Exploring minimally invasive approach to define stages of type 1 diabetes remotely

Helena Kontola[1,*], Inka Alanko[1,*], Jaakko Koskenniemi[1,2], MD, PhD, Eliisa Löyttyniemi[3], PhD , Saori Itoshima[1,2], MD, PhD, Mikael Knip[,4,5,6], MD, PhD, Riitta Veijola[7,8], MD, PhD, Jorma Toppari[1,2], MD, PhD, Jukka Kero[1], MD, PhD

[1]Department of Pediatrics, Turku University Hospital, Turku, Finland, [2]Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology, and Centre for Population Health Research, University of Turku, Turku, Finland, [3]Department of Biostatistics, University of Turku, Turku, Finland, [4]Pediatric Research Center, New Children's Hospital, Helsinki University Hospital, Helsinki, Finland, [5]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland, [6]Center for Child Health Research, Tampere University Hospital, Tampere, Finland, [7]Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland, [8]Department of Pediatrics, PEDEGO Research Unit, Medical Research Center, University of Oulu, Oulu, Finland, *These authors contributed equally to this work.

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Corresponding author:

Jukka Kero, MD, PhD

Department of Pediatrics, Turku University Hospital

Kiinamyllynkatu 4-8, FIN-20500 Turku, Finland

Phone: +358 50 4773365, E-mail : jukka.kero@utu.fi

ABSTRACT

Objective: New methods are pivotal in accurately predicting, monitoring, and diagnosing the clinical manifestation of type 1 diabetes in high-risk children. Continuous glucose monitoring (CGM) is a valuable tool for patients with type 1 diabetes, but there is still a knowledge gap regarding its utility in the prediction of diabetes. The current study explored whether 10-day CGM or CGM during an oral glucose tolerance test (OGTT) performed in the laboratory or at home (home-OGTT) could be accurate in detecting stages of type 1 diabetes.

Research design and methods: 46 subjects aged 4-25 years carrying genetic risk for type 1 diabetes were recruited and classified into the following groups: islet autoantibody (IAb) negative, one IAb, and stages 1-3 of type 1 diabetes, based on the laboratory OGTT and IAb results at baseline. A 10-day CGM was initiated before the OGTT.

Results: Here, we showed that CGM was sensitive in detecting asymptomatic individuals at stage 3, and dysglycemic individuals in stage 2 of type 1 diabetes both during OGTT and the 10-day period. CGM also showed significant differences in several variables during the 10-day sensoring among individuals at different stages of type 1 diabetes. Furthermore, CGM showed different OGTT profiles and detected significantly more impaired glucose tolerance results when compared to plasma glucose.

Conclusions: CGM together with home-OGTT could detect stages of type 1 diabetes and offer an alternative method to confirm normoglycemia in high-risk individuals.

INTRODUCTION

Longitudinal follow-up studies have reported that children with multiple islet autoantibodies (IAbs) have a lifetime risk of more than 85% to develop type 1 diabetes (1–3) and develop alterations in glucose metabolism even several years before the clinical diagnosis of diabetes (4). For example, the first-phase insulin response (FPIR) is lower among progressors compared to controls as early as 4-6 years prior to the clinical presentation of diabetes (5,6). In addition, a delayed peak in C-peptide response to oral glucose, an abnormal oral glucose tolerance test (OGTT), increasing HbA1c, or an increased glycemic variability (7–9) can be detected 1-2 years prior to diagnosis. In general, children participating in prediction studies are diagnosed at an early stage of type 1 diabetes. Therefore, the frequency of ketoacidosis at the time of diagnosis is lower compared to patients from the general population (10). Despite improvements in prediction measurements, it remains challenging to predict the impending manifestation of type 1 diabetes among high-risk children, and new methods are needed. Furthermore, frequent OGTTs, laboratory tests and study visits, are often challenging and burdensome for the individuals at risk and their families.

Continuous glucose monitoring (CGM) is a useful tool for diabetes management. CGM has been shown to improve glycemic control and may also reduce the risk of complications in patients with type 1 (11,12). However, there is a knowledge gap concerning the use of CGM in diabetes prediction. Previous studies have suggested that CGM can detect early hyperglycemia in children with multiple IAbs (13). In addition, evening glucose values measured with CGM appear to have a higher range in children with at least two IAbs than in children without IAb (8). Although HbA1c is a good indicator of long-term glucose levels, it provides no information about daily glucose variability in comparison to CGM (14). Further, CGM can detect increased glucose variability even before abnormal results in the standard

OGTT in IAb-positive children (8). However, more information is needed to validate CGM in the detection of different stages preceding the clinical presentation of type 1 diabetes (15).

The goal of this study was to explore whether minimally invasive continuous glucose monitoring together with home-OGTT could be a safe and accurate alternative to reliably detect impaired glucose tolerance, make a diagnosis of early type 1 diabetes and its different stages during the follow-up of children with increased genetic susceptibility to type 1 diabetes.

RESEARCH DESIGN AND METHODS

Source cohort of the study

Study subjects were recruited from the prospective Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study. Briefly, in the DIPP-study children born in three Finnish university hospitals (Turku, Tampere, Oulu) are screened for HLA-conferred susceptibility to type 1 diabetes, and those at increased risk are invited to the follow-up, which includes regular blood sampling for measurement of IAbs against insulin (IAA), protein tyrosine phosphatase-related IA-2 antigen (IA-2A), glutamic acid decarboxylase 65 (GADA) and zinc transporter 8 (ZnT8A) every 3-6 or 12 months as described previously (10,16). IAA, GADA, IA-2A, and ZnT8A were analyzed using specific radiobinding assays (17–20). HLA-DQB1 alleles were analyzed from cord blood using lanthanide-labeled oligonucleotide probe hybridization and time-resolved fluorometric detection as previously described (21). The classification and selection of the children with HLA-based genetic susceptibility to the DIPP-study has been described previously (22). The study was approved by the Ethics Committee of the Hospital District of Northern Ostrobothnia, Oulu, Finland. Separate written informed consents were obtained for genetic screening, follow-up, and for this study.

