

**Cardiff University School of Medicine**

**Continuous Subcutaneous Glucose  
Monitoring (CGM) to Predict Progression  
from Abnormal Glucose Tolerance (Pre-  
diabetes) to Type 2 Diabetes Mellitus**

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MD Thesis

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## Summary

The global incidence of Type 2 Diabetes Mellitus (T2DM) is increasing rapidly. Many people with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) will however not progress to T2DM but appear to spontaneously revert to normal glucose homeostasis, others however will progress slowly and in some cases rapidly progress towards diabetes. Therapeutic interventions will reduce the risk, or at least the pace, of deterioration from IFG and IGT to T2DM. However, in order to target interventions appropriately, to prevent progression in those at greatest risk further information as to which individuals are most likely to progress is needed. There is a variable rate of progression from either IFG, IGT or combined IFG and IGT to T2DM and in general, progression rates are lowest in the general population and highest in target “at-risk” group. Age, body mass index (BMI), fasting and 2 hour plasma glucose concentrations, elevated fasting pro-insulin, low 2-hour insulin and fasting triglyceride levels are known to be associated with a greater risk of progression and in order to maintain normoglycemia, adequate quantitative and qualitative moment-by-moment pancreatic beta-cell secretion and action is essential. A marker of deteriorating carbohydrate homeostasis would be increased fluctuations in blood glucose levels and continuous glucose monitoring (CGM) is an ideal method to look at just this. The use of CGM to quantify the fluctuations was proposed to assess whether CGM can help identify people with abnormal glucose tolerance that progress to T2DM. In this study, CGM profiles inspected by eye for variability appeared to correlate well with mathematically devised CGM parameters based on CGM data, both at baseline and at Year 1. However, neither the subject CGM profiles nor the CGM parameters at baseline were significant in predicting progression to diabetes (T2DM) at Year 1 or Year 3 from a pre diabetic state at baseline. However, when one looked at progression from pre diabetes to diabetes, with regard to CGM profiles and CGM parameters, the interval period between study baseline and Year 1 appeared to be when most variation in glucose levels occurred; this was especially the case for those subjects with IFG, compared to subjects with IGT or IFG+IGT mix, respectively. This effect was diluted at Year 3 and not observed. In conclusion, this study demonstrated that CGM did not predict progression from pre diabetes to diabetes (T2DM), but did however, correlate well by eye with mathematical assessments models of the same CGM data and identify an at risk IFG group that could be targeted at baseline with more intensive therapy.

## Statement

- (1) I declare that, except where indicated by specific reference, the work submitted is the result of my own investigation and the views expressed are my own.

Candidate: Sally Ann Price

Signature:

Date:

- (2) I declare that no portion of the work presented has been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Candidate: Sally Ann Price

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Date:

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## Abbreviations

ACS	Acute Coronary Syndrome
ADA	American Diabetes Association
ANOVA	Analysis of Variance
App	Application
BP	Blood Pressure
BMI	Body Mass Index
CF	Cystic Fibrosis
CF	CF related diabetes
CGMS	Continuous glucose monitoring system
CGM	Continuous glucose monitoring
Chi-sq	Chi-Square
CI	Confidence Interval
DPP	Diabetes Prevention Programme
DoF	Degree of Freedom
DM	Diabetes Mellitus
EVA	Equal Variances Assumed
EVNA	Equal Variances Not Assumed
FHx	Family history
FPG	Fasting plasma glucose
FSIVGTT	Frequently Sampled Intravenous Glucose Tolerance Test
GAD Ab	Glutamic acid decarboxylase antibody
GP	General Practitioner
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
Kg	Kilogram
LADA	Latent autoimmune diabetes of adults
M	Meter
Max	Maximum
Mmol/l	Millimol per litre
Mmol/mol	Millimol per mole
Min	Minimum
Mins	Minutes
mU/L	Milliunits per Litre
N	Number

NGT	Normal glucose tolerance
NHS LREC	NHS Local Research Ethics Committee
NODAT	New onset of diabetes after transplantation
PI	Principle Investigator
Pmol/ml	Pico mol per millilitre
Pmol/L	Pico mol per litre
P-P plots	Probability - Probability plots
<i>r</i>	Pearson's Correlation Coefficient
SMBG	Self monitoring of blood glucose
SG	Sensor glucose
SEWREC	South East Wales Research Ethics Committee
Sig	Significance
SNPs	Single- nucleotide polymorphisms
Std Dev	Standard deviation
Std Err	Standard Error
T2DM	Type 2 diabetes mellitus
T1DM	Type 1 diabetes mellitus
TM	Thalassemia Major
U/ml	Units per millilitre
WHO	World Health Organisation
%	Percentage

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## 1.0 Introduction

The term diabetes mellitus (DM) describes a metabolic disorder with heterogeneous aetiologies which is characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism. It consists of an array of Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7]) (1). Bearing in mind this study was initiated in 2009, the outcome indicator in this study was Type 2 diabetes mellitus (T2DM), which was diagnosed using the criteria set by the World Health Organisation (WHO) (2006) (2). In this document, the WHO published its guidelines on definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. In this guideline document, T2DM is diagnosed when a person is found to have symptoms of diabetes (polyuria, polydipsia and unexplained weight loss for Type 1 Diabetes (T1DM)) plus a fasting plasma glucose concentration of  $\geq 7.0$  mmol/l or a random venous plasma glucose concentration  $\geq 11.1$  mmol/l, as described by Alberti *et al* (1998) (3) or two hour plasma glucose concentration  $\geq 11.1$  mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT). These values are based on evidence which suggests the risk of complications increases significantly amongst people with plasma glucose levels consistently above these levels, as described by Gabir *et al* (2000) (4). Fasting and post-prandial (or random) plasma glucose levels broadly follow a normal distribution in the general population, as described by Lim *et al* (2000) (5) and the pre-diabetes categories of impaired fasting glycaemia (IFG)/ impaired glucose tolerance (IGT) stated below are an attempt to recognise this.

IFG:               fasting glucose  $>6.1$  mmol/l

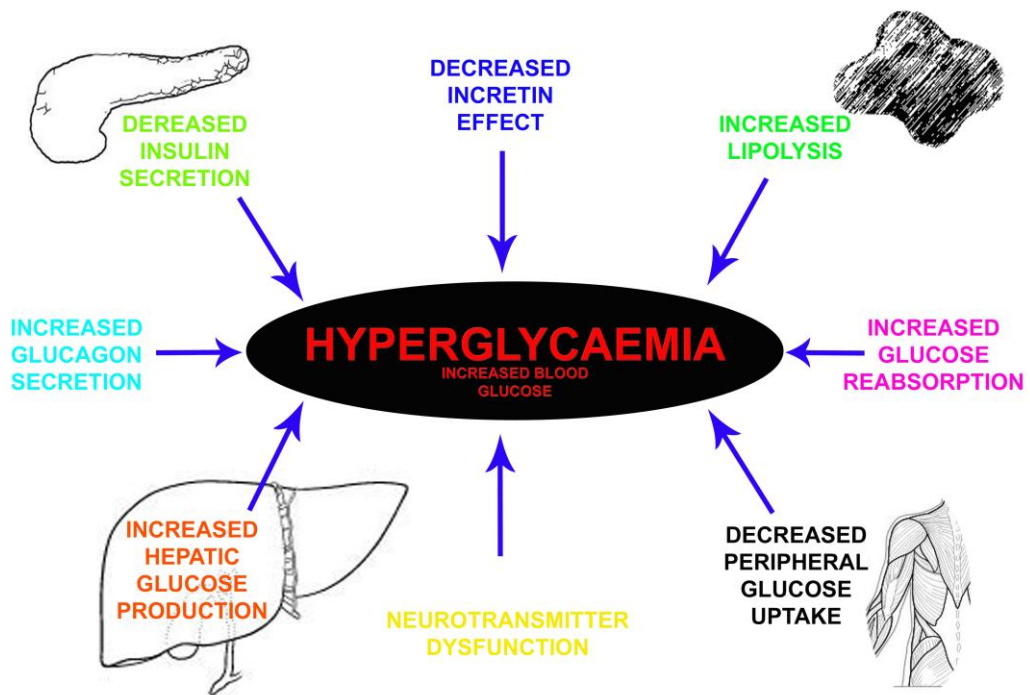
IGT:               fasting glucose  $<7$  mmol/l; 2 hour post-challenge 7.8-11.1 mmol/l

IFG and IGT are not clinical entities in their own right, but rather risk categories for cardiovascular disease and/or future diabetes. IFG has been introduced to classify individuals who have fasting glucose values above the normal range but below those diagnostic of diabetes while IGT is a stage of impaired glucose regulation. IFG and IGT define individuals who may develop progressive deterioration in glucose homeostasis, leading to frank T2DM, as described by Alberti *et al* (1996) (6). Many people with IFG or IGT will not progress to T2DM but appear to spontaneously revert to normal, as

described by Riccardi *et al* (1985) (7). Others may progress only very slowly, whereas in some cases the progression may be rapid, as described by Ferrannini *et al* (2004) (8).

