and rate ratio for minutes in hypoglycemia by treatment (relative to metformin), unadjusted and with adjustment for HbA1c (model 2) and HbA1c, age, diabetes duration, BMI and sex (model 3). In unadjusted analysis, the mean number of minutes <3 mmol/L per week for SU and metformin treatment did not substantially differ (duration ratio SU vs metformin 1.4 (95% CI 0.69 to 2.91), p=0.35), but duration in hypoglycemia substantially higher with insulin than metformin (duration ratio 2.5 (95% CI 1.3 to 5.0), p=0.009). After adjusting for HbA1c, differences between therapies were accentuated, with minutes

Table 1 Characteristics of CGM-assessed and self-reported hypoglycemia in type 2 diabetes according to treatment

	Median (IQR) for continuous variables, n (%) for proportions		
Variable	**Metformin group**	**SU group**	**Insulin group**
Number	28	100	51
Female, n (%)	18 (64.3)	57 (57.0)	31 (60.8)
Age, years	56.5 (49.5–61.5)	55.5 (50.0–62.0)	55.0 (49.0–64.0)
Diabetes duration, years	5.0 (2.0–8.0)	6.0 (3.0–9.0)	10.0 (8.0–17.0)
BMI, kg/m^2	26.9 (24.2–29.9)	26.7 (23.7–30.1)	25.8 (23.1–30.2)
eGFR	113.4 (96.8–123.7)	112.8 (93.8–121.0)	110.8 (92.3–121.8)
Renal impairment, n (%)	0 (0)	6 (6.0)	4 (7.8)
Glycemic control			
CGM duration	14 (13–14)	14 (13–14)	14 (13–14)
Average CGM glucose (mmol/L)	6.8 (5.4–9.9)	8.5 (7.0–12.0)	10.1 (8.2–14.5)
HbA1c (%)	6.4 (5.8–8.0)	8.2 (6.9–9.6)	9.8 (8.2–11.3)
HbA1c (mmol/mol)	46 (40–64)	66 (52–83)	84 (67–102)
Fasting glucose	7.2 (5.5–10.2)	8.2 (6.2–10.7)	9.3 (7.0–12.3)
Glucose variability (cv)	0.29 (0.26–0.33)	0.34 (0.29–0.39)	0.39 (0.33–0.47)
SD	2.06 (1.65–2.93)	3.16 (2.59–3.85)	4.0 (3.3–5.2)
Percent time spent in optimal range	78.1 (55.3–86.4)	60.1 (33.8–73.9)	40.1 (22.2,–55.4)
Percent time above 10	10.9 (1.3–35.3)	31.9 (14.3–66.0)	49.3 (30.8–74.2)
CGM hypoglycemia per week			
Episodes <3 mmol/L	1 (0–2.3)	0.5 (0–3.0)	2 (0–6.0)
Total time/week <3 mmol/L, min	39.2 (0–174.8)	17.0 (0–229.3)	127.5 (0–637.5)
Per cent time <3 mmol/L (%)	0.39 (0, 1.74)	0.17 (0, 2.26)	1.27 (0, 6.42)
Self-reported hypoglycemia, n (%)			
History of hypoglycemia events, n (%)	7 (25.0)	28 (28.0)	23 (45.1)
Hospitalized for hypoglycemia in the previous 12 months, yes	1 (3.6)	3 (3.0)	6 (11.8)
Hospitalized for hypoglycemia in the previous 12 months, % (95% CI)	3.6 (0.1 to 18.3)	3.0 (0.6 to 8.5)	11.8 (4.4 to 23.9)

BMI, body mass index; CGM, continuous glucose monitoring; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; SU, sulphonylurea.

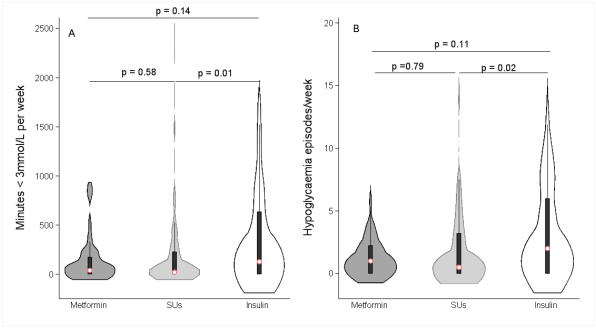

Figure 1 The distributions of hypoglycemia measured by CGM in individuals treated with metformin only, or sulphonylureas (SU) (with or without metformin) and insulin (with or without metformin and/or sulfonylureas). CGM, continuous glucose monitoring.