Study design and definition of study groups

The subjects were classified into the following five groups based on the presence of islet autoantibodies and stage of type 1 diabetes defined at the first study visit as described in Figure 1 and Table 1. The classification of the stages has been described previously (15) and was based on the number of islet autoantibodies (IAA, IA-2A, ZnT8A and GADA), HbA1c and laboratory OGTT using the following definitions: 0 IAb, (islet autoantibody negative children with normoglycemia), one IAb, (children with a single islet autoantibody and normoglycemia),

Stage 1 type 1 diabetes (children with two or more islet autoantibodies and normoglycemia), Stage 2 type 1 diabetes (children with two or more islet autoantibodies and dysglycemia), and Stage 3 type 1 diabetes (autoantibody-positive children who developed diabetes during the study period and had two diabetic OGTTs defined according to the ADA and WHO criteria (23,24) or having at least twice fasting plasma glucose ≥7 mmol/l fasting or random plasma glucose ≥11.1 mmol/mol at the study visit or during the CGM period). Dysglycemia was defined by one or more of the following findings: fasting glucose ranging between 6.1 and 6.9 mmol/L, any glucose value ≥11.1 mmol/l at 15, 30, 60 or 90 min time points during the OGTT, 120 min plasma glucose 7.8-11.0 mmol/l or HbA1c over 39 mmol/mol (5.7%). All study subjects were asymptomatic, and the early diabetes diagnosis was based on OGTT, except for one individual whose diagnosis was prompted by a high CGM value in the beginning of the CGM period and was confirmed by two high random plasma glucose values. If a subject had shown a dysglycemic OGTT prior to the start of the study, two normal OGTTs were required to be classified into the normoglycemic group. Individuals with ISO-BMI ≥ 30 kg/m^2 (or BMI for subjects over 18-years-old) and pregnant subjects were excluded from the study (25). Tanner-staging (26,27) was applied at the study visit for pubertal evaluation. During our study period, none of the study participants was on any form of treatment that would affect glucose metabolism.

Continuous glucose monitoring (CGM)

A Dexcom G6 continuous monitoring system (Dexcom, Inc., San Diego, CA) sensor was placed on the subject´s lower abdomen at least 12 hours before the first OGTT performed at the DIPP-study laboratory or at home. The CGM was continued up to 10 days, and data were excluded if less than 24 hours were recorded, or if the subjects developed an infection during the sensor use. The Dexcom G6 CGM sensor recorded glucose values ranging between 2.2 and

22.2 mmol/l. The mean absolute relative difference (MARD) for the Dexcom G6 CGM device in accuracy studies comparing sensor glucose values with reference venous blood glucose values has been reported to be 9.0% (28). If a subject was diagnosed with type 1 diabetes and insulin treatment was started during the period of sensor use, the CGM data after the initiation of insulin treatment were excluded. CGM was masked for the majority of subjects (65%) but was unblinded for 35% of subjects upon request by the subjects or their guardians. Furthermore, if a subject had a diabetic OGTT prior to or during the study period the CGM sensor was unmasked.

Oral Glucose Tolerance Test (OGTT)

A standard six-point OGTT (23) was performed between 8-10 am 1-3 days after the beginning of the CGM. Blood samples were drawn at the following time points: 0-, 15-, 30-, 60-, 90- and 120-min. Subjects were advised to fast for at least 10 h prior to the OGTT. Fasting plasma glucose was measured before the subjects drank the glucose solution within 5 min. The starting time for the OGTT was collected from the CGM sensor. Plasma glucose and HbA1c levels were analyzed using standard assays in the Clinical Chemistry Laboratory Turku University Hospital. An enzymatic assay with absorbance measurement was used for plasma glucose with a Cobas c 702 (Roche Diagnostics, Basel, Switzerland) and an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics) for HbA1c with a Cobas c 501 analyzer (Roche Diagnostics). The glucose drink (Glucosepro, Mediq, Finland) contained 75g glucose in 250 ml liquid. The dose administered was 1.75g glucose/kg body weight up to 43 kg and 75g of glucose for participants with \body weight > 43kg.

Home-OGTT

Participants were advised to perform a home-OGTT with the same instructions as for the laboratory OGTT (i.e. fasting for at least 10 hours and performing the test between 8-10 am) as in the laboratory OGTT. The participants were advised to measure self-monitored blood glucose (SMBG) before (0 min) and after (120 min) the home-OGTT. Home-OGTT was not performed if the participant had had diabetic plasma glucose values during the laboratory OGTT at the study visit or if the fasting blood glucose concentration was ≥7.0 mmol/l prior to the home-OGTT measured with SMBG. The same amount of glucose was used for the home-OGTT and the laboratory OGTT. Home-OGTT CGM data and all CGM data were retrieved using the Dexcom Clarity program (Dexcom).

Statistical analyses

Power calculations suggested that a sample size of 5-10 subjects per stage was needed to detect a 10% difference in mean evening plasma glucose values in CGM recordings (4.4-4.8 mmol/l) between IAb negative and positive subjects with 80% probability assuming a 5% type one error rate using a two-tailed t-test. Unless stated otherwise, analyses were performed using GraphPad Prism version 9 for Windows, (GraphPad Software, San Diego, CA). Analyses of differences in categorical variables between groups were carried out with the JMP® version 16 (SAS Institute Inc., Cary, NC) using Fisher's exact test, whereas calculations of metrics of glucose variability in 10-day CGMs including MAGE (mean amplitude of glucose excursions), HBGI (high blood glucose index) were done in R statistical environment (R Foundation for Statistical Computing, Vienna, Austria) using a package 'iglu'(29).

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The normal distribution assumption was checked using D´Agostino Pearson´s or Shapiro-Wilks tests, and if needed, data were logarithm or square root transformed to meet the assumption of normality. Differences between means were tested using a one-way analysis of variance (ANOVA) if a variable satisfied the assumption of normal distribution and the equality of variances, and Kruskal-Wallis if it didn´t. Tukey´s test, Dunn's test and Dunnett´s T3 test were used for post-hoc comparisons. (if possible please give references to these methods that are not as well known as many others)

Percentages each subject spent above each 0.1 mmol/l strata between 6 and 12 mmol/l were calculated. The differences in mean percentages of time between groups above 7.8 and the stratum that differed the most between groups were reported. Comparisons between plasma and sensor glucose values during the OGTT were analyzed with paired t-test in each group. Changes in mean glucose values over time points were compared between groups using a mixed-effects model including time and groups as within and between factors, respectively. The subjects were included as a random effect. The correlation between plasma and sensor glucose values during the OGTT was tested using Pearson's or Spearman's correlation tests. The AUCs were calculated using the trapezoidal method.