### **1.1 Pathophysiology**

T2DM is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids and pro-inflammatory cytokines in plasma leads to decreased glucose transport into muscle cells, elevated hepatic glucose production and increased fat breakdown. In T2DM, the reciprocal relationship between the glucagon- secreting alpha cell and the insulin- secreting beta cell is lost, resulting in hyperglucagonemia and consequentially hyperglycaemia, as described by Unger and Orchi (2010) (9). Beta cell dysfunction is a major factor across the spectrum of pre-diabetes to diabetes, with beta cell dysfunction happening early in the pathological process and not necessarily following the stage of insulin resistance. In the progression from normal glucose tolerance to abnormal glucose tolerance, postprandial blood glucose levels increase first; eventually, fasting hyperglycemia develops as suppression of hepatic gluconeogenesis fails. During the induction of insulin resistance, such as is seen after high-calorie diet, increased glucagon levels and increased glucose-dependent insulinotropic polypeptide (GIP) levels accompany glucose intolerance; however, postprandial glucagon like peptide-1 (GLP-1) response is unaltered, as described by Hansen *et al* (2011) (10). Genome wide association studies of single- nucleotide polymorphisms (SNPs) have also identified a number of genetic variants that are associated with beta-cell function and insulin resistance. Some of these SNPs have been shown to increase the risk for T2DM, as described by Billings and Florez (2010) (11). As patients with T2DM retain the ability to secrete some endogenous insulin, they are not absolutely dependent upon insulin for life, however many patients with T2DM will ultimately require insulin (Figure 1).



**Figure 1: Pathophysiology of T2DM**

-The pathophysiology of T2DM is illustrated in the above diagram. It is characterised by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells.

## 1.2 Aetiology

T2DM develops when a diabetogenic lifestyle (i.e. excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed upon a susceptible genotype, as described in Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7] (1). When testing for T2DM and pre-diabetes in asymptomatic individuals, T2DM screening should be performed in adults of any age who are overweight or obese, and who have one or more diabetic risk factors. The major risk factors for T2DM are the following, as described in the American Diabetes Association (ADA) Guidelines (2016) (12):

- Age greater than 45 years.
- Physical inactivity.
- Family history of T2DM in a first-degree relative (eg, parent or sibling).
- High risk race or ethnicity: African-American, Latino, Native American, Asian-American, Pacific Islander descent.
- History of previous IGT or IFG.
- Hypertension (>140/90 mm Hg) or on treatment.

- Dyslipidemia (high-density lipoprotein [HDL-C] cholesterol level < 35 mg/dL and / or a triglyceride level > 250 mg/dL).
- History of gestational diabetes mellitus (GDM) or of delivering a baby with a birth weight of >9 lb.
- Conditions associated with insulin resistance i.e polycystic ovarian syndrome (PCOS), a weight greater than 120% of desirable body weight (severe obesity) or acanthosis nigricans.
- A HbA1c  $\geq$ 5.7%.
- A history of cardiovascular disease.

In addition, the body mass index (BMI) at which excess weight increases risk for diabetes varies with different racial groups, as described by Barnes (2015) (13). Hypertension and pre-hypertension are also associated with greater risk of developing diabetes in whites compared with African-Americans, as described by Wei *et al* (2011) (14).

### **1.3 Epidemiology**

The global incidence of T2DM is increasing rapidly. At least 250 million people currently have diabetes and this figure is likely to more than double to 366 million by 2030 unless appropriate action is taken, as described in Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7] (1). The top 10 countries, in numbers of people with diabetes, are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh. It is anticipated that the greatest percentage increase in rates of diabetes will occur in Africa over the next 20 years, as people adopt Western lifestyles, gain weight. In 2011, the Centers for Disease Control and Prevention (CDC) estimated that nearly 26 million Americans of all ages have diabetes (90-95% being Type 2), a quarter of which are unaware of this fact, as described in the Statistics Report (2014) (15). It is also estimated that 79 million Americans have pre-diabetes, affecting 35% of adults aged 20 years and older. In 2014, the CDC reported that about 40% of USA adults will develop diabetes, primarily T2DM in their lifetime and more than 50% of ethnic minorities will be affected. These figures are higher than previous estimates and are due to the increase in obesity, as described by Gregg *et al* (2014) and Lipscombe (2014) (16) (17).

The prevalence of T2DM varies widely among various racial and ethnic groups. It is more prevalent among Hispanics, Native Americans, African-Americans and Asians/Pacific Islanders than in non Hispanic whites, as described in Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7] (1). With regard to age, T2DM occurs most commonly in adults aged 40 years or older, the prevalence of the disease increasing with advancing age, as described in Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7] (1). However, more recently T2DM has also been observed to be increasing in younger persons, particularly in highly susceptible racial and ethnic groups and the obese, as described in Type 2 Diabetes Mellitus: Practice Essentials, Background, Pathophysiology [Internet]. [cited 2016 Apr 7] (1).

#### **1.4 Morbidity and Mortality**

Diabetes mellitus (DM) is one of the leading causes of world morbidity and mortality because of its role in the development of cardiovascular, renal, neuropathic, and retinal disease. In patients with T2DM, the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated an association with a raised microvascular risk, as described by UKPDS (1998) (18), with other studies reporting an increased macrovascular risk, as described by Gabir *et al* (2000) and Lim *et al* (2000) (4) (5). This has an effect on morbidity and premature mortality of patients with DM, with the prognosis being strongly influenced by the degree of disease control and risk factor management. To date, some studies have demonstrated an early multifactorial intensive management approach is key to managing patients with T2DM, in order to reduce the incidence of microvascular, as described by UKPDS (1998) (18) and macrovascular events, as described by Group: The Action to Control Cardiovascular Risk in Diabetes Study (2008) (19).