<3 mmol/mol 2.1 (95% CI 0.9 to 4.7, p value=0.067) and 5.5 (95% CI 2.4 to 12.6, p value=<0.001) times greater than metformin with SU and insulin, respectively. Findings were not substantially altered by further adjustment for age, BMI, diabetes duration, renal impairment and sex.

When adjusting to HbA1c of 53 mmol/mol (7%), an internationally recognized target for glycemic control, estimated minutes in hypoglycemia (per week) were 137.2 (95% CI 49.6 to 224.7), 290.9 (168.8 to 413.0) and 751.9 (433.9 to 1070.0) with metformin, SU and insulin, respectively (online supplemental material 3). Findings were similar for hypoglycemia rates per week, with rates approximately two and five times higher with SU and insulin than metformin after adjustment for HbA1c (table 3). Estimated adjusted mean rates of hypoglycemia at a range of clinically relevant HbA1c (and FPG) thresholds are shown in online supplemental figure 4).

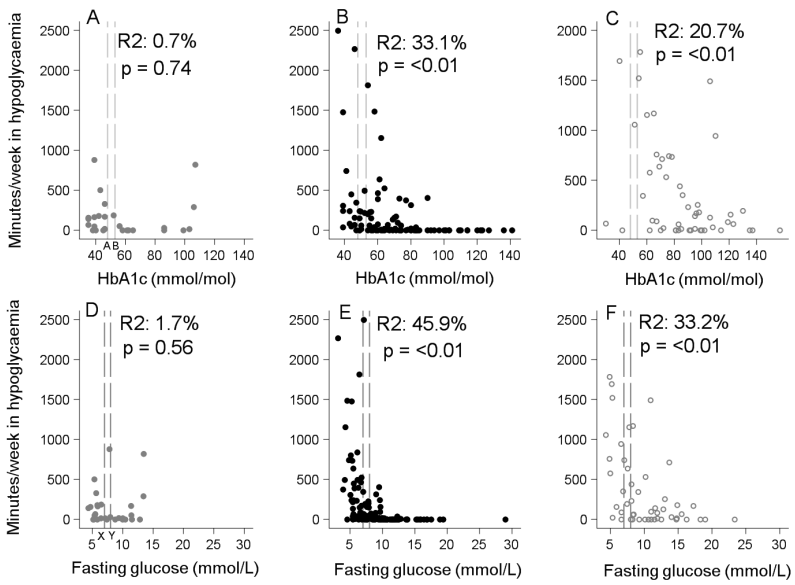

Figure 2 Comparison of glycemic control and hypoglycemia duration (minutes per week <3 mmol/L). Graphs in the top row show the relationship between HbA1c and the number of minutes spent in hypoglycemia per week for metformin (A), sulphonylureas (B), and insulin (C) treated participants, respectively. The bottom row shows the relationship between fasting glucose and number of minutes spent in hypoglycemia per week for metformin (D), sulphonylurea (E) and insulin (F) treated participants, respectively. The long-dashed lines denote glycemic thresholds, HbA1c 6.5% (48 mmol/mol) and 7.0% (53 mmol/mol) (top row), fasting glucose 7.0 mmol/L and 8.0 mmol/L (bottom row). HbA1c, glycated haemoglobin.

DISCUSSION

This study has demonstrated that both CGM assessed and self-reported clinically significant hypoglycemia in participants treated with SUs in Uganda is infrequent among patients who receive SU treatment. While observed hypoglycemia rates and duration were similar in those treated with metformin and SU, hypoglycemia risk was strongly associated with glycemic control, and after adjusting for differences in HbA1c, the risk of hypoglycemia doubled and quintupled in those treated with SUs and insulin, respectively. The modest hypoglycemia excess associated with SUs in comparison with metformin occurred predominantly in those with tight glycemic control. Hypoglycemia was more common in insulin treated diabetes than those treated with SU, further increasing on adjustment for glycemic control.