RESULTS

Demographics and classification of study participants into different groups and stages of type 1 diabetes

As described in Figure 1, a total of 46 children with HLA-conferred risk for type 1 diabetes participated in the study. Six-point laboratory OGTT data were available from 45 individuals, CGM data from 40 individuals and 24 subjects performed home-OGTT during the sensoring. There were no significant sensor-related complications such as local skin reactions in any of the subjects.

Figure 1 summarizes the study overview and Table 1 shows the population characteristics including the number of IAb, laboratory OGTT, and HbA1c results at the baseline. The median age of all participants was 11.7 years (range 3.9-25.4). There were no significant differences between groups for age, sex, Tanner stage, BMI, or time from seroconversion. Twenty percent of all participants reported a first-degree relative with type 1 diabetes. As expected, the HbA1c was lowest in subjects without IAb and highest in subjects at stage 3 DM (Table 1).

Correlation between venous plasma and sensor glucose values during OGTT

To test the accuracy of the CGM during a standard OGTT, laboratory venous plasma and sensor glucose values during laboratory OGTT were compared in subjects with 0 or one IAb, and at Stages 1-2 (N=34) in figure 2A and 2B and supplement table 1. Stage 3 individuals did not undergo a home-OGTT were not included in figure 2C. As shown in Figure 2A, the means of plasma and sensor glucose during the laboratory OGTT overlapped, but sensor glucose values were significantly higher than plasma glucose after the 30 min time point. When all 25 CGM time points were included, the CGM OGTT curve showed a different shape compared to the

standard six-point venous plasma OGTT (Fig, 2B). The peak value was observed approximately 15 min later in the CGM than in the plasma glucose during the OGTT. Comparison between the home-OGTT and laboratory OGTT using the CGM device showed almost identical curves (Fig. 2C). The correlation between the sensor glucose and plasma glucose values during the laboratory OGTT at the 60 min time point is shown in Figure 2D. Overall, the correlation varied between moderate to strong (r=0.41-0.82) at different (0, 15, 30, 60, 90 and 120 min) OGTT time points and a stronger correlation was observed in later (60, 90 and 120 min) as opposed to earlier time points (0, 15 and 30 min) of the OGTT as described more detail in Supplemental Figure 1.

Sensor and venous plasma glucose variations in individuals at different stages of type 1 diabetes during OGTT

The graphical and statistical comparisons of sensor and plasma glucose values between the study subjects during laboratory OGTT with different IAb profiles and stages of type 1 diabetes during OGTT are presented in Figure 3 and Supplemental Table 1. An illustration of the plasma and CGM glucose curves during the laboratory OGTT of each individual in different groups is shown in Supplemental Figure 2S. Overall, sensor and plasma glucose values were highly comparable among individuals without IAb or with an early diagnosis of type 1 diabetes. Among individuals with one IAb or at stages 1 and 2 sensor glucose values were in general significantly higher than the plasma glucose between 30-60 min time points (Fig. 3). There was a difference between groups in the shape of the CGM glucose patterns during the OGTT ($p<0.0001$ for interaction between time and study group in the mixed-effect model). As expected, the most prominent differences were observed between stage 3 and other groups at or after 30 min. At 0 min the only statistically significant difference was found between stages 1 and 3 (Supplemental Table 1). Both the 6- and 25-point CGM AUC values during the

laboratory OGTT were higher than the AUC calculated using plasma glucose values. The AUC for the six-point OGTT was generally higher in the subjects with a higher numerical stage of type 1 diabetes for both CGM and plasma glucose. However, rather unexpectedly, the one IAb group had higher glucose AUCs than the stage 1 group (Supplemental Table S1).

In the laboratory OGTT, the average peak of plasma glucose was detected in 86% at 30 min and in 14% at 60 min, while the peak was detected at a mean of 45 min (SD 9 min) when using CGM, in individuals with 0 IAb, one IAb or stage 1. In contrast, in individuals at stages 2 and 3, the peak glucose value was detected significantly later during a 2h OGTT in both laboratory plasma and CGM values (Supplemental Figure S3 and Supplemental Table S2).

Next, we evaluated the accuracy of the CGM in defining stages of diabetes during the laboratory OGTT. The number of individuals classified into different stages with a standard plasma glucose OGTT at baseline visit was compared to the CGM-based classification using the same criteria. As shown in Supplemental Figure S4, in 5/10 and 2/8 individuals in the stage 1 and 2 groups, sensor glucose values during OGTT fulfilled criteria for dysglycemia or diabetes, respectively. In all state 3 subjects who started CGM monitoring before the laboratory OGTT (N=5), CGM values also fulfilled the OGTT criteria for type 1 diabetes. Unexpectedly, 5/6 subjects with one IAb presented dysglycemic CGM values during OGTT with either fasting sensor glucose values > 6.1 mmol/l or a 120 min glucose value > 7.8 mmol/l. One of nine individuals in the 0 IAb group presented with dysglycemic sensor glucose values.

10-day CGM variability in individuals at different stages of type 1 diabetes

Glucose variability over the whole 10-day CGM was compared between the different stages of type 1 diabetes (Figure 4 and Table 2). As expected, the most obvious significant differences were observed between stage 3 subjects and the other study groups. The mean and range of sensor glucose values gradually increased from stages 1 to 3. Similarly, the average CV%

values increased gradually from the 0 IAb group to stage 3 subjects: 0 IAb: CV% =15.5%, one IAb: 17.3%, stage 1:19.8%, stage 2: 22.6% and stage 3: 29.8%, respectively (Table 2). There were also statistically significant differences in several CGM variables including the nocturnal range, CGM-estimated HbA1c, HBGI, MAGE and the time (%) spent > 7.8 mmol/l between stage 3 and the other stages of type 1 diabetes. However, unexpectedly, the median time % spent over 7.8 mmol/l was higher in the one IAb group than in the stage 1 group. As expected, the highest mean in time spent > 11.0 mmol/l and > 13.9 mmol/l was observed in stage 3. The mean percentage of time spent in the 3.9-7.8 mmol/l range decreased progressively from 94% ± 2.7% (SD) to 68% ± 13.4% in subjects with 0 IAb to stage 3. There were no statistically significant differences in time % spent < 3.9 mmol/l ($TBR_{<3.9\ mmol/L}$) or < 3.0 mmol/l ($TBR_{<3.0\ mmol/L}$) between any of the study groups. The CGM data of the eight asymptomatic individuals with diabetic OGTT results are shown in Table 2. For any cut-point between 6 and 12 mmol/l, the groups differed the most in time spent above 9.1 mmol/l (Supplemental Figure S5). However, significant pairwise differences were found only between stage 1 and stage 2 and not between the other adjacent groups.

