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Comparison of Mean Plasma Glucose Measured using Self-Monitoring and Continuous Glucose Monitoring – a Simulation-Based Study.

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Abstract

Introduction: Patients with type 2 diabetes mellitus (T2DM) are likely to experience short-term oscillations in plasma glucose (PG) and the mean plasma glucose (MPG) can provide an average of these events. For this reason, the MPG is the biomarker for this study. The methods used to measure MPG are the seven-point self-monitoring of blood glucose (SMBG) which is sampled 7 times daily and the continuous glucose monitoring (CGM) which uses a device to sample every 5 minutes. The aim of this study is to compare the calculations of MPG using both CGM and SMBG to the ‘true' MPG and to also assess the impact that different dietary scenarios have on these methods ability to calculate the MPG.

Methods: 500 individuals with T2DM were simulated using the integrated glucose insulin (IGI) model. The area under the curve (AUC) was calculated for each method CGM, SMBG 15.5 and SMBG 24. The MPG calculations for each method is the AUC divided by the time duration. In this analysis the IGI model was used as it is fitted to both insulin and glucose observations and was used to calculate the ‘true' MPG. The different dietary scenarios were simulated (using the IGI model), these included the standardised, intermittent fasting and increased glucose load.

Results: The calculations of MPG conducted using the CGM method appeared to highly correlated to the ‘true' MPG and was unaffected by the dietary scenarios. The calculations of MPG using SMBG 15.5 illustrated an overestimation in calculations of MPG. The calculations of MPG using the SMBG 24 method was further investigated and appeared to have some sensitivity to the absence of snacks. The SMBG 24 calculations for Scenario 6 (additional late-night snack) appeared to be lower in MPG compared to the early, late, and standard scenarios. The reason for this result could be due to the incretin effect, where the glucose levels are lowered by an increased insulin response.

Conclusion: The calculations of MPG using the CGM method is the most reliable as it appears to be closely aligned to the ‘true' MPG and the calculations are not influenced by the different dietary scenarios. The calculations of MPG using SMBG 15.5 in majority of cases overestimated the calculations of MPG appeared to be overestimating. The calculations of MPG for SMBG 24 appeared to be influenced by the scenarios with the absence of snacks.

Keywords: Type 2 diabetes mellitus (T2DM) , mean plasma glucose (MPG), continuous glucose monitoring (CGM) , self-monitoring of blood glucose (SMBG)

Popular Science Summary

Modelling approaches and techniques in the pharmaceutical industry have improved its relevance to contribute to the drug development processes throughout the past decade. A systematic view of modelling and simulations can aid to identify and examine the potential risks for a specific drug and can also permit an investigation of the disease type in order to provide more information later on in the stages of drug development [1].

The pharmacometrics field is a branch within drug development which utilises computational modelling and simulation to clinical pharmacology. It is also defined as the science of mathematical models of biological, physiological, pharmacological systems used to explain and quantify interactions between the drug and the patient and overall allows a comprehension of the disease [2].

In this study, the disease investigated was diabetes mellitus (DM) with the patient population being those who suffer from type 2 diabetes mellitus (T2DM), as this is the most common type. The study assesses the mean plasma glucose (MPG) as the biomarker. A biomarker can be defined as a specific feature that is measured as an indicator of typical biological processes or a response to an exposure [3]. In this study, the MPG is assessed as a biomarker as patients with T2DM are highly likely to experience short-term oscillations in plasma glucose (PG) and the MPG can provide an average of these events [5]. The methods that are typically used to measure the PG in patients are the continuous glucose monitoring (CGM) which is done via a device attached to the upper arm and samples PG every 5 minutes, and the self-monitoring of blood glucose (SMBG) which is done by patients sampling PG via finger pricks (7 times daily) [6]. In this analysis these two methods were used to compare the calculations of MPG (through their sampling of PG) to the ‘true’ MPG which was calculated by the integrated glucose insulin (IGI) model. The IGI model was used throughout this study as it is fitted to both glucose and insulin observations and was therefore used to calculate the ‘true’ MPG and was also used to simulate the patient’s different dietary scenarios.

The purpose of this study was to compare the calculations of MPG done using CGM and SMBG to the ‘true’ MPG and also examined the influence that different dietary scenarios could potentially have on the ability to calculate the MPG from use of both methods.

1. Introduction

Diabetes mellitus (DM) is considered as chronic hyperglycaemia when the reduced metabolism is triggered by a deficiency in insulin secretion, as well as in insulin action, or both [4]. Non-insulin-dependent, type 2 diabetes mellitus (T2DM) is the most common type with approximately 90% of patients with diabetes suffering from this type [4]. It is also recognised that patients with T2DM have increased risk of developing micro- and macro-vascular diabetic complications. Although, reliable evidence supports helpful effects of long-term exercise interventions on glycated haemoglobin A1c (HbA1c) in patients with T2DM. The measurement of HbA1c mirrors the mean plasma glucose (MPG) level and is presently the primary target in the clinical management of hyperglycaemia in T2DM [5]. Patients with T2DM are highly likely to experience short-term oscillations in plasma glucose (PG) throughout the day defined as glycaemic fluctuations, and the MPG can provide an average of these events. As increased MPG levels are highly linked with micro-vascular and macro-vascular diabetic complications [5].

There are two main approaches to measure the MPG, these are the self-monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM) [6]. The SMBG approach is done by measuring the blood glucose with finger pricks 7 times daily – before and after every meal (6 times in total) and once before going to bed. The CGM approach is done by collecting recordings of glucose levels every 5 minutes during the day with the device attached to the upper arm [6].

The SMBG approach is considered a standard method of glucose measurement, since it is easy to perform, reliable, and does not require an additional device, and calibration period [6]. The main disadvantage of this approach is that only the glucose measurements at specific time points are recorded, and the PG fluctuations that could occur between measurements might be missed. The evidence from type 1 diabetes mellitus (T1DM) studies suggests that the CGM approach is superior to the conventional method [7] since it detects all the PG fluctuations throughout the day. Therefore, the comparison of the two approaches is required to draw any conclusion on the appropriate method to measure the MPG for patients with T2DM.

The measure of MPG as a biomarker will allow the capability to study the disease mechanisms and facilitate an assessment of therapeutic measures by delivering surrogate endpoints for intervention studies [8]. It is also important to recognise the role of MPG as it pertains to the glycated haemoglobin (HbA1c), MPG is highly correlated with HbA1c, however without the time-delay that HbA1c undergoes. Therefore, due to this it could possibly be an alternative surrogate endpoint as it may allow shorter study durations compared to HbA1c [8].

Model-based techniques provide the ability to predict clinical efficacy from data for drug development processes [9]. In this analysis the integrated glucose insulin (IGI) model was used as it is simultaneously fitted to both insulin and glucose observations, and this is done by including the control mechanisms between both entities. The purpose of this was to develop a model with reasonable predictive properties that can allow drug development for clinical optimisation and assessments from a mechanistic perspective [10].