Table 2 Number of minutes <3 mmol/L per week in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Minutes <3 mmol/L (95% CI)	Duration ratio (vs metformin)	P value
Model 1 R^2=0.05	Metformin (Ref)	146.0 (60.6 to 231.3)	1.0	
	SU	206.7 (119.2 to 294.2)	1.4 (0.7 to 2.9)	0.345
	Insulin	365.9 (229.9 to 501.9)	2.5 (1.3 to 5.0)	**0.009**
Model 2 R^2=0.23	Metformin	74.0 (14.6 to 133.4)	1.0	
	SU	156.9 (97.6 to 216.3)	2.1 (0.9 to 4.7)	0.067
	Insulin	405.7 (262.1 to 549.3)	5.5 (2.4 to 12.6)	**<0.001**
Model 3	Metformin	96.4 (20.2 to 172.6)	1.0	
R^2=0.30	SU	157.5 (97.6 to 217.4)	1.6 (0.7 to 3.6)	0.230
	Insulin	355.0 (212.7 to 497.2)	3.7 (1.5 to 9.3)	**0.006**

Model 1: unadjusted; model 2: adjusted for HbA1c; model 3: adjusted for HbA1c, age, diabetes duration, BMI, sex, and renal impairment. Adjusted minutes <3 mmol/L are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73.2 mmol/mol). 95% CIs are shown in the parentheses. Renal impairment was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.
Values shown are mean (95 % CIs) and p-value. Bold values denote statistical significance at the p < 0.005 level.
BMI, body mass index; HbA1c, glycated haemoglobin; SU, sulphonylurea.

Table 3 Hypoglycemia rates in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Rates (95% CI)	Rate ratio (vs metformin)	P value (verses metformin)
Model 1 R^2=0.03	Metformin (reference)	1.3 (0.7 to 1.9)	1.0	
	SUs	2.1 (1.4 to 2.8)	1.6 (0.9 to 2.7)	0.108
	Insulin	3.2 (2.1 to 4.2)	2.4 (1.4 to 4.2)	**0.002**
Model 2 R^2=0.21	Metformin (reference)	0.6 (0.3 to 1.0)	1.0	
	SUs	1.5 (1.1 to 2.0)	2.4 (1.4 to 4.1)	**0.001**
	Insulin	3.8 (2.3 to 4.6)	5.4 (3.0 to 9.9)	**<0.001**
Model 3 R^2=0.24	Metformin (reference)	0.7 (0.3 to 1.1)	1.0	
	SUs	1.6 (1.1 to 2.0)	2.1 (1.2 to 3.6)	**0.006**
	Insulin	3.2 (2.0 to 4.4)	4.4 (2.2 to 8.7)	**<0.001**

Model 1: unadjusted; model 2: adjusted for HbA1c; model 3: adjusted for HbA1c, age, diabetes duration, BMI, sex and renal impairment. Adjusted rates are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73 mmol/mol). Renal impairment was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.
Values shown are mean (95% CIs) and p-value. Bold values denote statistical significance at the $p < 0.05$ level.
BMI, body mass index; HbA1c, glycated haemoglobin; SUs, sulphonylureas.

Studies comparing hypoglycemia risk across different treatments in type 2 diabetes are limited in LMICs, especially sub-Saharan Africa. The few hypoglycemia-related studies among people with type 2 diabetes patients in sub-Saharan Africa that have assessed the incidence and prevalence of hypoglycemia have predominantly used self-reported hypoglycemia and documented increased risk with insulin use.[21] The majority of these studies either included only patients on insulin and or grouped SUs together with other oral glucose-lowering agents.[11 21 22] Our finding that SU treatment is associated with a modest risk of clinically significant hypoglycemia among those with type 2 diabetes is consistent with studies in other popualtions.[23 24] However, it should be noted that the SUs in these studies are of newer generation, like gliclazide and glimepiride, that are known to have a lower hypoglycemia risk compared with glibenclamide.[7] The present study, although not designed to compare intra-SU class differences, showed a modest hypoglycemia risk even when majority (two out of three) of our patient population were taking glibenclamide, an older agent with higher hypoglycemia risk.[7] Moreover, the modest hypoglycemia excess in the SUs group mainly occurred in a small proportion of patients with tightly controlled diabetes, below international glycemic targets.[25–27]