CONCLUSIONS

In this study, we evaluated the accuracy and variability of CGM during OGTT performed in the laboratory or at home in individuals at risk for developing type 1 diabetes. Furthermore, we tested the accuracy of CGM in defining the type 1 diabetes stages. Our study demonstrated that CGM either during OGTT or during a 10-day period is sensitive in detecting asymptomatic individuals at stage 3 of type 1 diabetes, and also showed significantly different glucose AUCs during OGTT, an increase in CV% and a decreased proportion of the time in range (%TIR i.e glucose between 3.9-7.8 mmol/l) during 10-day monitoring among individuals at different stages of type 1 diabetes. Importantly, CGM did not miss any dysglycemic or diabetic glucose values among the 34 subjects who underwent CGM during the OGTT. Accordingly, CGM seems to be a reliable method for the early detection of asymptomatic type 1 diabetes. However, our results also showed that use of CGM during OGTT in diabetes staging would lead to changed classification of individuals in early stages to more advanced stages, and thus permit earlier diabetes diagnosis if the ADA dysglycemia criteria are applied without any correction for CGM levels that are generally higher than plasma levels in our experience. Compared to laboratory OGTT, CGM is less invasive and offers more information and provides the ability to assess glycemic variability in real time. CGM-based home-OGTT would offer an alternative method to confirm normal glucose metabolism in high-risk research subjects, for example, during a pandemic or in the presence of other challenges related to travelling to the research laboratory.

During the OGTT sensor and plasma glucose correlated best in individuals without islet autoantibodies or at stage 3 of type 1 diabetes. In general, CGM during standard OGTT showed

higher values compared to plasma glucose, specifically in stage 2 and 3 individuals. Interestingly, when comparing full 25-point CGM OGTTs to a standardized 6-point laboratory OGTT curves, CGM revealed higher and slightly different timing of the peak glucose values. It appears that peak glucose values are better captured with CGM than with discontinuous measurements of a regular laboratory OGTT. However, the CGM estimates the plasma glucose by measuring the interstitial fluid glucose concentration using an electrochemical sensor inserted subcutaneously, and the sensors, in general, have a short time lag (3-12 min) when compared to blood glucose (30). The Dexcom G6 used in this study applies a predictive algorithm which reduces the time lag between plasma glucose and interstitial tissue glucose values to 4 minutes (31–33). The clinical significance of different OGTT-profiles or higher peak glucose values is less clear than the standard 0 and 2h time points, but glucose values above 11.0 mmol/l at any time point between 0 and 2h are associated with an increased risk of type 1 diabetes in genetically high- risk children (4). In accord with these findings, a decreased early (30 min) C-peptide response to oral glucose and an increased later response has been described to occurring at least 2 years before the diagnosis of type 1 diabetes (9). One could speculate that in our study, individuals with one or two IAb and a high peak (> 11.0 mmol/l) sensor glucose value but normal 0 and 2h glucose values might have a different prognosis than those without such a high peak. This profile might also be associated with lower first-phase insulin response (FPIR), which is associated with earlier β-cell dysfunction and eventually to progression to type 1 diabetes, and can also be seen in individuals with two or more IAbs (5). Long-term prospective studies using CGM during OGTT together combined with FPIR measurements would be needed to clarify this question.

Previous CGM studies have demonstrated a statistically significant difference in time spent > 7.8mmol/l between 0 IAb controls and patients with multiple IAbs who progressed to type 1 diabetes (8,13). Steck et al. (2019) showed that during CGM an 18% cutoff value for time spent

> 7.8 mmol/l predicts progression to type 1 diabetes. Similarly, using the same 18% cutoff value in our study, all asymptomatic subjects at stage 3 who developed type 1 diabetes during the study period and two subjects from the stage 2 group would have been predicted to develop type 1 diabetes.

Differences in the day and night mean and range glucose values between islet autoantibody-negative and positive cases have been described (8). Supporting this finding, in our CGM analysis, the daytime and nighttime means, and ranges increased gradually from stages 1 to 3. In addition, a clear increase in CV% was detected between study groups indicating that these parameters could serve as early markers of deterioration of glucose variability before the progression towards overt type 1 diabetes.

Can CGM be safely used to diagnose diabetes in individuals at risk? Here, we present CGM data at the time of diagnosis in asymptomatic individuals between the age of 4.5-19.7 who fulfilled the standard OGTT diabetes criteria during the study. All seven subjects who had a laboratory OGTT that was diagnostic of type 1 diabetes at baseline also confirmed the result based on a CGM-based OGTT. The diabetes diagnosis for one asymptomatic individual was confirmed by elevated random plasma glucose values measured because of the high sensor glucose values obtained early in the study. In the 10-day CGM all these individuals showed significant differences in nearly all key CGM variables compared to the control group. Thus, the CGM values above or below (e.g., proportion of time in range) observed for our stage 3 group strongly suggest the manifestation of diabetes and prompted a confirmatory OGTT or laboratory plasma glucose measurement. The establishment of CGM reference values for people with diabetes would help clarify the diagnosis in some cases. CGM would be less invasive and time-consuming than having to repeat the standard OGTT a second time. OGTT adherence even among high-risk individuals is only around 60% for multiple reasons (34), and

it may not be feasible for young children. Larger cohorts with different age groups would be required to revise the criteria for type 1 diabetes and dysglycemia by CGM (14,28).