Both IFG and IGT are considered to be a pre-diabetic state, associated with insulin resistance, increased risk of cardiovascular pathology and increased mortality (although IGT is of lesser risk than IGT). Over 10 years, there is a 50% risk of IFG patients progressing to overt diabetes. However, some newly identified IFG patients progress to T2DM in less than three years and IGT may precede T2DM by many years, as discussed by Barr *et al* (2007) (20). Results from studies by De Fronzo *et al* (2010) (21), using frequently sampled intravenous glucose tolerance test (FSIVGTT), strongly suggested that progressive  $\beta$  cell failure is the main determinant of progression of NGT



to IGT; Suzuki *et al* (2003) (22) also supported this view. Accordingly, targeted pathophysiologic therapy based on oral OGTT-derived measures of insulin sensitivity and  $\beta$ -cell function can be implemented in medical practice, which is associated with marked improvement in glucose tolerance and reversion of pre-diabetes to normal glucose tolerance in more than 50% of patients Armato *et al* (2012) (23). In 2007, Gillies *et al* (2007) (24) performed a systemic review and meta-analysis, of randomised controlled trials that evaluated interventions to delay or prevent T2DM in individuals with IGT. The conclusion from this analysis was that lifestyle and pharmacological interventions reduce the rate of progression to T2DM in people with IGT. Da Qing IGT and Diabetes Study also investigated subjects with IGT who were randomised to a control group or one of three active treatment groups: diet only, exercise only or diet plus exercise. After a 6 year follow up period, results demonstrated that all interventions were associated with a reduction in risk of developing diabetes by 31%, 46% and 42%, respectively (Li *et al* 2008) (25). The Finnish Diabetes Prevention Study (DPS) also showed that it was possible to achieve primary prevention of T2DM by changing lifestyle (diet and exercise) in subjects with IGT (Tuomilehto *et al* 2001) (26). When The Diabetes Prevention Program (DPP) Research Group randomized over 3000 individuals with IFG and IGT, to placebo, metformin or a lifestyle intervention program, after 2.8 years, the incidence of T2DM was 11.0, 7.8 and 4.8 cases per 100 person-years, respectively (Knowler *et al* 2002) (27); the DPP reduced the development of T2DM by 31% and has been recommended by the ADA for treating high risk individual with IGT. The WHO recommendation on physical exercise for adults in this context is at least 150 minutes per week of moderate- intensity aerobic physical activity, or 75 minutes per week of vigorous- intensity aerobic physical activity, or an equivalent combination of moderate and vigorous- intensity activity (WHO 2010) (28). These studies demonstrate the potential to prevent T2DM in high risk individuals, reducing the conversion rate of IGT to T2DM, through lifestyle intervention, focusing on achievement and maintenance of weight reduction by having a healthy diet and increasing physical activity. In order to achieve this, it is important to tailor lifestyle interventions within distinct populations and with each health care encounter, patients should be educated and encouraged to follow an appropriate management plan. IFG, IGT and diabetes can now be found in almost every population in the world and therapeutic interventions have been shown to reduce the risk, or at least the pace, of deterioration from IFG and IGT to T2DM, as described by the Statistics Report (2014) (15), Gregg *et al* (2014) (16) and Lipscombe (2014) (17).

Epidemiological evidence suggests that without effective prevention and the control programmes discussed above, the burden of diabetes is likely to continue to increase globally, as described by Griffin *et al* (2011) (31) and Alberti *et al* (2007) (32). The consequence of this is an escalating financial healthcare burden with allocation of healthcare resources becoming increasingly difficult, as described by Ferrannini *et al* (2004) (8), which will be a challenge in the current economic climate. Therefore early identification of individuals at increased risk of developing T2DM is crucial. If individuals at increased risk of developing T2DM can be identified early on, healthcare interventions or lifestyle changes can be targeted appropriately, as discussed above. In order to target interventions to prevent progression in those at greatest risk, further information as to which individuals are most likely to progress is needed. There is a variable rate of progression from IFG and IGT to T2DM and in general, progression rates are lowest in the general population and highest in target “at-risk” groups, as described by Group: The Action to Control Cardiovascular Risk in Diabetes Study (2008) (19), Barr *et al* (2007) (20), De Fronzo *et al* (2010) (21) and Suzuki *et al* (2003) (22). Age, BMI, fasting and 2 hour plasma glucose concentrations, elevated fasting pro-insulin, low 2-hour insulin and fasting triglyceride levels are associated with a greater risk of progression, as described by Alberti *et al* (1996) and Group: The Action to Control Cardiovascular Risk in Diabetes Study (2008) (6) (19). Adequate moment-by-moment insulin secretion and action being essential for glycaemic control.

### **1.5 Diagnostic Testing for Diabetes Mellitus**

As discussed in section 1.0, T2DM is diagnosed in clinical practice, as per the WHO definition (2006) (bearing in mind the start date of the study) when a person is found to have a fasting plasma glucose  $\geq 7.0$  mmol/l or a random glucose  $\geq 11.1$  mmol/l, as described by Albeti *et al* (1998) (3). Pre-diabetes or intermediate diabetes is diagnosed when IFG (fasting glucose  $> 6.1$  mmol/l) or IGT (fasting glucose  $< 7$  mmol/l; 2 hour post-challenge 7.8-11.1 mmol/l) occurs. As a basic screening test, OGTT is cheap and fairly straightforward to perform, however it is performed during a single two hour period, it is a non-physiological stimulus and individuals show day to day variability in glucose tolerance.

In 2011, The World Health Organisation (WHO) published a report (37), which was an addendum to the diagnostic criteria published in by WHO (2006) (2) and Alberti *et al*

(1998) (3) and addressed the use of glycated haemoglobin (HbA1c) in diagnosing diabetes mellitus. The WHO Consultation accepted that HbA1c can be used as a diagnostic test for diabetes. A HbA1c =  $\geq 48$  mmol/mol is recommended as the cut off point for diagnosing T2DM, but a value of less than that does not exclude diabetes diagnosed using glucose tests. A HbA1c = 42-47 mmol/mol can also indicate people with pre-diabetes, with a HbA1c =  $< 42$  mmol/mol being classed as normal. The use of HbA1c can be used as a diagnostic test for diabetes on condition that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, with no conditions present which preclude its accurate measurement. In patients without symptoms of diabetes the laboratory venous HbA1c should be repeated. If the second sample is  $< 48$  mmol/mol the person should be treated as at high risk of diabetes and the test should be repeated in 6 months or sooner if symptoms develop. The benefits of using HbA1c for the diagnosis of DM are that it is a simple test that can be performed at any time of the day, reflects average plasma glucose over the previous eight to 12 weeks, as described by Nathan *et al* (2007) (38), has less biological variability and does not require any special preparation such as fasting. These advantages have implications for early identification and treatment which have been strongly advocated in recent years. These properties have made it the preferred test for assessing glycaemic control in people with diabetes. However, there are many situations where HbA1c is not appropriate for diagnosis of diabetes. For example, HbA1c is not suitable in all children and young people, in patients of any age suspected of having Type 1 diabetes mellitus (T1DM) and in patients with symptoms of diabetes for less than 2 months. It is also not suitable in patients at high diabetes risk who are acutely ill (e.g. those requiring hospital admission), patients taking medication that may cause rapid glucose rise e.g. steroids, antipsychotics or patients with acute pancreatic damage, including pancreatic surgery. Pregnancy or the presence of genetic, haematologic and illness-related factors that influence HbA1c and its measurement (for example. erythropoiesis, altered haemoglobin glycation and erythrocyte destruction) also make HbA1c an inappropriate diagnostic tool for DM diagnosis. In addition, there are aspects of the measurement of HbA1c that are problematic and the utility and convenience of HbA1c compared with measures of plasma glucose for the diagnosis of diabetes needs to be balanced against the fact that (i) in some countries it is unavailable and not well enough standardized, despite being a recognized valuable tool in diabetes management; (ii) it does not measure day-to-day variability in glucose per se, as it reflects average plasma glucose over the previous eight to 12 weeks (Nathan *et al* 2007) (38) and (iii)

many people identified as having diabetes based on HbA1c will not have diabetes by direct glucose measurement.

In 2016, the ADA published a set of diagnostic criteria for diabetes (12). There are four criteria options for diabetes diagnosis:

- (1) FPG  $\geq 7.0$  mmol/l\*
- (2) 2 hr PG  $\geq 11.1$  mmol/l during OGTT\*
- (3) HbA1c  $\geq 48$  mmol/mol\*
- (4) Random PG  $\geq 11.1$  mmol/l (in individuals with symptoms of hyperglycaemia or hyperglycaemia crisis)

\*In the absence of unequivocal hyperglycaemia, results to be confirmed by repeat testing.