The evaluation of MPG calculations using both SMBG and CGM compared with "true" MPG calculated by the IGI model will permit a further comprehension of the impact of glycaemic fluctuations and will give an insight on how to potentially prevent such variabilities, as it is crucial for reducing the risk to develop comorbidities in patients with T2DM [11].

The hypothesis for this analysis is that the MPG calculated using CGM will be closely correlated to the "true" MPG compared to the MPG calculated using SMBG. The AUC will also be underestimated in the case of the SMBG method as it does not capture a broader overview of the PG profile in comparison to the CGM method.

2. Aim

This study aims to assess the comparison of the calculation of the observable MPG from SMBG and CGM to the “true” MPG values, and to investigate the impact of different dietary scenarios have on these methods in calculating MPG.

3. Material and methods

3.1 The Data

500 individuals with T2DM were simulated using the IGI model approach [10]. Ethical considerations have not been observed for this study as it is not relevant. The IGI model was used to simulate the datasets as this model sufficiently describes the insulin and glucose profiles [10]. The data was simulated over a 4-day period.

3.2 Data Analysis and Calculations

The output dataset generated by the model was used to calculate the MPG for each method (CGM and SMBG) which is calculated through the area under the curve (AUC) over a time duration. The model also calculated the 'true' MPG.

Day 1 was not included in calculations of the AUC as the patient's PG had not yet reached steady state. The MPG was calculated for days 2, 3 and 4 where the MPG was compared between the days and the average was taken between days 3 and 4 as the differences between these days were small in comparison to day 2 which showed to have a slight deviation from days 3 and 4.

Equation 1 is the trapezoidal rule which operates on approximating the AUC of the function *f(x)* as a trapezoid, where $\Delta x = \frac{b-a}{n}$ is the width (x-axis) such that a = $x_0 < x_1 < x_2 ... < x_n$ = b and the height is *f(x)* (y-axis) [11].

Equation 2 is the calculation of the delta of each method to the 'true' MPG values, which is the average MPG that was calculated for days 3 and 4 then according to the method for CGM and SMBG 24 was divided by 24 hours whereas for SMBG 15.5 was divided by 15.5 hours.

$$\int_a^b f(x)dx \approx Tn\frac{\Delta x}{2}[f(x_0) + 2f(fx_1) + 2f(x_2) + \cdots + 2f(x_{n-1}) + f(n)] \quad (1)$$

$$Delta = \frac{MPG\ for\ each\ method\ -'true'model\ calculated\ MPG}{('true'model\ calculated\ MPG)} \quad (2)$$

The AUC calculations were done using the trapezoidal rule (equation 1). For the CGM AUC calculations the PG was sampled every 5 minutes using a device attached to the patient. Whereas for the SMBG calculations the PG was sampled 7 points throughout the day, 5 minutes before a meal, 2 hours after a meal and once before bed. The AUC calculations for the SMBG method were conducted in two ways, one with the time duration of 15.5 hours which is the time from the first morning sample (5 minutes before breakfast) to the last (5 minutes before bed) and the other being the time duration of 24 hours re-using the first morning sample in order to account for the overnight.

The comparison between the methods was also determined by evaluating the proximity of the calculated MPG to 'true' MPG using equation 2. The statistical analysis for each method and scenario was performed, primarily the unpaired t-test which compared the averages of two independent groups to determine the difference between them. The p-value is a description of how probable it is to find a distinct set of observations if the null hypothesis were true, where the null hypothesis is that the difference in group means is zero [12]. If the p value is greater than 0.05 then this indicates that the null hypothesis is false as there is a difference between the groups [13].

3.3 Scenarios

The scenarios consisted of three different dietary scenarios which were standardised, intermittent fasting and increased glucose load. These different scenarios allow an understanding of the effect different diets have on calculating MPG. This investigated the change in meal and snack size, the change of the meal and snack times and the absence of snacks. This varied dependent on the scenario, for an example with the intermittent fasting, the meals and snacks were consumed within an 8-hour window and a fasting period of 16 hours.

3.3.1 Standardised Scenarios

Table 1 Summary of the Standardised Scenarios

Scenario	No. of Meals (mg)	No. of Snacks (mg)	Meal Size (mg)	Snack Size (mg)	Meal schedule	Snack schedule	Description
Standard	3	3	62500	12500	8:30,14:30,20:30	11:30,17:30,23:30	All meals and snacks
Early	3	3	62500	12500	6:00,12:00,18:00	09:00,15:00,21:00	Last sample 21:55 (for 22:00)
Late	3	3	62500	12500	9:00,15:00,21:00	12:00,18:00,23:30	Last sample 00:55 (for 01:00)
S1	3	2	62500	12500	8:30,14:30,20:30	11:30,17:30	Removal of last snack
S2	3	2	62500	12500	8:30,14:30,20:30	11:30,17:30	last snack added to meal (75000)
S3	3	0	62500	-	8:30,14:30,20:30	-	Removing all snacks
S4	3	0	75000	-	8:30,14:30,20:30	-	Adding snacks to meals
S5	3	3	62500	12500	7:00,13:00,19:00	10:00,16:00,22:00	Earlier meals and snacks
S6	3	4	62500	12500	8:30,14:30,20:30	11:30.17:30,23:30, 03:00	One added late-night snack

The standardised scenarios consisted of three meals and three snacks. Firstly, a standard scenario was simulated for comparative purposes followed by scenarios as shown in table 1 which explored; the removal of the last snack, adding the last snack to the last meal, removing all snacks, adding all snacks to the meals, earlier meals and snacks, and adding a late-night snack.

3.3.2 Intermittent Fasting

Table 2 Summary of the Intermittent Fasting Scenarios

Scenario	No. of Meals (mg)	No. of Snacks (mg)	Meal Size (mg)	Snack Size (mg)	Meal schedule	Snack schedule	Description
Standard	3	2	62500	12500	8:30,12:30,16:30	10:30,14:30	Standard 3 meals and 2 snacks
S1	3	1	62500	12500	8:30,12:30,16:30	10:30	Removing last snack
S2	3	1	62500	12500	8:30,12:30,16:30	10:30	Adding last snack to last meal (75000)
S3	3	0	62500	-	8:30,12:30,16:30	-	Removing all snacks
S4	3	0	75000	-	8:30,12:30,16:30	-	Adding snacks to the meals
S5	3	2	62500	12500	7:00,11:00,15:00	9:00,13:00	Earlier snacks and meals

The scenarios for the intermittent fasting followed a similar pattern as the standardised scenarios instead with a standard scenario of three meals and two snacks as the last snack could not fit the narrow 8- hour window, and the subsequent scenarios as displayed in table 2 with the adding and removal of snacks and change in meals and snacks times.