A key strength of this study is the objective assessment of hypoglycemia through use of blind CGM monitoring. This removed potential biases that could arise from patient reactivity to glucose measurements, differences in glucose testing by treatment, hypoglycemia unawareness and recall bias that may affect studies assessing self-reported hypoglycemia or using medical records. An additional strength is comparison across therapies. It is well known that CGM can report occurrence of hypoglycemia in those who do not have diabetes, or are treated with medications not associated with hypoglycemia risk,[28 29] meaning the absolute risk of meaningful hypoglycemia by CGM will be overestimated. By including a metformin 'control' arm in our study, we ensured to avoid this overestimation by assessing the excess risk. A notable limitation of our study was that routine capillary glucose monitoring is not available to the vast majority of people with diabetes in Uganda, due to cost. Therefore, self-reported hypoglycemia is very unlikely to have been confirmed by glucose testing and is likely to be inaccurate in a population like ours where healthy literacy including hypoglycemia education is not good. Such testing may even be limited in a healthcare setting. Additionally, the modest number of participants treated with only metformin will have impacted our ability to detect modest differences in hypoglycemia risk in comparisons against metformin, as shown by the large CIs of estimates for metformin treated participants. Lastly, the majority of participants with SU and insulin treated diabetes had poor glycemic control, while this reflects current practice in this region, given the strong relationship between glycemic control and hypoglycemia risk, it is likely that hypoglycemia rates would be substantially higher were glycemic control improved in this population, as suggested by our adjusted analysis.

Glycemic control is the cornerstone of lowering microvascular complications among people living with diabetes. While there is no doubt that there is an association between SUs (especially the older agents like glibenclamide) and insulin treatment and hypoglycemia, the high rates of poor glycemic control in type 2 diabetes patients and relatively low hypoglycemic events among patients taking SUs suggest that there is room for optimizing glycemic control using these cheap, readily available and effective agents, despite the specific challenges of food insecurity and lack of glycemic monitoring in many LMIC populations. This supports the recommendations

to optimize glycemic control using these readily available and affordable agents including metformin and SUs.[8 30] The modest excess of hypoglycemia was predominantly seen in a small proportion of patients taking SUs whose fasting glucose was less than 7 mmol/L or HbA1c <7% (53mmol/mol) (thresholds often recommended by international guidelines) suggesting caution is needed when treating below these levels.[27]

In conclusion in a low resource sub-Saharan African setting, clinically significant hypoglycemia is infrequent among people with type 2 diabetes receiving SU treatment, and the modest excess occurs predominantly in those with tight glycemic control.

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Contributors AJN, BMS, MJN, and AGJ conceptualized and designed the study. AJN researched the data with assistance from PAB, RM, MJN, and AGJ. AJN and LRR conducted data cleaning. AJN analyzed the data with assistance from ALJC, LRR, BMS, and AGJ. AJN drafted the paper, which was critically revised by all authors. AJN, BMS and AGJ were fully responsible for the work and conduct of the study, had full access to all of the data and took responsibility for the accuracy and the integrity of the data of analysis. All authors approved the final manuscript.