The ADA suggested that if there is no clear clinical diagnosis, to repeat the same test immediately using a new blood sample. The same test with same or similar results, confirms the diagnosis and diagnosis is confirmed with different tests above diagnostic threshold. Discordant results from two separate tests require a repeat test with a result above diagnostic cut point. The ADA also suggested that screening for pre-diabetes can be done using HbA1c, FPG or 2 hr PG after OGTT criteria. When testing for T2DM and pre-diabetes in asymptomatic individuals, if the test is normal it should be repeated every 3 years.

This study was initiated in 2009 and used the WHO 2006 criteria for diabetes diagnosis (2). If this study was being initiated in current times, then perhaps the more modern approach [according to WHO (2011) criteria] of using HbA1c as a diagnostic test for diabetes would be used (37). The use of HbA1c as a diagnostic test for diabetes is also a favoured option of the ADA (2016) (12). However, using HbA1c alone in initial diabetes screening identifies approximately 20% fewer cases of diabetes than diagnosis based on fasting and 2 hr postload glucose levels, as described by Wang *et al* (2011) (39).

### **1.6 Glucose Biomarkers - History**

An attempt was made to identify any biomarkers that predict progression from abnormal glucose tolerance to T2DM. Glycaemic control and beta cell function were

further assessed in addition to the repeat OGTT. Glucose (fasting indicator of glucose homeostasis), HbA1c (intermediate term glycaemic control) and biomarkers of beta cell function, C-peptide and Insulin were assayed (Varvel *et al* 2014) (40). A glutamic acid decarboxylase autoantibody test (GAD antibody test) was also conducted to look for T1DM or latent autoimmune diabetes of adults (LADA) in any of study subjects.

The antigens recognised by these antibodies include insulin, glutamic acid decarboxylase (GAD65 kDa isoform) and an islet cell antigen IA-2 or ICA-512. C-peptide is a peptide composed of 31 amino acids and is produced from the pancreatic beta cells during enzymatic cleavage of proinsulin. Proinsulin is the precursor of C-peptide and insulin, which are produced in equal amounts during enzymatic cleavage. C-peptide has negligible extraction by the liver and constant peripheral clearance. It is mainly excreted by the kidney, and its half-life is 3-4 times longer (20-30 v 3-5 minutes) than that of insulin. It therefore circulates at concentrations approximately five times higher than insulin in the systemic circulation and can therefore be used to assess endogenous insulin secretion Jones *et al* (2013)((41). Insulin is an anabolic hormone that promotes glucose uptake, glycogenesis, lipogenesis, and protein synthesis of skeletal muscle and fat tissue through the tyrosine kinase receptor pathway. Insulin is the most important factor in the regulation of plasma glucose homeostasis, as it counteracts glucagon and other catabolic hormones—epinephrine, glucocorticoid, and growth hormone (42). Insulin resistance is a condition in which the body produces insulin but does not use it effectively. When people have insulin resistance, glucose builds up in the blood instead of being absorbed by the cells, leading to T2DM or pre-diabetes. Insulin testing can be used to assist in diagnosing early T2DM, where there is a relatively increased production of insulin with a concurrent increase in blood glucose levels (43).

### **1.7 Continuous Glucose Monitoring Systems (CGMS)**

To potentially diagnose and target interventions to prevent progression to DM in those at greatest risk, further information as to which individuals are most likely to progress is needed. Adequate moment-by-moment insulin secretion and action is essential for glycaemic control and a marker of deteriorating carbohydrate homeostasis would be increased fluctuations in blood glucose levels. Health care professionals are increasingly

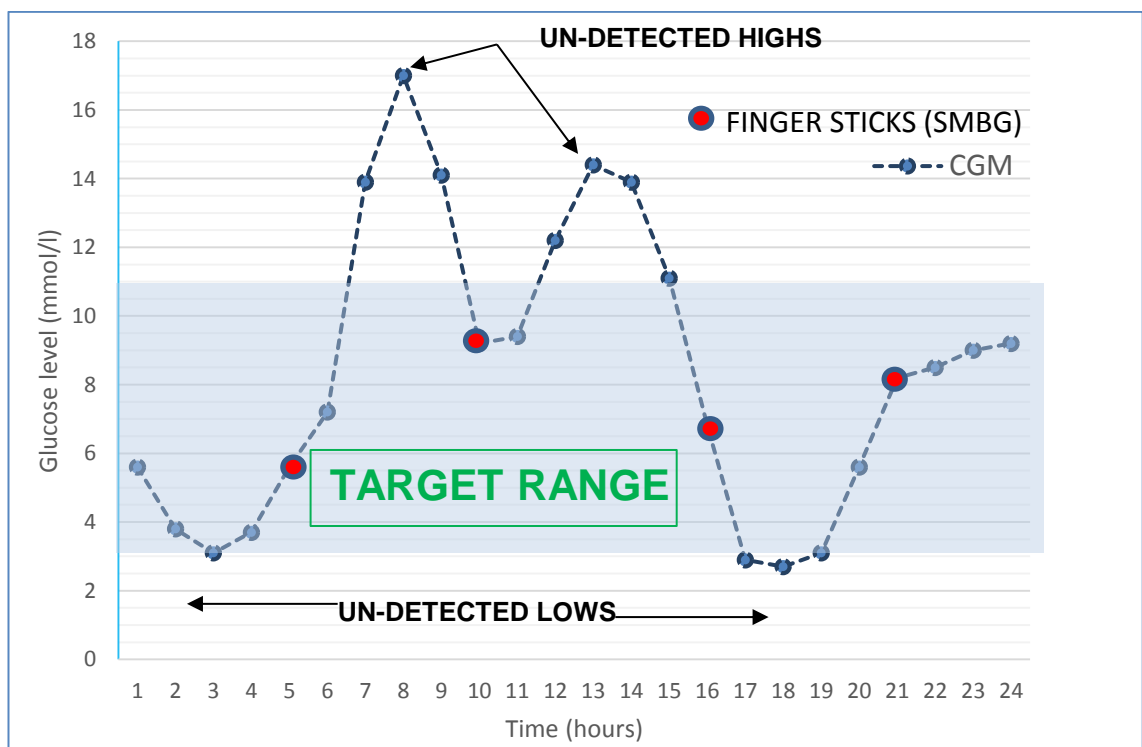
searching for tools to evaluate their patients' glucose control in a quick and easy way to optimize their diabetes management. CMGS in the form of an *iPro*<sup>TM</sup> allows just this and provides an integrated measure of glucose monitoring. This is a small, discreet device that measures 24 hour continuous glucose levels via a glucose sensor applied transcutaneously (Figure 2). CGM is mostly used by diabetologists as a monitoring tool, used to look at interstitial glucose profiles and identify trends; it is also particularly useful in adults and children to solve specific clinical questions. Clinically it has many uses and currently, it can be used for the detection of hypoglycaemia, as described by Schopman (2013) (44), with post-hypoglycaemic hyperglycaemia as a cause of sub-optimal glycaemic control. It can also be used to help elucidation the cause of nocturnal seizures in patients with insulin treated diabetes as well as in the determination of glycaemic excursions above and below ideal in individuals with satisfactory HbA1c levels, as described by Buckingham (2008) (45). CGM is used as a tool to examine the benefits (or otherwise) of a change from multiple daily insulin injections to insulin pump therapy and treatment adjustment – e.g. moving from oral anti-diabetic drugs to insulin or basal-bolus adjustments; it is also used to look for hyperglycaemic peaks during pregnancy. CGM has also been used as a research tool (46). It has been demonstrated that CGM can be associated with reducing A1C without increasing the risk of hypoglycemia vs finger stick testing alone, as described by Chiasson *et al* (2002) (29), Torgerson *et al* (2004) (30) and Griffin *et al* 2011 (31).



**Figure 2: CGM *iPro*<sup>TM</sup> unit**

-The CGM *iPro*<sup>TM</sup> was a small, discreet device that measured 24 hour continuous glucose levels via a glucose sensor applied transcutaneously.