3.3.3 Increased Glucose Load

Table 3 Summary of the increased glucose load Scenarios

Scenario	No. of Meals (mg)	No. of Snacks (mg)	Meal Size (mg)	Snack Size (mg)	Meal schedule	Snack schedule	Description
Standard	3	3	62500	12500	8:30,14:30,20:30	11:30,17:30,23:30	All meals and snacks
S1	3	3	75000	42200	8:30,14:30,20:30	11:30,17:30,23:30	Increased the meal size
S2	3	3	75000	54700	8:30,14:30,20:30	11:30,17:30,23:30	Increased meal and snack size
S3	3	2	75000	12500	8:30,14:30,20:30	11:30,17:30	Removing the last snack
S4	3	3	87500	42200	8:30,14:30,20:30	11:30,17:30	Increased meal size
S5	3	3	87500	54700	8:30,14:30,20:30	11:30,17:30,23:30	Increased meal and snack size
S6	3	0	87500	54700	8:30,14:30,20:30	11:30,17:30	Removing the last snack
S7	3	-	87500	-	8:30,14:30,20:30	-	Removing all snacks
S8	3	2	75000	42200	8:30,14:30,20:30	11:30,17:30	Removing the last snack
S9	3	2	87500	42200	8:30,14:30,20:30	11:30,17:30	Removing the last snack
S10	3	-	75000	-	8:30,14:30,20:30	-	Removing all snacks

The increased glucose load includes increased meals and snacks sizes where the meals are increased by 12500mg and the snacks 42200mg is the snack size of a can of Fanta which is ~ 32g per 250 mL serving assuming a can of Fanta is 330mL (0.32 x 0.33) / 0.25 ~ 42200mg [11], the size increase is by 12500mg to 54700mg.

3.4 Software

R Studio software version 4.0 was used to produce the input dataset [14]. To generate an output dataset using Nonlinear Mixed Effects Modelling (NONMEM), version 7.5.0 [15] with Pearl-speaks-NONMEM (PsN, version 5.2.0) [16] was used. The data analysis was also performed in R.

4. Results

The main findings of the comparison of the calculation of the MPG from CGM and SMBG to the "true" MPG values are summarised in this section. This also included an examination of the effect that different dietary scenarios have on these calculations of the MPG for both CGM and SMBG. The data visualisation provided a comprehension of the data and allowed a strategic plan on how to proceed with the calculations and data analysis. The findings of the different scenarios depicted a variation in the delta (between each method to the 'true' model) between the methods (CGM, SMBG 15.5 and SMBG 24).

4.1 Visualising the Data

The data of the standard scenario of 3 meals and 3 snacks with meal and snacks sizes of 62500mg and 12500mg respectively, was analysed. The 'true' MPG was calculated by the model and was plotted in a boxplot and violin plot to assess the data and to determine how to conduct the calculations and data analysis. Then the PG and HbA1c was investigated with the calculations of the AUC over the time duration for each method (for CGM, SMBG 15.5 and SMBG 24) and this was visualised using boxplots of the deltas for each method.

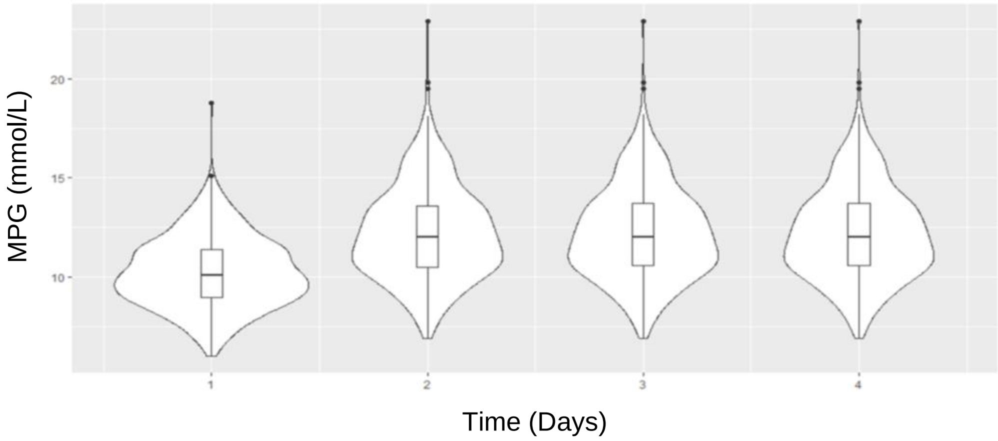


Figure 1.0: Boxplot and violin plot of the 'true' MPG plotted against the duration of the study (4 days).

This illustrates the 'true' MPG calculated by the model over the study duration of 4 days, where day 1 appears to not reach steady state. Day 1 was removed from further analysis for this reason.

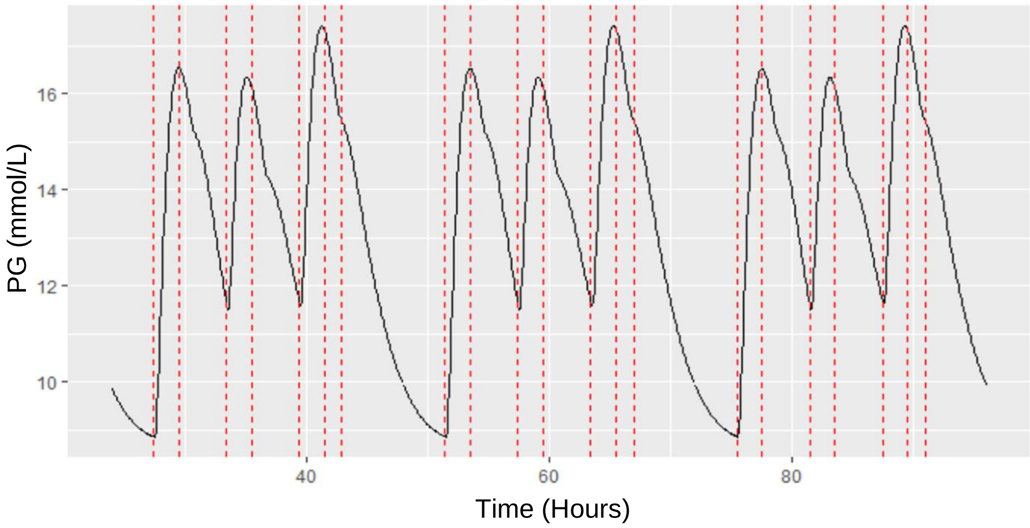


Figure 1.1: PG vs Time plot for days 2, 3 and 4. The red dashed vertical lines represent the SMBG sampling times for patient with ID 1.

This displays the PG profile for the remaining days of the study, along with the sampling times for SMBG which were sampled 5 minutes before every meal, 2 hours after every meal and 5 minutes before bed. This figure provides an understanding of the PG events that is captured by using the SMBG method.