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REFERENCES

1 International Diabetes Federation. *IDF diabetes atlas*. 10th edn. Brussels: International Diabetes Federation, 2021. https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf
2 Stratton IM, Adler AI, Neil HA, *et al*. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321:405–12.
3 Rawshani A, Rawshani A, Franzén S, *et al*. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2018;379:633–44.
4 Aschner P, Gagliardino JJ, Ilkova H, *et al*. Persistent poor glycaemic control in individuals with type 2 diabetes in developing countries: 12 years of real-world evidence of the International Diabetes Management Practices Study (IDMPS). *Diabetologia* 2020;63:711–21.
5 Barendse S, Singh H, Frier BM, *et al*. The impact of hypoglycaemia on quality of life and related patient-reported outcomes in type 2 diabetes: a narrative review. *Diabet Med* 2012;29:293–302.
6 Leiter LA, Boras D, Woo VC. Dosing irregularities and self-treated hypoglycemia in type 2 diabetes: results from the Canadian cohort of an international survey of patients and healthcare professionals. *Can J Diabetes* 2014;38:38–44.
7 Kalra S, Bahendeka S, Sahay R, *et al*. Consensus Recommendations on Sulfonylurea and Sulfonylurea Combinations in the Management of Type 2 Diabetes Mellitus - International Task Force. *Indian J Endocrinol Metab* 2018;22:132–57.
8 Chow CK, Ramasundarahettige C, Hu W, *et al*. Availability and affordability of essential medicines for diabetes across high-income, middle-income, and low-income countries: a prospective epidemiological study. *Lancet Diabetes Endocrinol* 2018;6:798–808.
9 Atun R, Davies JI, Gale EAM, *et al*. Diabetes in sub-Saharan Africa: from clinical care to health policy. *Lancet Diabetes Endocrinol* 2017;5:622–67.
10 Khunti K, Gomes MB, Pocock S, *et al*. Therapeutic inertia in the treatment of hyperglycaemia in patients with type 2 diabetes: a systematic review. *Diabetes Obes Metab* 2018;20:427–37.
11 Kaplan H, Amod A, van Zyl FH, *et al*. Incidence of hypoglycaemia in the South African population with diabetes: results from the IDMPS wave 7 study. *J Endocrinol Metabol Diabetes South Afr* 2019;24:58–64.
12 Alwafi H, Alsharif AA, Wei L, *et al*. Incidence and prevalence of hypoglycaemia in type 1 and type 2 diabetes individuals: a systematic review and meta-analysis. *Diabetes Res Clin Pract* 2020;170:108522.
13 Dunkley AJ, Fitzpatrick C, Gray LJ, *et al*. Incidence and severity of hypoglycaemia in type 2 diabetes by treatment regimen: a UK multisite 12-month prospective observational study. *Diabetes Obes Metab* 2019;21:1585–95.
14 Hemmingsen B, Schroll JB, Wetterslev J, *et al*. Sulfonylurea versus metformin monotherapy in patients with type 2 diabetes: a Cochrane systematic review and meta-analysis of randomized clinical trials and trial sequential analysis. *CMAJ Open* 2014;2:E162–75.
15 Edridge CL, Dunkley AJ, Bodicoat DH, *et al*. Prevalence and incidence of hypoglycaemia in 532,542 people with type 2 diabetes on oral therapies and insulin: a systematic review and meta-analysis of population based studies. *PLoS One* 2015;10:e0126427.
16 Wright AD, Cull CA, Macleod KM, *et al*. Hypoglycemia in type 2 diabetic patients randomized to and maintained on monotherapy with diet, sulfonylurea, metformin, or insulin for 6 years from diagnosis: UKPDS73. *J Diabetes Complications* 2006;20:395–401.
17 Niwaha AJ, Rodgers LR, Greiner R, *et al*. HbA1c performs well in monitoring glucose control even in populations with high prevalence of medical conditions that may alter its reliability: the OPTIMAL observational multicenter study. *BMJ Open Diabetes Res Care* 2021;9:e002350.
18 Danne T, Nimri R, Battelino T, *et al*. International consensus on use of continuous glucose monitoring. *Diabetes Care* 2017;40:1631–40.