Although CGM seems to safely detect the manifestation of diabetes and demonstrate differences between different stages of development there is a risk of overestimating glucose values and thus the stage of diabetes. Many of the normoglycemic subjects would have been considered dysglycemic or as having type 1 diabetes if only CGM values had been used, suggesting that the CGM criteria need to be adjusted. It is possible that by measuring interstitial glucose more frequently (every 5 min) during the OGTT or evaluating several hours of glucose variability in real-life, it will become easier and more sensitive to identify individuals in the one IAb or stage 1 groups who are progressing towards diabetes. Thus, our observation that several indicators of glucose metabolism in the one IAb group were higher than in the stage 1 group and actually closer to the stage 2 group, would be explainable if some of these individuals were progressing towards diabetes. Unfortunately, the relatively small number of subjects (6-12) per group make the results sensitive to random sampling errors. Further long-term prospective follow-up studies with CGM are warranted to confirm these findings. All of these study subjects carry an increased genetic risk of type 1 diabetes due to their HLA genotypes, thus limiting the generalization of these results. However, in our control group the mean percentage of time spent in the a range 3.9-7.8 mmol/l exceeded 93% and several other CGM parameters were consistent with metrics previously reported for a healthy normoglycemic population (28).

One limitation of our study, was that we did not exclude data obtained during the first 24 hours of CGM data, but some of our subjects performed the laboratory OGTT during the first 24 hours. Higher mean absolute relative difference (MARD) has been reported for the first 24 hours (9.3%) compared to second (8.4%) or days thereafter (MARD for 4-5, 7 and 10 days is 9.4%, 8.7% and 9.0% respectively) when using Dexcom G6 sensor (33).

In summary, our findings suggest that the CGM-based evaluation of IAb-positive individuals could be a powerful alternative tool to confirm normal glucose metabolism in individuals at high risk for type 1 diabetes and could provide improved accuracy and novel insights into the prediction of type 1 diabetes and its presymptomatic staging.

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Conflict of interest

The authors state no potential conflicts of interest.

Author Contributions

HK, IA, JKe, JT and RV designed the study, H.K.,I.A. and J.Ke. collected and analyzed the data and wrote the manuscript. J.Ko, J.T., R.V., M.K., S.I. and E.L reviewed/edited the manuscript.

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		Stage of Diabetes Development			
Clinical Characteristics	**0 IAb**	**1 IAb**	**Stage 1**	**Stage 2**	**Stage 3**
N	9	6	12	11	8
Male, N (%)	2 (22%)	2 (33%)	6 (50%)	2 (18%)	4 (50%)
Age median (range)	9.3 (3.9-15.2)	12.3 (7.4-13.1)	9.7 (5.1-25.4)	15.1 (4.0-20.2)	9.7 (4.5-19.7)
ISO-BMI[1] (kg/m^2), mean (SD)	22.1 (2.5)	22.3 (2.9)	22.8 (3.0)	22.7 (2.3)	22.2 (2.8)
HbA1c (mmol/mol), mean (SD)	32.0 (7.2)	35.2 (1.8)	32.3 (1.1)	37.0 (4.1)	41.4 (2.0)
Years from seroconversion, median (range)	na	5.1 (1.5-11.4)	7.95 (2.0-20.9)	8.4 (0.8-15.0)	6.8 (0.0-20.9)
IAbs, N (% of the group) 0	9 (100%)	0	0	0	0
1	0	6 (100%)	0	2 (18%)	2 (33%)
2	0	0	6 (50%)	2 (18%)	3 (50%)
3	0	0	1 (8%)	6 (55%)	1 (13%)
4	0	0	5 (42%)	1 (9%)	2 (25%)
IAb type, N (%) GADA	0	2 (33%)	11 (92%)	9 (82%)	4 (50%)
IA-2A	0	1 (17%)	10 (83%)	6 (55%)	5 (63%)
IAA	0	3 (50%)	7 (58%)	6 (55%)	5 (63%)
ZnT8	0	0	7 (58%)	7 (64%)	5 (63%)
Tanner stage 1	5 (56%)	2 (33%)	5 (42%)	2 (18%)	5 (63%)
2	1 (11%)	0	0	2 (18%)	0
3	0	0	0	2 (18%)	0
>3	2 (22%)	1 (17%)	3 (25%)	5 (45%)	2 (25%)
FDR with T1D, N (%)	4 (44%)	1 (17%)	2 (17%)	0	2 (25%)

595 **Table 1.** Demographics of the study participants. [1]ISO-BMI, age-, and sex-adjusted body
596 mass index, FDR, first degree relative with type 1 diabetes, 0 and 1 IAb, children without and
597 with 1 type of islet autoantibodies, and na, not applicable.
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	Stage of Diabetes Development				
CGM variables	**0 IAb**	**1 IAb**	**Stage 1**	**Stage 2**	**Stage 3**
Mean (SD)[A]	**5.80 (0.35)[E]**	**6.35 (0.35)[E]**	**5.87 (0.53)[E]**	**6.43 (0.54)[E]**	**7.35 (0.76)**
Range mean (min-max)[B]	**1.00 (0.80-1.30)[E,F]**	**0.98 (0.80-1.10)[E,F]**	**1.16 (1.00-1.4)[E]**	**1.46 (1.00-1.90)**	**2.21 (1.30-3.4)**
CV% (SD)[A]	**15.50 (1.80)[E,H]**	**17.29 (2.97)[E,H]**	**19.77 (1.93)[E]**	**22.64 (3.94)[G]**	**29.77(6.49)[H]**
MAGE mean (min-max)[c]	**2.6 (2.2-2.8)[E,H]**	**2.6 (2.1-3.2)[E,H]**	**3.1 (2.3-4.3)[G,H]**	**4.1 (3.2-5.7)**	**4.8 (3.3-6.2)**
HBGI mean (min-max)[C]	**0.46(0.19-0.69) [G,H]**	**0.24 (0.12-0.39) [E,F]**	**0.47 (0.12-1.0) [G,H]**	**1.3 (0.62-2.7)**	**2.2 (0.41-5.9)**
Day mean (SD)[B]	**5.73 (0.34)[G]**	6.47 (0.69)	5.88 (0.44)	**6.39 (0.46)**	7.23 (1.17)
Day range mean (min-max)[B]	**0.88 (0.66-1.12)[E,F]**	**0.95 (0.80-1.11)[E,F]**	**1.12 (0.91-1.46)[E]**	**1.31 (0.89-2.00)**	**1.87 (1.25-2.58)**
Night mean (SD)[A]	**5.75 (0.38)[E]**	6.44 (0.40)	**5.98 (0.59)[E]**	6.57 (0.74)	**7.30 (0.76)**
Night range (min-max)[B]	**0.69 (0.51-0.86)[E,F]**	**0.70 (0.54-0.86)[E]**	**0.79 (0.36-1.11)[E]**	**0.99 (0.73-1.45)[E]**	**1.62 (0.99-2.21)**
CGM estimated HbA1c (%) mean (SD)[A]	**5.28 (0.24)[E]**	**5.62 (0.23)[E]**	**5.31 (0.32)[E]**	**5.68 (0.35)[E]**	**6.37 (0.50)**
CGM estimated HbA1c (mmol/mol) mean (SD)[A]	**34.38 (2.88)[E]**	**38.20 (2.28)[E]**	**34.50 (3.66)[E]**	**38.67 (3.67)[E]**	**46.00 (5.44)**
Measured HbA1c mean (SD)[A]	**36.00 (2.94)[E]**	**35.00 (2.83)[E]**	**32.10 (1.73)[E]**	**36.00 (3.16)[E]**	**46.00 (4.69)**
Time (%) of glucose <3,0 mmol/l median (95% CI)[C]	0.00 (0.00-0.36)	0.00 (0.00-0.00)	0.10 (0.03-0.29)	0.0 (0.00-0.69)	0.10 (0.03-0.35)