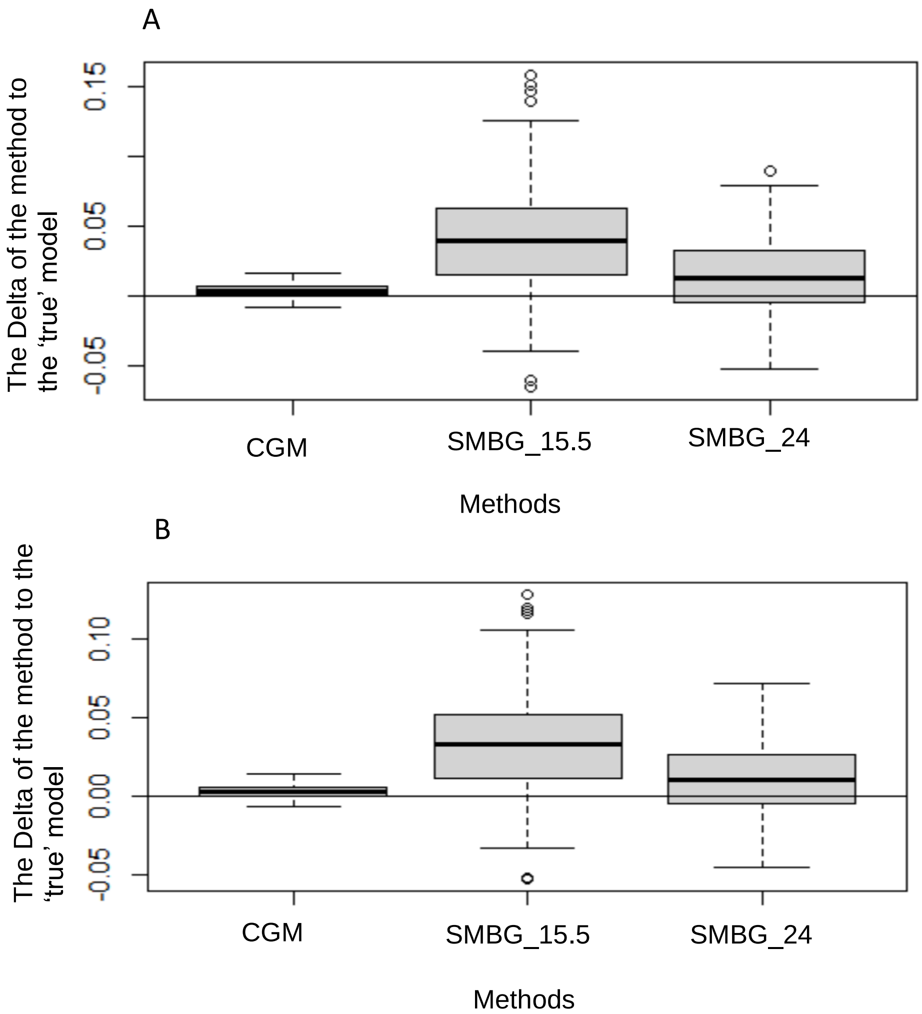


Figure 1.2: (A) is a plot of the delta of the methods to the 'true' model assessing MPG, (B) is the delta of the methods to the 'true' model assessing HbA1c

Both the MPG and HbA1c exhibit very similar results as presented in figure 1.3, which is to be expected as MPG is highly correlated to MPG. Therefore, the analysis continued with just the assessment of MPG.

4.2 Scenarios

The scenarios were evaluated with boxplots of the delta of each method to the ‘true’ MPG for all sets of scenarios. This allowed an assessment of the performance of each method and the influence various scenarios have on these methods.

As seen in the appendix 1 (figure A1), for a vast majority of the scenarios the calculations of MPG using the CGM method consistently remains highly correlated to the ‘true’ MPG, the narrow boxplot depicts that the method allows relatively good calculations of the MPG on an individual level and as a population. The calculations of MPG using SMBG 15.5 however seems to be overestimating the MPG calculations. The calculations of MPG using SMBG 24 in comparison to calculations of MPG using SMBG 15.5 does not show such a large deviation from the ‘true’ MPG and generally is able to calculate the MPG adequately. However, the calculations of MPG using SMBG 24 does appear to vary depending on the scenario as seen in (B) where scenario 2 was the removal of the last snack and adding this to the last meal and (C) where scenario 4 was the removal of all snacks and adding them to the 3 meals, the SMBG 24 boxplot shows a slight deviation from the ‘true’ MPG in comparison to the standard in (A). This suggests that the calculations of MPG using SMBG 24 is sensitive to the snacks in particular the absence of snack(s).

The calculations of MPG using SMBG 24 was further investigated due to its sensitivity with the alterations of the snacks, as seen in appendix 2 (figure A2) displays boxplots of SMBG 24 for each scenario for the standardised, the intermittent fasting and the increased glucose load along with table 1 (as seen in the appendix 4) which shows the results of corresponding unpaired t-test compared with to the standard scenario and the ‘true’ MPG. These findings illustrate a trend with scenario 4 from the intermittent fasting scenarios and the standardised scenarios, when all the snacks are added to the meals (equal to 75000mg). Where a statistical difference is demonstrated for the t-test compared to the standard scenario and with the t-test compared to the ‘true’ MPG, for the standardised scenario both t-tests displayed a difference for scenario 4. However, for the intermittent fasting scenario the statistical difference was only displayed from the result of the t-test compared to the ‘true’ MPG for scenario 4. In the case of the increased glucose load scenario 7 appears to have a statistical difference based on the results of the t-test compared to the ‘true’ model. However, when compared to the standard scenario all the scenarios 1-10 appear to be impacted, illustrating statistical differences.

The results of the standardised scenarios as seen in appendix 3 (figure A3) displays the majority of the scenarios appearing to have a large deviation from the ‘true’ MPG than with the exception of scenario 5. For the intermittent fasting the majority of the scenarios align with the ‘true’ MPG with the exception of scenarios 4 and 5.
The results of the increased glucose show scenarios 3, 6 and 7 appear to have a large deviation to the ‘true’ model, whereas scenarios 2 and 5 appear to have a close correlation to the ‘true’ MPG, and this coincides with the statistical analysis. In the case of scenarios 8, 9 and 10 for the increased glucose load it does not appear to have a large deviation from the ‘true’ model, however scenario 10 appears to have a slight deviation in comparison to scenario 8 and 9.