As OGTT and HbA1c are not ideal in capturing moment-by-moment glycaemic variability, we propose the use of continuous glucose sensing to quantify this. CGMS (Medtronic) are accurate, as described by Guerci *et al* (2003) (50) and measure interstitial glucose levels every 10 seconds, the results of which are stored as a smoothed average over 5 minutes. This can occur continuously for up to 3 days with a single sensor, as described by Sachedina *et al* (2003) (51). Monitors are externally calibrated by the subject who performs self monitoring of blood glucose (SMBG) four-point testing in each 24 hour period. A typical CGM tracing with four-point testing can be seen in Figure 3 which illustrates how much more information on the glucose profile can be obtained (hyperglycemia, hypoglycemia and variability patterns) and how excessive glucose variability can be even in patients that appear to be well controlled from SMBG four-point and HbA1c testing, respectively (46) (52). CGMS documents continuous glucose variations throughout the day, rather than during a short OGTT or discontinuous home blood glucose testing, thereby offering a definite advantage.



**Figure 3: Typical CGM Tracing with four-point SMBG testing**

-Monitors were externally calibrated by the subject who performed SMBG four-point testing in each 24 hour period. A typical CGM tracing with four-point testing is seen above and illustrates the added information on the glucose profile that can be obtained. Glucose variability can also be observed, even in patients that appear to be well controlled from SMBG testing.

CGM is one of the latest advancements in diabetes management. A traditional glucose meter uses the information from a finger stick test for a blood glucose value that represents only a snapshot in time. CGM monitors continuously, to let you see what's happening between finger stick tests. That way, one can watch glucose levels and patterns that you may not have been able to see before.

### **1.8 Aims and Objectives: Study Hypothesis**

**Aim:** To assess whether CGMS can help identify people with abnormal glucose tolerance who progress to Type 2 diabetes mellitus (T2DM).

**Objectives:** The following research objectives were compiled in order to facilitate the study aim.

- (1) To obtain ethical approval for the study, via the NHS Local Research Ethics Committee (NHS LREC) mechanism, South East Wales Research Ethics Committee (SEWREC) and from the School, Cardiff University.
- (2) To obtain statistical advice from the Professor of Statistics, Professor Robert Newcombe, Cardiff University School of Medicine, regarding study sample size.
- (3) To collate a study sample of fifty individuals from the general population, composed of subjects who have had an abnormal OGTT and consented to study participation.
- (4) To obtain demographic data and a thorough medical history, medication history, family history and social history on all study subjects at baseline, Year 1 and Year 3.
- (5) To obtain GAD antibody status, C-peptide and Insulin levels on all study subjects at baseline.
- (6) To perform OGTT, check HbA1c and perform CGM on all study subjects at baseline, Year 1 and Year 3.
- (7) To construct glycaemic excursion variables to reflect glucose fluctuations observed in CGM and apply them in Excel 2007 to the raw CGM sensor data.
- (8) To analyse baseline, Year 1 and Year 3 data.
- (9) To separate by inspection, CGM profiles for each study subject into 3 groups based on variability (least variability, medium variability and most variability).



- (10) To use the Paired Sample  $t$ -Test to compare differences in two means of the set parameters as the study progressed with time.
- (11) To use independent sample  $t$  Test to compare the means of two independent groups (diabetes and non diabetes) at Year 1 and Year 3, in order to determine any significant difference between them, when tested against a number of baseline parameters.
- (12) To use one way analysis of variance (ANOVA) at baseline, to evaluate any significant difference between the CGM profiles by inspection and the statistically constructed CGM parameters.
- (13) To use binary logistic regression analysis to estimate the relationships among variables. Baseline parameters were tested for Year 1 and Year 3 outcome, using binary logistic regression analysis, to see if any of them affected the outcome or influenced each other.

**Null Hypothesis:** CGM can be used to predict progression from abnormal glucose tolerance (pre-diabetes) to T2DM.

### **1.9 Study Timeframe**

This study began in 2009 and continued until 2012. The parameters chosen for analysis and the outcome indicators together with the technologies used were a reflection of this time. If this study had been conducted in more recent times, then some of the parameters chosen for analysis and outcome indicators selected would be in line with current guidelines. The technologies employed would also be more modern and at the cutting edge of diabetes care, in this regard.

## **2.0 Materials and Methods**

### **2.1 Ethics**

Cardiff University School of Medicine requires that all research involving human participants, human material or human data is subject to formal ethical review and approval before such work can be started. This study had been granted approval via the NHS LREC mechanism SEWREC and from the School, Cardiff University. With regard to data storage and security, hard copies of any study data were kept securely at a NHS research location, lock coded and could only be accessed by agreed members of the research team. Any computer files that contained personal or identifier data were password protected and only accessed by agreed members of the team (53).

### **2.2 Statistical Advice**

Statistical advice regarding sample size was obtained from the Professor of Statistics, Robert Newcombe, Cardiff University School of Medicine. A proposed sample size of 50 subjects ( $n = 50$ ) with confirmed IFG/IGT on OGT testing was considered justifiable. This was based on the assumption that 30% of subjects will have a diagnosis of definite diabetes by 2 years and that half the remaining subjects, i.e. 35% will have reverted to normal. Comparing those who become diabetic with those who revert to normal would have a power of 80% to detect a shift of exactly 1 standard deviation in the mean value for any parameter tested, using a test at the conventional two-sided alpha level, as described by McCrum- Gardner (2010) (54) and Cohen (1977) (55).

The effect size (ES) in a population is intrinsically linked to three other statistical properties, as described by Cohen (1977) (55) and Cohen (1992) (56):

- (i) The sample size on which the sample effect size is based.
- (ii) The probability level at which we will accept an effect as being statistically significant (the  $\alpha$  level); typically an  $\alpha$  level of 0.5 is used.
- (iii) The ability of a test to detect an effect of that size (known as the statistical power). The power of a test is the probability that a given test will find an effect assuming that one exists in the population.

The probability of failing to detect an effect when one genuinely exists is  $\beta$ , the probability of a Type II error. It follows that the probability of detecting an effect if one exists must be the opposite of not detecting that effect i.e.  $(1-\beta)$ . Cohen (1977) (55)

suggested that we would hope to have a 0.2 probability of failing to detect a genuine effect, and so the corresponding level of power he recommended was  $1-0.2 = 0.8$ . Therefore, a power of 0.8 or an 80% chance of detecting an effect if one genuinely exists should be aimed for and is the minimum accepted level.

### **2.3 Recruitment Population**

The recruitment of subjects for the study took place over a 20 month period between October 2009 and June 2011. In total, 486 patients referred for out-patient Oral Glucose Tolerance Testing (OGTT), from either their General Practitioner or a Secondary Care Physician, respectively, were screened. Patients either attended The University Hospital of Wales, Cardiff or Llandough Hospital, Penarth for testing. All patients referred for OGTT were felt to have 'a priori' - a reason to undergo testing and thus were deemed as intermediate risk with regard to progression to DM.

### **2.4 OGTT**

Patients who were referred for OGTT attended out-patients phlebotomy suite at The University Hospital of Wales, Cardiff or Llandough Hospital, Penarth between 08.45 and 09.30am for testing. The OGTT test was conducted by the by UHW phlebotomy staff. Three days prior to the test, the patients were asked to have a normal diet containing more than 150g carbohydrate daily and were instructed to fast from 10pm the previous evening. This gave a minimum 10 hour fasting period, where the patients refrained from eating or drinking anything other than water, until the test was completed. For an OGTT, the WHO recommends 75g of anhydrous glucose (or its equivalent) in a final volume of 300 ml is used (57). In our OGTT protocol, 113 mL Polycal was poured into a designated beaker and water was added to the 200 mL mark and the contents of the beaker were mixed. This was consumed within 5 minutes and then the patients drank a further 100 mL of water to make the final volume 300 mL. The patients were warned of the possible side effects of nausea, vomiting, diarrhoea, as the glucose drink was hyperosmolar. The patients were allowed to drink additional water during the test if needed and were asked to sit quietly throughout the test, smoking not being permitted. The test was not performed during intercurrent illness. Venous blood was sent for laboratory glucose analysis before taking the glucose load (zero minutes) and 120 minutes after consumption of the glucose load.