<3,9mmol/l median (95% CI)[D]	0.33 (0.00-3.17)	0.15 (0.01-0,58)	0.85 (0.53-2.84)	0.40 (0.01-2.09)	1.10 (0.49-2.93)
3,9-7,8 mmol/l mean (SD)[B]	**93.95 (2.66)[E]**	**91.48 (3.22)[E]**	**90.89 (4.87)[E]**	83.84 (10.18)	**67.76 (13.44)**
>7,8 mmol/l median (95% CI)[D]	**3.95 (2.21-6.19)[E,F]**	**8.35 (4.82-11.94)[E]**	**5.95 (3.60-10.27)[E]**	**19.14 (7.63-21.33)[E]**	**44,48 (17.02-43.15)**
>11,0 mmol/l median (95% CI)[C]	**0.0 (0.00-0.28)[E,F]**	**0.20 (0.04-0.43)[E]**	**0.30 (0.09-0.81)[E]**	**1.30 (0.62-2.96)**	**7.55 (2.13-13.45)**
>13,9 mmol/l median (95% CI)[C]	**0 (0.0-0.21)[E]**	**0.00 (0.00)[E]**	**0.00 (0.0-0.05)[E]**	0.30 (0.13-0.51)	**1.65 (0.24-4.16)**

Table 2. 10-day CGM variables. Difference between groups tested using [A]ANOVA and Tukeys test, [B]Welch Anova and Dunnett´s T3 test, or [C]Nonparametric Kruskal-Wallis and Dunn´s test. Superscripts indicate statistically significant differences to [E and G]stage 3 (E $p<0.001$ and G $p<0.05$), [F and H] stage 2 (F $p<0.001$ and H $p<0.05$). CV% indicates coefficient of variation in percentage, MAGE, mean amplitude of glucose excursions, and HBGI, high blood glucose index.

FIGURE LEGENDS

Figure 1. Study design and demographics of the study participants. Study design and classification of the subjects into the different groups and stages of type 1 diabetes. CGM was started before the OGTT performed in the laboratory and at home and then compared to the staging (Stage 1-3) done at baseline visit based on islet autoantibodies (IAb) and laboratory OGTT.

Figure 2. Comparison of venous plasma and sensor glucose values during OGTT performed in the laboratory or at home. Venous plasma (P-gluc, black circle) and sensor glucose (sensor gluc, white square) curves and values during OGTT performed in the laboratory (lab-OGTT, panels A, B and D) or at home (home-OGTT, panel C). **A)** Shows six and **B)** all 25 sensor glucose values. **C)** Shows six sensor glucose values from the laboratory test (black circle) and home-OGTT (white square). **D**) Pearson correlation of the sensor and plasma glucose values at the 60-minute time point during the OGTT (Other time points shown in Supplemental figure 1). Only subjects with data from both CGM and plasma glucose during a laboratory OGTT are included in all figures. N = Number of individuals per group, r = Pearson correlation coefficient, symbols indicate means and whiskers show SD. Dotted lines show the 2h decision threshold for diagnosis of type 1 diabetes (11.1 mmol/l) and normal (7.8 mmol/l) 2h OGTT limit.

Figure 3. Sensor and venous plasma glucose variation in individuals with and without islet autoantibodies (1 and 0 IAb), and at stages 1-3 of type 1 diabetes. Mean (SD) curves of sensor (white square) and venous plasma (black circle) glucose during OGTT. N= Number of individuals, *P<0.05 between plasma and sensor glucose values using unpaired t-test (two-tailed). Dotted lines show the 2 h OGTT limit for the diagnosis of diabetes (11.1 mmol/l) and for normoglycemia (7.8 mmol/l).

Figure 4. CGM variability in individuals with and without islet autoantibodies (1 and 0 IAb) and at stages 1-3 of type 1 diabetes. A) Violin plot of 10-day sensoring period showing the distribution of glucose values, the bolded line indicates mean and dotted line SD. **B)** A scatter plot of time (%) of glucose values over 7.8 mmol/l with mean and SD. Dotted line indicates an 18% cut-off level, previously shown to predict the progression to clinical diabetes in high-risk children (11).