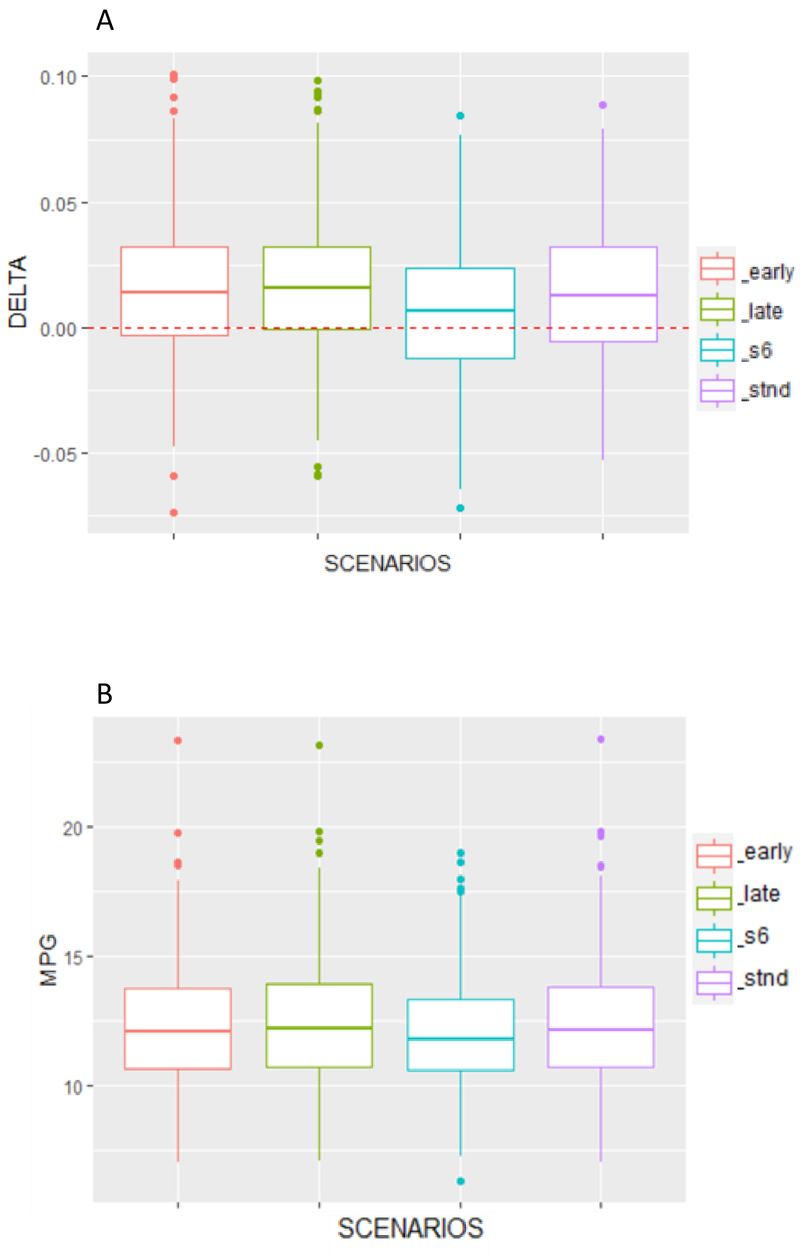
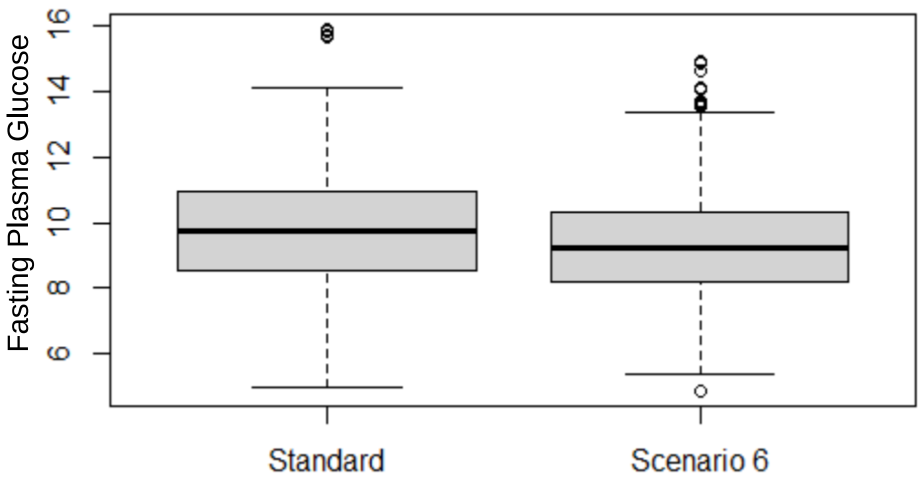


Figure 2.0: (A) is the boxplot of the delta of the methods to the 'true' for the early, late, scenario 6 and the standard. (B) is the boxplot of the MPG for the early, late, scenario 6 and the standard

These extended scenarios from the standardised scenarios appear to not have a significant impact on the calculations of the MPG with the exception of scenario 6. Scenario 6 is the additional late-night snack at, and this displays a statistical difference as shown in the results of the unpaired t-test compared to the standard scenario (table 1 in the appendix 4). This result was further assessed and compared to the standard scenario in order to comprehend this finding.



Standard:

Mean = 9.793649

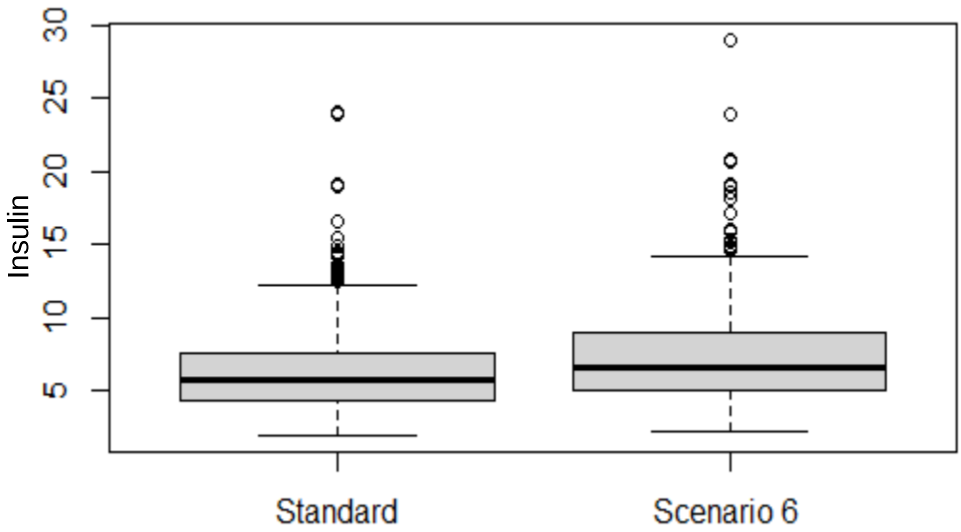
IQR = 2.37355

Scenario 6:

Mean = 9.344359

IQR = 2.082525

Figure 2.1: The boxplot of fasting PG for the standard scenario with 3 meals and 3 snacks of meal size 62500mg and snack size 12500mg and for the scenario 6 with 3 meals of size 62500mg and 4 snacks of 12500mg and the last snack at 03:00. Both scenarios presented with the mean and interquartile range.



Standard:

Mean = 6.364289

IQR =3.226375

Scenario 6:

Mean = 7.326689

IQR = 3.80795

Figure 2.2: The boxplot of fasting insulin for the standard scenario with 3 meals and 3 snacks of meal size 62500mg and snack size 12500mg and for the scenario 6 with 3 meals of size 62500mg and 4 snacks of 12500mg and the last snack at 03:00. Both scenarios presented with the mean and interquartile range.