## **2.5 Screening Population**

As stated in Section 2.2, 486 patients in total were screened over a 20 month period. All subjects between the ages of 18-80 years were considered. Subjects unable or unwilling to give informed consent or unable or unwilling to comply with research requirements were excluded. In addition, pregnant females, an intercurrent illness with prognosis of < 2 years and subjects with a known previous allergic reaction to adhesive plasters were also excluded. Gender, ethnic origin, nationality, religion, belief or sexual orientation was irrelevant in the decision to recruit (see APPENDIX 1: CGM Study Protocol).

When the patients attended for their clinically indicated OGTT, if they met the study inclusion criteria as stated above (see APPENDIX 1: Study Protocol), they were invited to participate in the study. Eligible candidates were given a patient information sheet (see APPENDIX 1: Patient Information Sheet) and were able to make an informed decision at this point as to whether they wished to participate in the study or not. Those patients who agreed to participate in the study then completed and signed a study consent form and subject contact details were obtained (see APPENDIX 1: Patient Consent Form). Informed consent was obtained from the Principle Investigator (PI). All patients were free to leave the hospital after the OGTT and the result of the test was automatically sent to the referring Physician.

Subjects who had consented and wanted to participate in the study were contacted and invited to participate in the study if (i) they met the study inclusion criteria, (ii) had given informed consent and (iii) had a positive OGTT - found to have IFG or IGT, as per the diagnostic criteria below (58). If subjects did not meet the study inclusion criteria - were normal glucose tolerant (NGT) or had T2DM, they were excluded and not invited to participate in the study. A study information letter was sent to the GPs of participating subjects (see APPENDIX 1: GP Letter).

### Diagnostic Criteria (58)

#### **Normal Glucose Tolerance (NGT)**

Both of the following criteria must be met:

- Fasting glucose  $\leq 6.0$  mmol/l
- 2 hour GTT glucose  $< 7.8$  mmol/l

### **Diabetes Mellitus**

Either of the following is diagnostic:

- Fasting glucose  $\geq 7.0$  mmol/l
- Random glucose  $\geq 11.1$  mmol/l
- 2 hour GTT glucose  $\geq 11.1$  mmol/l

### **Impaired Fasting Glucose (IFG)**

Both the following criteria must be met:

- Fasting glucose  $>6.1$  - $6.9$  mmol/l
- 2 hour GTT glucose  $<7.8$  mmol/l

### **Impaired Glucose Tolerance (IGT)**

Both of the following criteria must be met:

- Fasting glucose  $<7.0$  mmol/l
- 2 hour GTT glucose  $\geq 7.8$  mmol/l but  $< 11.1$  mmol/l

## **2.6 Study Schedule**

### **2.6.1 Baseline Schedule**

OGTT positive subjects attended at baseline ( $t = 0$  months) where informed signed consent was re-obtained by the PI (see APPENDIX 1). Demographic data was obtained, including: height, weight, body mass index (BMI), together with past medical history, drug history, smoking status and any family history of diabetes. A blood test was also obtained for HbA1c analysis. Subjects were then instructed as described in Section 2.7 in self monitoring of blood glucose (SMBG) and the CGM was fitted, as described in Section 2.8 following education of its use. A single CGM sensor was worn by each subject for a period of up to three whole days (maximum of six whole days via two sensors in total) and subjects were asked to perform meter tests of their blood glucose levels (SMBG) at least four specified times of the day (four point testing) day while undergoing CGM.

### **2.6.2 Year 1 Follow Up**

At  $t = 1$  year, subjects returned following an overnight fast, for repeat testing. Those that did not respond were presumed to have withdrawn consent. Informed signed consent was re-obtained by the PI, using the original consent form (see APPENDIX 1) and demographic data was obtained, including: height, weight, body mass index (BMI),

together with past medical history, drug history, smoking status and any family history of diabetes. Subjects then underwent an OGTT, as described in Section 2.4 and blood samples were also obtained for HbA1c analysis, anti –GAD antibody, C-peptide and insulin. Subjects were then instructed as described in Section 2.7 in SMBG and the CGM was fitted, as described in Section 2.8 following education of its use. A single CGM sensor was worn by each subject for a period of up to three whole days and subjects were asked to perform meter tests of their blood glucose levels (SMBG) at least four specified times of the day (four-point testing) day while undergoing CGM. The OGTT and HbA1c results obtained at this stage were forwarded to the GPs to ensure continuity of care of the subjects and implementation of treatment where necessary (see APPENDIX 1).

### **2.6.3 Year 3 Follow Up**

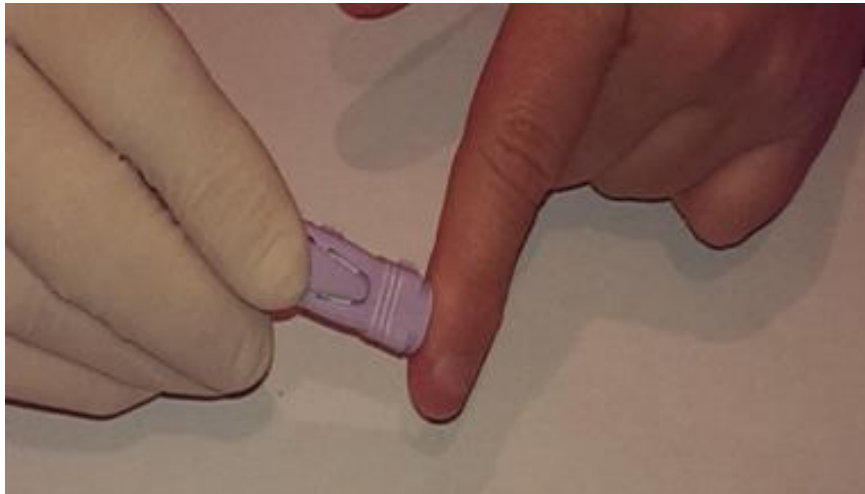
At  $t = 3$  years, subjects returned following an overnight fast, for repeat testing. Those that did not respond were presumed to have withdrawn consent. Informed signed consent was re-obtained by the PI, using the original consent form and demographic data was obtained, including: height, weight, BMI, together with past medical history, drug history, smoking status and any family history of diabetes. Patients were given a standardised questionnaire regarding implemented change post initial positive OGTT testing. Subjects then underwent a fasting blood test and samples were obtained for analysis of FPG and HbA1c. The FPG and HbA1c results obtained at this stage were forwarded to the GPs to ensure continuity of care of the subjects and implementation of treatment where necessary (see APPENDIX 1).

At Year 3, FPG was performed and used as outcome indicator, instead of OGTT. Although ethical approval was obtained for a OGTT at Year 3, when the study subjects contacted and invited back, they were not keen to have OGTT; however, they were happy to attend for a FPG. Therefore, in order to gain a Year 3 outcome FPG was used.

### **2.7 Self Monitoring of Blood Glucose (SMBG)**

Blood glucose monitoring is a way of testing the concentration of glucose in the blood. All subjects enrolled in the study were taught SMBG using the OneTouch® Ultra ® blood glucose meter and were educated with regard to finger prick testing and the use of test strips. The blood glucose test was performed by piercing the skin of the finger,

lateral to the nail base, as illustrated in Figure 4, with a single use safety lancet (Unistick® 3 Comfort) (59).



**Figure 4: Single use safety lancet: Unistick® 3 Comfort (59)**

-The blood glucose test was performed by piercing the skin of the finger, lateral to the nail base with a single use safety lancet (Unistick® 3 Comfort).

The small drop of blood obtained was then applied to a disposable ‘One Touch® Ultra® single coded test strip’ as illustrated in Figure 5 (60), which was pre- inserted into the OneTouch® Ultra ® blood glucose meter, an electronic device for quantitatively measuring glucose in whole blood.