Added notes:

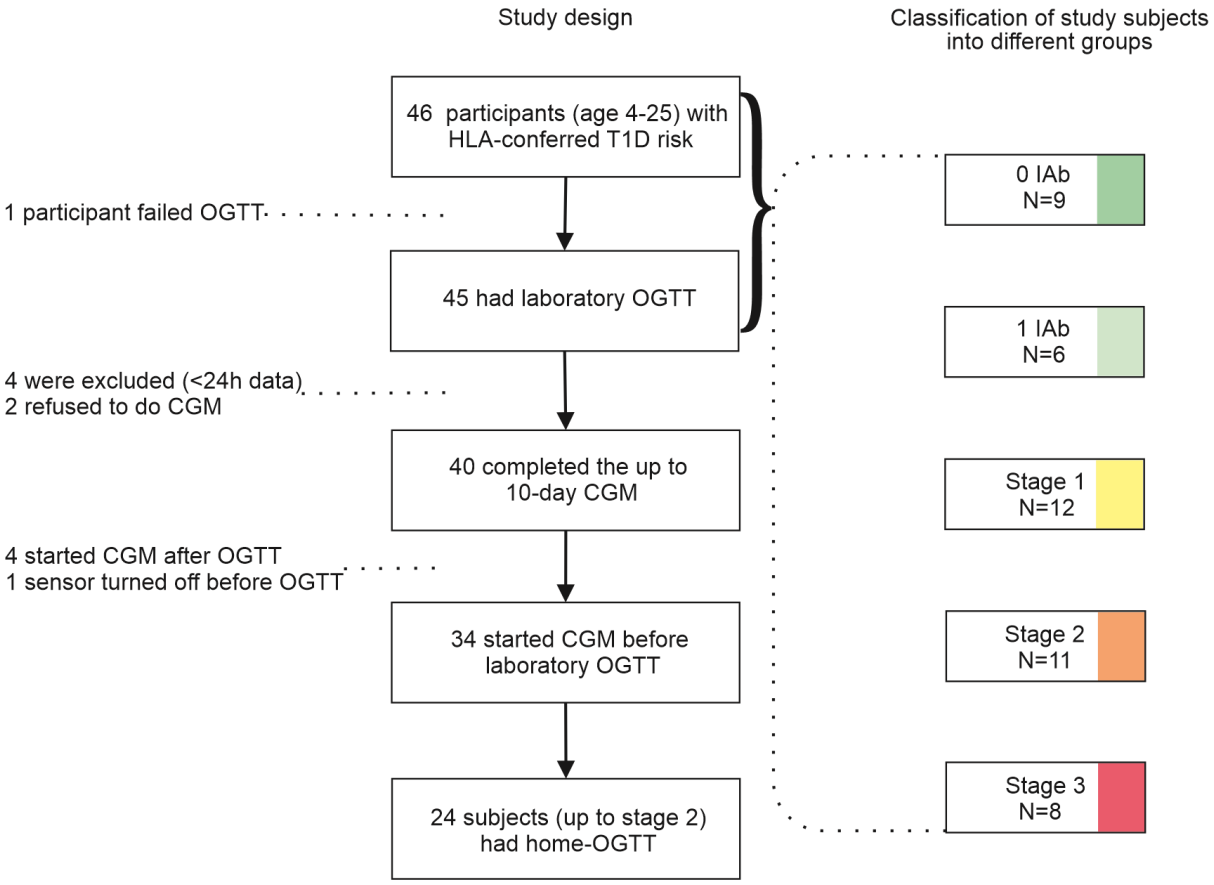
Re Supplementary Figures and tables (call these S1, S2 S3 etc.)

In addition to the violin ;plots for mean glucoe, as show them for several other CGM metrics including SD IQR %CV, average ADRR, LBGI, HBGI, BGRI for each of the 5 categories of subjects.

In the second figure showing %time with glucose > 7.8 mmol/L: the yellow color for the symbols is almost impossible to identify. Need another color or show the data points with borders identified with black borders. (for all groups).

Suppl. Table S5: explain all abbreviations in much greater detail. Very difficult to understand what is being shown. I finally figured it out. Need some accompanying text to make it more readily understandable.

The differences between plasma glucose and CGM glucose for all six time points and for all five groups are small. It is good to show these data relating to accuracy and comparability but make sure that it is adequately explained. Also, recall that some of this might be due to lags, and are likely to be larger when glucose is rapidly rising or falling. Actually I still do not understand the entries in the last 2 rows of this table What does AUC/d mean in the last row? I suggest that you have two or three of your colleagues read this table to be sure that it is understandable. Not all explanations need to be crammed into the table – there can be explanatory text included as well.



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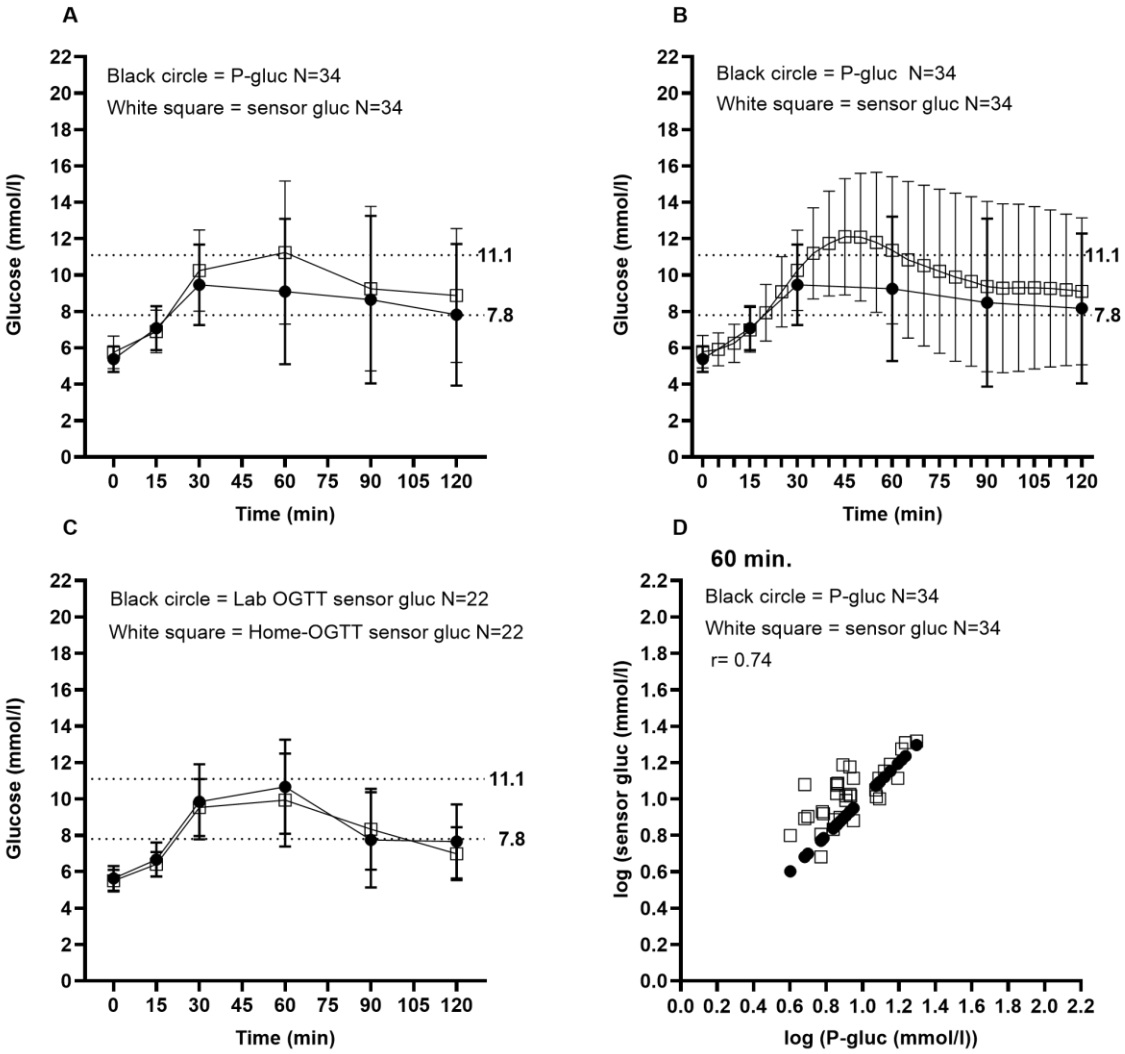
686 Figure1.