The results of the fasting plasma glucose (FPG) illustrated in figure 2.1 shows that the standard scenario appears to be higher in FPG in comparison to scenario 6, from the first sample taken 08:25, 5 minutes before the first meal. Whereas the insulin levels appear to be higher in scenario 6 in comparison to the standard scenario from the same first sampling time of 08:25, 5 minutes before the first meal.

5. Discussion

This study provides an assessment of the comparison of MPG calculated using CGM and SMBG to the 'true' MPG, and the impact different scenarios have on this calculation. The hypothesis for this study was that the MPG calculated using the CGM method would be closely correlated to the 'true' MPG values, whereas the MPG calculated using the SMBG method would appear to be underestimated as the method does not capture a broader overview in comparison to CGM.

The results of the data suggest that the CGM method is highly correlated to the 'true' MPG and that the SMBG method calculated in both ways (SMBG 15.5 and SMBG 24) appeared to deviate from the 'true' MPG with the SMBG 15.5 overestimated in all cases. The SMBG 24 appeared to show less of a deviation from the 'true' MPG in comparison to SMBG 15.5, however this method showed some sensitivity to the scenarios that present changes in the snacks in particular adding the snacks to the meals and removing the last snack.

The visualisation of the data allowed an understanding of the standard scenario simulated for the calculations and the data analysis. The calculations and data analysis in this section focused on the comparison of PG and HbA1c which appeared fairly similar, as seen in figure 1.2. This result was as expected as MPG is highly correlated to HbA1c. Therefore, for this reason the analysis of the calculations that involved the assessment of HbA1c was excluded from further investigation.

The data analysis consisted of comparing the calculations of MPG using CGM, SMBG 15.5 and SMBG 24 with the introduction of different dietary scenarios. These findings illustrated that the calculations of MPG using CGM appeared to be closely correlated to the 'true' MPG as the boxplot is narrowly distributed (seen in figure 2.0) which indicates that the method is able to calculate the MPG well on an individual level and as a population, this was the case for the majority of scenarios. This is as predicted as previous studies have concluded that the CGM method is the preferred method as it captures the patient's PG levels every 5 minutes giving a very detailed profile [6]. The calculations of MPG using SMBG 15.5 however, appeared to overestimate the MPG. This method deviated from the 'true' MPG and showed a wide variability in the calculations of MPG and was therefore discounted from further analysis. The calculations of MPG using SMBG 24 in comparison to SMBG 15.5 appeared to be improved in calculations, as it did not deviate as much from the 'true' MPG and the method adequately calculates the MPG.

The calculations of MPG using SMBG 24 was further investigated as the results of the different scenarios displayed a sensitivity to the snacks, this is evident with the example of scenario 4 in the standardised and intermittent fasting (where the snacks were added to the meals) as this showed a statistical difference between the scenario and the standard scenario. Although, the intermittent fasting scenarios generally appeared to be closely correlated to the 'true' MPG as it shows less deviation from the 'true' MPG as compared to standardised and increased glucose load. In the case of increased glucose load scenario 7 which did not include any snacks and had 3 meals of meal sizes of 87500mg, appeared to be statistically different from the other scenarios from the results of the unpaired t-test compared to the 'true'. These results from different dietary scenarios displayed that the calculations of MPG using SMBG 24 are impacted by the absence of snacks.

An interesting outcome was exhibited with scenario 6 which included an additional late-night snack. This result demonstrated statistical difference in the calculations of MPG for scenario 6 compared to the early, late, and standard scenarios, with the unpaired t-test. The reason for this result could be due to the incretin effect which is triggers the insulin response and for this reason PG levels are lowered [19]. This is shown in figures 2.1 and 2.2, where the insulin levels are higher for scenario 6 and the FPG levels are lowered as the increased insulin regulated the glucose in this case. This finding is interesting and could also be debated whether or not this would occur in reality in patients with chronic T2DM, as this might be a potential flaw in the IGI model.

This study provided an in-depth insight into the impact that different dietary scenarios have on the ability to calculate the MPG using both CGM and SMBG methods. However, there were some limitations to the analysis, as the ‘true’ MPG was calculated over the 24-hour period and to draw a comparison of the calculation of MPG using SMBG 15.5 to the ‘true’ MPG cannot be done in this way completely as it is disproportionately measured. Another limitation to consider is with the intermittent fasting scenarios where the maximum of 3 meals and 2 snacks were explored, but a scenario that could have been investigated for an example having 2 large meals and 3 snacks as this is potentially more common in reality for those who partake in this diet. This could not be tested as the calculations of MPG for the SMBG exclusively depend on the 3 meals, in terms of the sampling for this method.

For this study the further consideration can be made for investigating the results of scenario 6 by including a sensitivity analysis, exploring this late-night snack at different times for an example if a patient had a snack at 01:00, 02:00 or 04:00. To examine if this would have an effect on the MPG and would this exhibit the same result as in the case of scenario 6.

6. Conclusion

This study investigated the assessment of the calculations of MPG using the methods CGM, SMBG 15.5 and SMBG 24 and explored the impact different dietary scenarios have on these methods ability to calculate MPG. The calculations of MPG using CGM, is the method that is closely correlated with the 'true' MPG and appeared to be unaffected by the different dietary scenarios. The calculations of MPG using SMBG 24 appeared to have less deviation from the 'true' MPG in comparison to the calculated MPG using SMBG 15.5 which showed to be overestimating. The calculations of MPG using SMBG 24 illustrated a sensitivity to the snacks, this had an effect on the ability to calculate the MPG for this method.