**Figure 5: One Touch® Ultra® test strips**

-The small drop of blood obtained from finger prick testing for SMBG was then applied to a disposable ‘One Touch® Ultra® single coded test strip’.

Prior to being used, the One Touch®Ultra® blood glucose meter was plasma calibrated, thus allowing easy comparison with laboratory methods. After 5 seconds the level of blood glucose was shown on the meters digital display. In this study, the blood glucose

meters were One Touch®Ultra® blood glucose meters, which had an inbuilt data download system which connected to the PC via an external cable.

## 2.8 Continuous Glucose Monitoring System (CGMS)

CGMS is a continuous glucose monitoring system which measures the glucose levels in interstitial fluid. CGMS measures the glucose level of interstitial fluid every 10 seconds and the results are stored as a smoothed average over 5 minutes, as described by Boyne *et al* (2003) (61). A typical CGM system usually consists of three components: a disposable sensor that measures glucose levels, a transmitter that is attached to the sensor and a receiver that displays and stores glucose information (Figure 6). The information in the receiver is then converted into estimated mean values of glucose standardized to capillary blood glucose levels measured during calibration.

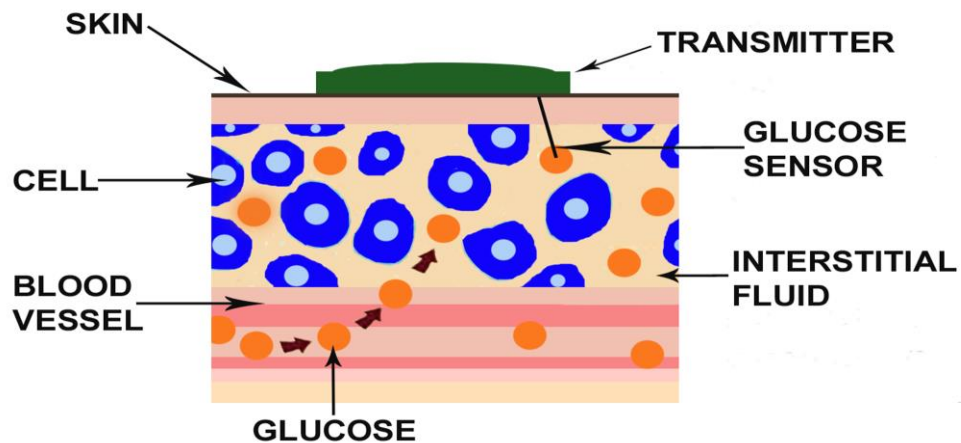


Figure 6: Schematic diagram illustrating the theory behind CGM (62)(63)

-The CGM system comprised of three components: a disposable sensor that measured interstitial glucose levels, a transmitter that was attached to the sensor and sat on the surface of the skin and a receiver that displayed and stored glucose information. The information in the receiver was then converted into estimated mean values of glucose standardized to capillary blood glucose levels measured during calibration.

It's known that glucose travels first from the blood vessels and capillaries into the interstitial fluid. As CGMS sensor glucose (SG) readings measures interstitial glucose while SMBG meter measures glucose levels in the blood, the BG meter readings from SMBG and SG readings from CGMS rarely match exactly i.e. there is a lag in real time glucose levels by 5 to 10 minutes. This is normal and should be expected. However, when glucose levels are rising or falling quickly, there is a larger difference between the BG meter values and the SG readings i.e. typically post-prandially. When the BG value is rising, its value is greater than the SG that follows behind it, but when the BG falls



the BG in front is now less than the SG value. Clinically, knowing the direction and speed of glucose changes is useful and can be more useful than individual BG or sensor readings, which are momentary snapshots of glucose measurement. When using CGM trends are the key (63). The CGMS used in this study was a Medtronic CGM device; a typical Medtronic CGM kit is illustrated in Figure 7.



**Figure 7: Typical Medtronic CGM kit (64)**

-A typical Medtronic CGM kit used for each study subject consisted of a re-usable Medtronic sensor inserter, a single use glucose Sof-sensor®, a re-usable Medtronic iPro™ charger, a re-usable Medtronic iPro™ CGM transmitter and a single use IV3000 Smith and Nephew transparent adhesive dressing.

### **2.8.1 CGM Sensor and Sensor Insertion**

Currently, available CGM devices are considered minimally invasive enzyme-coated electrodes. These devices measure interstitial glucose concentrations and convert these values to blood glucose levels. The sensor catheter has electrodes impregnated with glucose oxidase (the same enzyme used to measure glucose levels as a test strip), which is introduced into the subcutaneous tissue. The reaction between interstitial fluid glucose and glucose oxidase located on the electrode produces hydrogen peroxide. This reaction converts the interstitial glucose into an electrical current proportional to the glucose concentration at the site of the catheter insertion, which travels to the transmitter attached. Devices using enzyme-coated catheters require frequent calibrations to correct variations in the reaction between the electrode and the subcutaneous tissue, as well as fluctuations in glucose and oxygen diffusion at the site of the electrode (65), as described by Burge *et al* (2008) (66). The CGM used the

Medtronic Sof-sensor® in this study (Figure 8). The Sof-sensor® was stored in the fridge at 4 °C (+2 °C to +27°C). Prior to using it the sensor package was removed from the fridge to warm up to room temperature for about 15 minutes before opening the sensor package to prevent condensation.



**Figure 8: The glucose Sof-sensor® (63)**

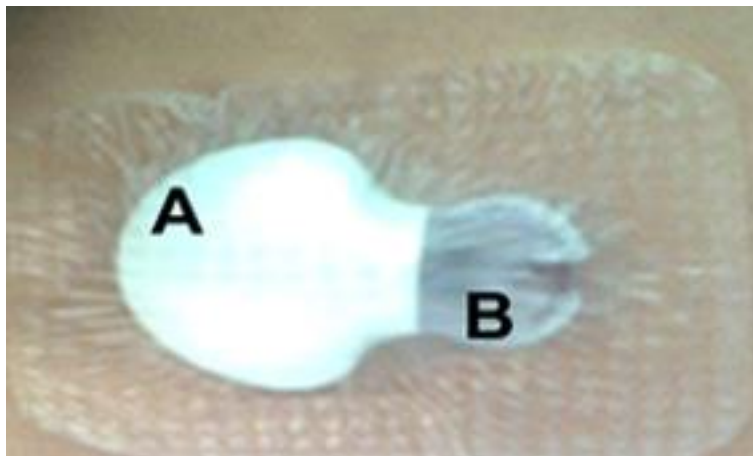
-The glucose Sof-sensor® had a gold catheter electrode impregnated with glucose oxidase, which was introduced into the subcutaneous tissue. The reaction between interstitial fluid glucose and glucose oxidase located on the electrode produced a reaction which converted the interstitial glucose into an electrical current proportional to the glucose concentration at the site of the catheter insertion, which travelled to the transmitter attached.

In order for the sensors to be inserted, the subjects were in a standing position. In each subject, the sensor was placed 2 inches from the umbilicus, after the area was cleaned with a steri-wipe (NICE-PAK International Ltd) and areas of the natural body bend were avoided. In order for the sensor to be inserted, the sensor was removed from the packaging and placed into an insertion device. With a push of a button the glucose sensor was inserted with a needle, via the insertion device. It was inserted anywhere between a 45 and 60 degree angle, just under the skin of the abdomen (67). The needle and insertion device were removed after the glucose sensor was in place. The sensors had a lifespan of 3 days and the subjects had to have the sensor replaced once during the six days they wore the sensor in total. With a push of a button the glucose sensor was inserted with a needle, via the insertion device. It was inserted anywhere between a 45 and 60 degree angle, just under the skin of the abdomen (67). The needle and insertion device were removed after the glucose sensor was in place

### **2.8.2 CGM Transmitter**

The Medtronic CGM transmitter [A] is a small lightweight device that attaches to the glucose sensor [B] and gathers glucose data (Figure 9). In this study, the CGM non

implanted Medtronic *iPro*<sup>TM</sup> transmitter was used. Prior to each sensor use, the transmitter was cleaned and charged and the transmitter was recharged after each sensor use i.e. every three days.