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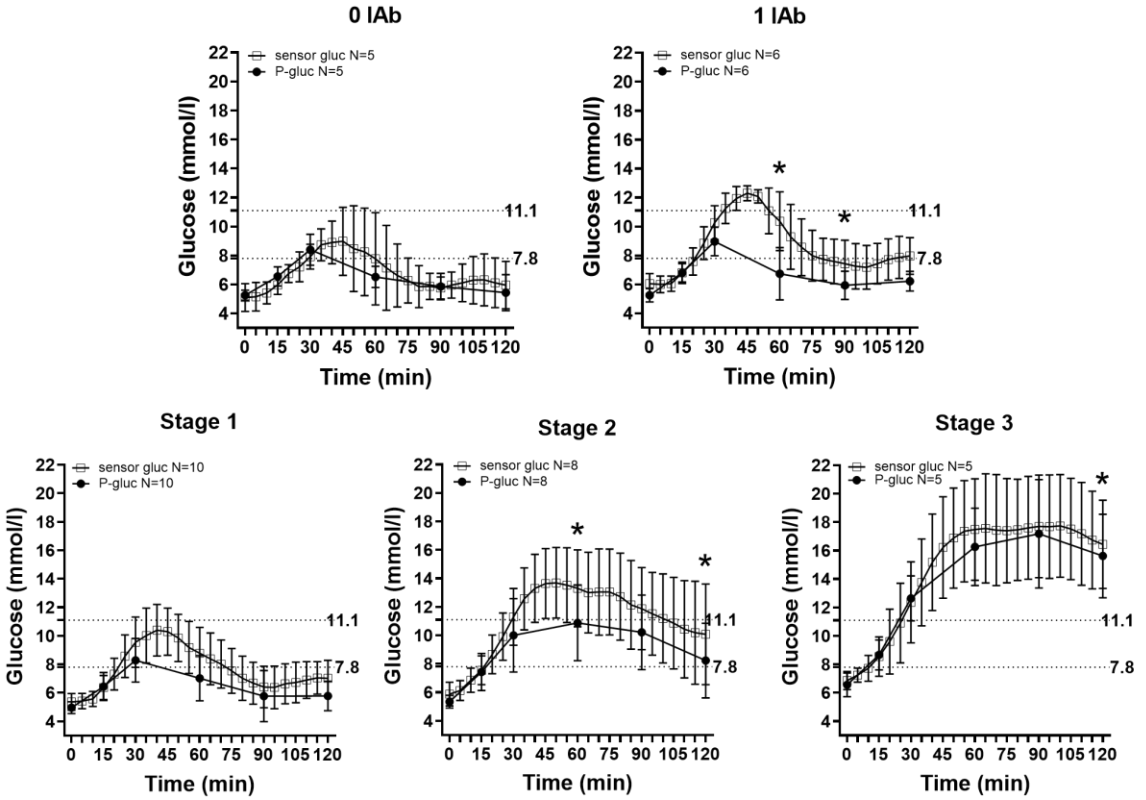
688 Figure 2.



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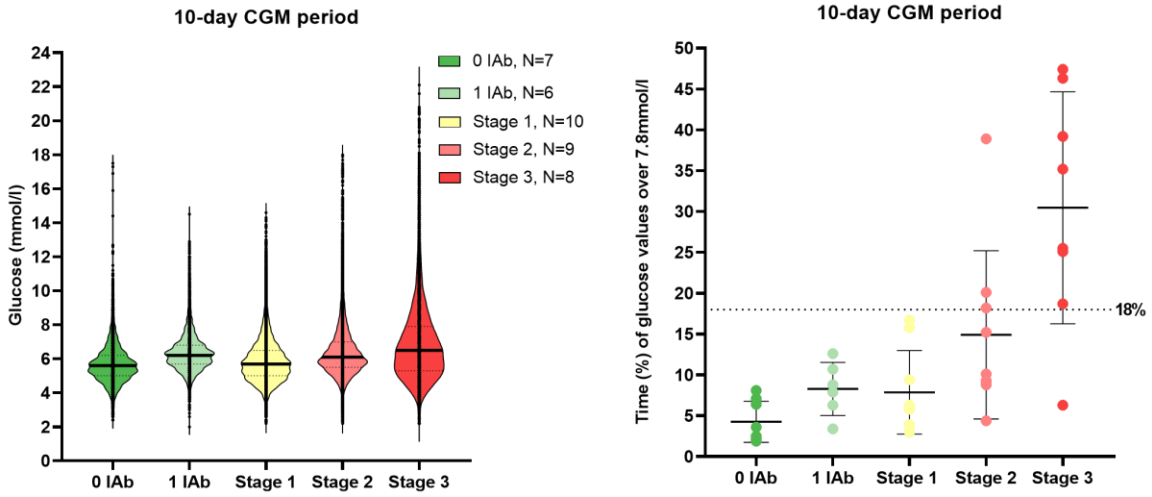
690 Figure 3.



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692 Figure 4.