19 Chen W, Qian L, Shi J, *et al*. Comparing performance between log-binomial and robust poisson regression models for estimating risk ratios under model misspecification. *BMC Med Res Methodol* 2018;18:63.
20 Motta V. Estimating Poisson pseudo-maximum-likelihood rather than log-linear model of a log-transformed dependent variable. *RAUSP* 2019;54:508–18.
21 Tiruneh GG, Abebe N, Dessie G. Self-reported hypoglycemia in adult diabetic patients in East Gojjam, Northwest Ethiopia: institution based cross-sectional study. *BMC Endocr Disord* 2019;19:17.
22 Khunti K, Alsifri S, Aronson R, *et al*. Rates and predictors of hypoglycaemia in 27 585 people from 24 countries with insulin-treated type 1 and type 2 diabetes: the global HAT study. *Diabetes Obes Metab* 2016;18:907–15.
23 Baretić M, Bralić Lang V, Lang B V. Hypoglycemia in patients with type 2 diabetes treated with oral antihyperglycemic agents detected by continuous glucose monitoring: a multi-center prospective observational study in Croatia. *BMC Endocr Disord* 2020;20:35.
24 Mbanya JC, Al-Sifri S, Abdel-Rahim A, *et al*. Incidence of hypoglycemia in patients with type 2 diabetes treated with gliclazide versus DPP-4 inhibitors during Ramadan: a meta-analytical approach. *Diabetes Res Clin Pract* 2015;109:226–32.
25 American Diabetes Association. 6. Glycemic targets: Standards of Medical Care in Diabetes-2019. *Diabetes Care* 2019;42:S61–70.
26 Qaseem A, Wilt TJ, Kansagara D, *et al*. Hemoglobin A1c targets for glycemic control with pharmacologic therapy for nonpregnant adults with type 2 diabetes mellitus: a guidance statement update from the American College of physicians. *Ann Intern Med* 2018;168:569–76.
27 WHO. *Diagnosis and management of type 2 diabetes (HEARTS D).No. WHO/UCN/NCD/20.1*. Geneva: World Health Organization, 2020.
28 Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group, Fox LA, Beck RW, *et al*. Variation of interstitial glucose measurements assessed by continuous glucose monitors in healthy, nondiabetic individuals. *Diabetes Care* 2010;33:1297–9.
29 Zhou J, Li H, Ran X, *et al*. Reference values for continuous glucose monitoring in Chinese subjects. *Diabetes Care* 2009;32:1188–93.
30 Mohan V, Khunti K, Chan SP, *et al*. Management of type 2 diabetes in developing countries: balancing optimal glycaemic control and outcomes with affordability and accessibility to treatment. *Diabetes Ther* 2020;11:15–35.

Appendix 5 – unpublished papers and other work

Paper: Routine islet autoantibody testing in clinically diagnosed adult-onset type 1 diabetes can help identify misclassification and, where negative, is associated with a high prevalence of treatment change & successful insulin cessation.

Authors: R. Eason*, N. Thomas*, A.V Hill, B.A Knight, A Carr, A.T Hattersley, T.J McDonald, B.M Shields, A.G Jones for the StartRight Study group.

Submission status: Submitted Diabetes Care 2022

Paper: Random c-peptide is a pragmatic measure of beta cell function, predicting glucose variability and hypoglycaemia risk

Authors: S. Brackley, N.Thomas, A. Carr, R. Andrews, S.V. Hope, A. Jones

Submission status: Diabetes UK abstract 2022

Paper: Milder Loss of Insulin Containing Islets in African American Individuals with Type 1 Diabetes and Type 2 Diabetes-Associated *TCF7L2* Genetic Variants

Authors: Maria J. Redondo, Sarah J. Richardson, Daniel Perry, Charles G. Minard, Alice L.J. Carr, Irina Kusmartseva, Alberto Pugliese, Mark Atkinson

Submission status: Planned submission Diabetes 2022

Paper: Larger time intervals in continuous glucose monitoring data lead to reduced detection of hypoglycemic episodes

Authors: Neil Vaughan*, Catherine L Russon*, Alice LJ Carr, Richard M Pulsford, Michael Allen, Robert C Andrews

Submission status: Under review Plos One 2022

Paper: High resolution analysis of insulitis profiles in the pancreata of young people with recent-onset Type 1 diabetes using a digital pathology platform

Authors: Rebecca C. Wyatt, Alice L.J. Carr, Pia Leete, Myriam Padilla, Mingder Yang, Marika Bogdani, Gail Deutsch, Christine Flaxman, Mark Atkinson , Irina Kusmartseva, Noel G. Morgan and Sarah J. Richardson

Submission status: Planned submission Diabetologia

Paper: Testing the EXTOD structured education programme for safe and effective exercise in type 1 diabetes: Results of a randomised controlled pilot trial.

Authors: Parth Narendran, Alice Carr, Richard Oram and Robert Andrews

Submission status: Diabetes UK abstract 2022, winning the Diabetes UK Education and Self-management Award. Planned submission of full paper.

Working closely with Professor Colin Dayan, Dr Peter Senior and Dr Peter Taylor as part of The Trial Outcome Markers Initiative in Type 1 Diabetes (TOMI-T1D) work package 5, part of the Critical Path (C-path) Institute.

Collaboration with Professor Rob Andrews and George Stoy, owner of Georges Surf School, Polzeath, to develop structured training for surf coaches to aid people with diabetes in surf lessons. This work is an extension of the EXTOD structured education program set up by Professor Rob Andrews and Dr Parth Nerandran, aiming to involve Surf England and the World Surf League.