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Appendix

Appendix 1

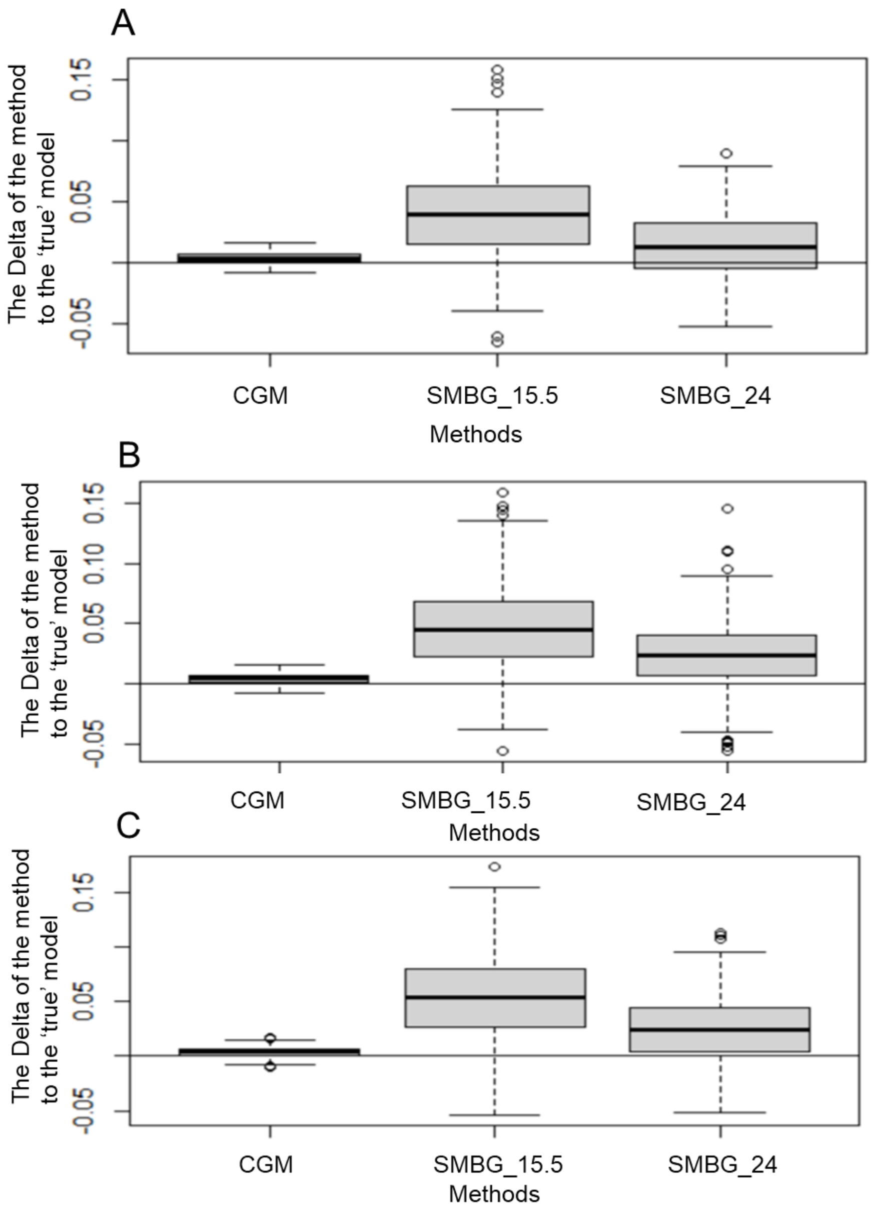

Figure A1: (A) is the boxplot of the delta of the methods for the standard scenario, (B) is the boxplot of the delta of the methods for scenario 2, and (C) is the boxplot of the delta of the methods for scenario 4.

Appendix 2

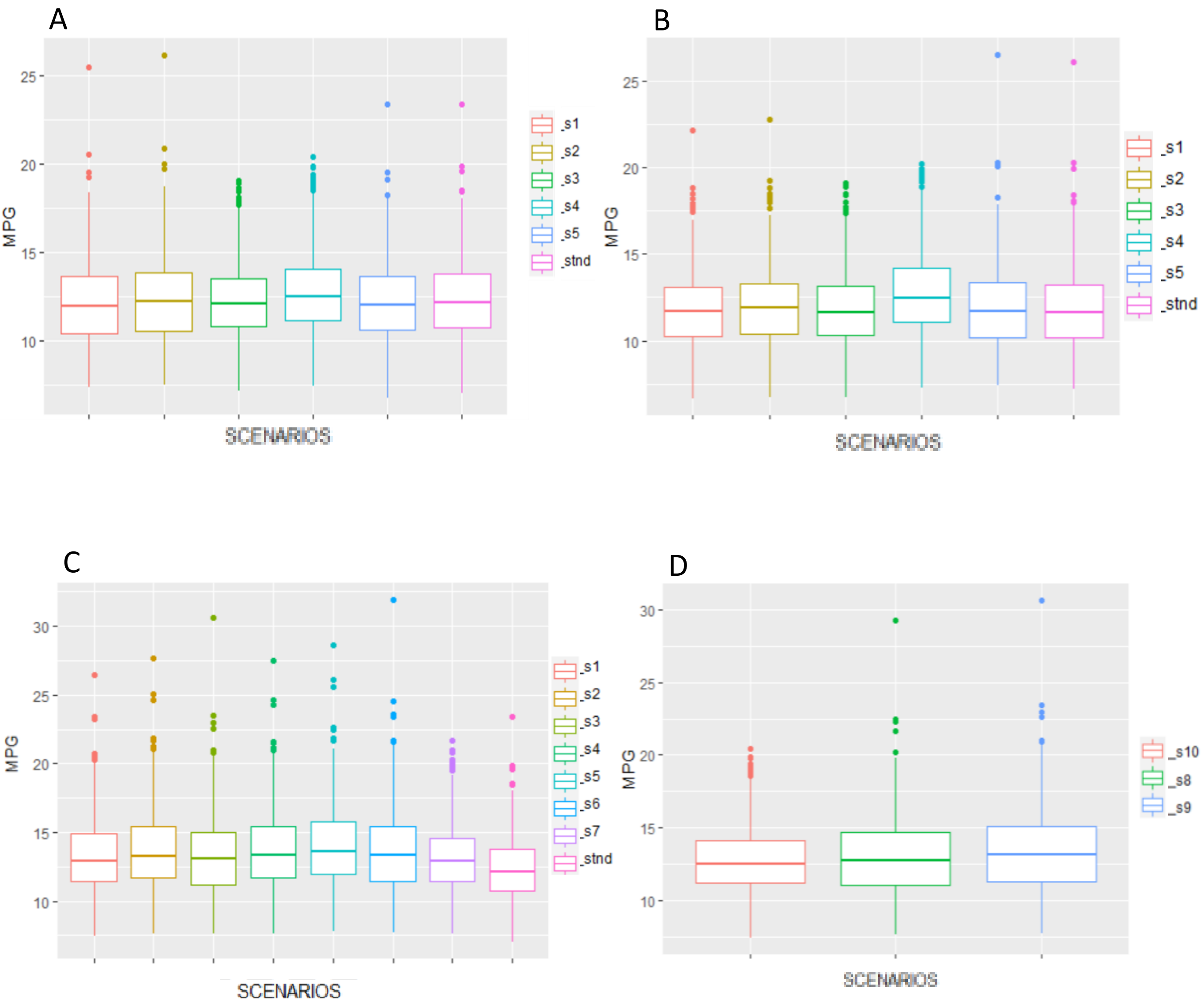

Figure A2: (A) is the boxplot of the MPG of standard scenarios. ***Standardised scenario:*** *S1 = 62500mg, 12500mg removing last snack, S2 = 62500mg, 12500mg adding last snack to meal (75000mg), S3 = 62500mg removing all snacks, S4 = 75000mg adding all snacks to the meals, S5 = 62500mg, 12500mg earlier meals and snacks. (B) is the boxplot of the MPG of the intermittent fasting scenarios.* ***Intermittent Fasting:*** *S1 = 62500mg, 12500mg removing last snack, S2 = 62500mg, 12500mg adding last snack to meal (75000mg), S3 = 62500mg removing all snacks, S4 = 75000mg adding all snacks to the meals, S5 = 62500mg, 12500mg earlier meals and snacks. (C) is the boxplot of the MPG of the increased glucose load.* ***Increased glucose load****: S1 = 75000mg, 42200mg, S2 = 75000mg, 54700mg, S3 = 75000mg, 54700mg, removing last snack, S4 = 87500mg,* *42200mg, S5 = 87500mg, 54700mg, S6 = 87500mg, 54700mg, removing last snack, S7 = 87500mg removing all snacks. (D) is the boxplot of the MPG of the increased glucose load. **Increased glucose load**: S8 = 75000mg, 42200mg, removing last snack, S9 = 87500mg, 42200mg, removing last snack, S10 = 75000mg, removing all snacks.*

Appendix 3

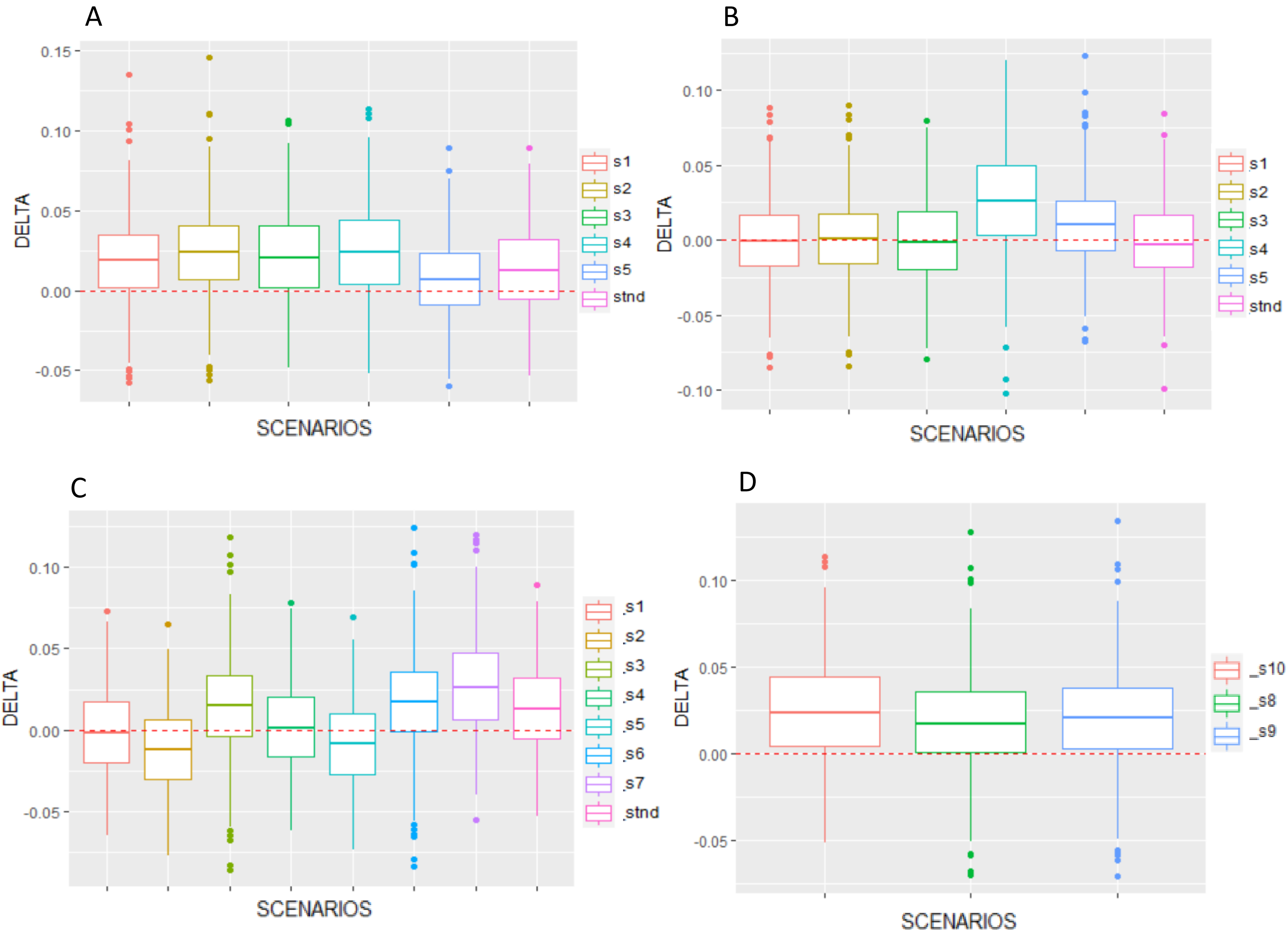

*Figure A3: (A) are the boxplots of the delta of the methods to the 'true' for the **Standardised scenario:** S1 = 62500mg, 12500mg removing last snack, S2 = 62500mg, 12500mg adding last snack to meal (75000mg), S3 = 62500mg removing all snacks, S4 = 75000mg adding all snacks to the meals, S5 = 62500mg, 12500mg earlier meals and snacks. (B) are the boxplots of the delta of the methods to the 'true' for the **intermittent fasting scenarios**: S1 = 62500mg, 12500mg removing last snack, S2 = 62500mg,* *12500mg adding last snack to meal (75000mg), S3 = 62500mg removing all snacks, S4 = 75000mg adding all snacks to the meals, S5 = 62500mg, 12500mg earlier meals and snacks and (C) and (D) are the boxplots of the delta of the methods to the 'true' for* ***the increased glucose load scenarios****: S1 = 75000mg, 42200mg, S2 = 75000mg, 54700mg, S3 = 75000mg, 54700mg, removing last snack, S4 = 87500mg, 42200mg, S5 = 87500mg, 54700mg, S6 = 87500mg, 54700mg, removing last snack, S7 = 87500mg, removing all snacks, S8 = 75000mg, 42200mg, removing last snack, S9 = 87500mg, 42200mg, removing last snack, S10 = 75000mg, removing all snacks.*

Appendix 4

Table 1 Summary of Statistical Analysis of Unpaired t-tests

Scenario	Unpaired t-test ('true' Model-scenario)	Unpaired t-test (Standard-scenarios)
Standardised		
Standard	0.328	-
S1	0.1273	0.1095
S2	0.06016	0.8239
S3	0.08066	0.5336
S4	0.05527	0.01518
S5	0.5949	0.4707
Early	0.2589	0.7506
Late	0.1998	0.621
S6	0.5783	0.02112
Intermittent fasting		
Standard	0.9877	-
S1	0.9948	0.7704
S2	0.9056	0.1732
S3	0.9715	0.6366
S4	9.81e-05	3.855e-10
S5	0.3856	0.7184
Increased glucose load		
Standard		-
S1	0.844	5.456e-09
S2	0.4914	9.326e-15
S3		

S4	0.2398	7.35e-08
S5	0.9688	3.897e-15
S6	0.6597	< 2.2e-16
S7	0.1909	3.221e-13
S8	0.03834	2.754e-07
S9	0.1882	0.0001637
S10	0.1456	3.27e-09
	0.05527	0.01518