**Figure 9: Medtronic CGM transmitter attaches to glucose Sof-sensor®**

-The Medtronic CGM non implanted *iPro*<sup>TM</sup> transmitter [A] was a small lightweight device that attached to the glucose sensor [B] and gathered glucose data.

In each subject, when the sensor had been inserted, the transmitter was then attached. This CGM unit was then taped to the abdomen with IV3000 Smith and Nephew transparent adhesive dressing as illustrated in Figure 2. The Medtronic transmitter used in this study was waterproof and could be worn while swimming or showering to a depth of three metres and the subjects were advised of this when they attended. The CGM monitors were externally calibrated by the subject who was advised to perform pre-meal finger prick home glucose testing at least four times in each 24 hour period, as described above. The BG meter readings used for calibration were essential in ensuring the glucose sensor maintained its accuracy over time. The best time to calibrate the Medtronic Sof-sensor® (63) is when glucose levels are least likely to be changing rapidly and this is why all subjects were requested to check BM pre-prandially.

The *iPro*<sup>TM</sup> transmitter gathered glucose data and stored it until the subject was instructed to return. After 3 days, the data was ready to be downloaded and Solutions 2.2A® Software for CGMS® *iPro*<sup>TM</sup> was used in conjunction with the Continuous Glucose Recorder to extract glucose data from the CGMS® *iPro*<sup>TM</sup> wirelessly or via the

ComLink™ connected to a PC. The Solutions® Software was pre installed on the PC and for each subject, glucose data was downloaded from the CGMS *iPro*™ Recorder and stored as a uniquely coded patient data file on the PC. The software applied a retrospective regression calibration algorithm to the CGMS *iPro*™ download data. The relative accuracy of the SG values produced by the regression algorithm was evaluated with the reference meter BG values used for the calibration. Each Meter BG used for calibration was paired with the corresponding sensor values generated by the calibration algorithm at the same point in time. The paired sensor and meter values were statistically evaluated for Coefficient of Correlation and Mean Absolute Difference (68).

### **2.8.3 CGM Result Profiles: An Example**

Prior to the study, the CGMS was trialled on a normoglycaemic test subject ‘SAP’. Five types of report were generated: Sensor Summary, Sensor Daily Details, Sensor Modal Day, Sensor Modal Time Periods and Sensor Data. Examples of these reports are demonstrated below, as generated for test subject ‘SAP’.







Sensor Summary Patient:

ID: sap

Medtronic Solutions: CGMS iPro

CGMS iPro 2.2A

Page 1

Date		9/15/2009	9/16/2009	9/17/2009	9/18/2009	9/19/2009	9/20/2009
Sensor	# of Sensor Values	202	288	288	282	288	288
	Average (mmol/L)	5.1	5.0	5.6	5.8	5.8	5.6
	Min - Max (mmol/L)	4.5-6.3	4.2-8.7	4.5-8.8	4.6-6.9	4.8-7.4	2.8-7.4
	STDev (mmol/L)	0.4	0.7	0.8	0.5	0.6	1.0
Meter	# of Meter Values	2	1	2	1	2	2
	Average (mmol/L)	5.2	5.0	4.9	4.8	5.7	5.6
	Min - Max (mmol/L)	5.1-5.2	5.0-5.0	4.7-5.1	4.8-4.8	5.4-6.0	4.8-6.4
Optimal Accuracy Criteria	Designation	X: Use Clinical Judgment	X: Use Clinical Judgment	X: Use Clinical Judgment	X: Use Clinical Judgment	X: Use Clinical Judgment	X: Use Clinical Judgment
	# of Paired Readings	0	0	2	1	1	2
	Mean Abs. Diff. [MAD%]	n/a	n/a	4.3	10.4	7.9	6.6
	Correlation Coeff. [R]	n/a	n/a	n/a	n/a	n/a	n/a
Excursions High > 11.1mmol/L Low < 7.8mmol/L	# of Excursions <sup>a</sup>	1	0	1	1	0	0
	# of High Excursions <sup>a</sup>	0	0	0	0	0	0
	# of Low Excursions <sup>a</sup>	1	0	1	1	0	0
	Duration Above High Limit	00:00 (0%)	00:00 (0%)	00:00 (0%)	00:00 (0%)	00:00 (0%)	00:00 (0%)
	Duration Within Limits	00:00 (0%)	00:25 (2%)	00:50 (3%)	00:00 (0%)	00:00 (0%)	00:00 (0%)
	Duration Below Low Limit	16:50 (100%)	23:35 (98%)	23:10 (97%)	23:30 (100%)	24:00 (100%)	24:00 (100%)
	Pie Chart Red: Above Limits Green: Within Limits Blue: Below Limits						
	Glucose Area Above High Limit (mmol/L*Day)	0.0	0.0	0.0	0.0	0.0	0.0
	Glucose Area Below Low Limit (mmol/L*Day)	2.7	2.8	2.3	2.1	2.0	2.2
	Breakfast	Pre-Meal (mmol/L) Avg (1 hour) Min/Max					
Post-Meal (mmol/L) Avg (3 hour) Min/Max							
Lunch	Pre-Meal (mmol/L) Avg (1 hour) Min/Max						
	Post-Meal (mmol/L) Avg (3 hour) Min/Max						
Dinner	Pre-Meal (mmol/L) Avg (1 hour) Min/Max						
	Post-Meal (mmol/L) Avg (3 hour) Min/Max						
Time Period 06:00-05:59	A (mmol/L) Avg Min/Max	5.2 4.5-6.3	5.3 4.2-8.8	5.5 4.5-6.7	5.6 4.6-7.4	6.0 4.8-7.4	5.2 2.8-7.4
Time Period 06:00-23:00	B (mmol/L) Avg Min/Max	5.1 4.5-6.0	4.8 4.2-5.7	5.3 4.5-6.6	5.6 4.6-6.9	5.7 4.8-7.3	5.3 2.8-7.3
Time Period 20:00-06:00	C (mmol/L) Avg Min/Max	5.5 4.9-6.3	6.0 4.5-8.8	5.9 4.8-6.7	5.8 5.3-7.4	6.4 4.9-7.4	5.0 3.7-7.4

X: Please use your clinical judgment - this day does not satisfy the optimal accuracy criteria according to set thresholds:  
 N>=3, R>=0.79 and MAD<=28% [or <=18% if the range (Min-Max) of meter values is less than 100mg/dL (5.6 mmol/L) - see Criteria Note below].  
 C: This day does not have any paired sensor/meter data and no sensor plot is provided. As a result, 'Meter Only' data is available.  
 S: Please use your clinical judgment - this day does not have any meter data. As a result, 'Sensor Only' data is available.  
 Criteria Note: If the range (Min-Max) of Meter Values is less than 100 mg/dL (5.6 mmol/L) then 'R' will be reported as 'N/A'.  
 In this case the optimal accuracy threshold set for MAD is <=18%.  
 Excursion Note: Excursions are counted in the day that the excursion event started.

Date Printed: 07-Sep-08 06:41

Figure 10: Example: Sensor Summary report (i) for test subject 'SAP'

-The Sensor Summary report displayed a tabular summary of statistical data from the CGMS iPro™ Recorder as well as the Meter Glucose data, with each column containing one 24 hour period of data.

Date		9/21/2009	Totals			
Sensor	# of Sensor Values	120	1756			
	Average (mmol/L)	4.2	5.4			
	Min - Max (mmol/L)	2.2-8.5	2.2-8.8			
	STDev (mmol/L)	0.9	0.8			
Meter	# of Meter Values	0	10			
	Average (mmol/L)		5.2			
	Min - Max (mmol/L)		4.7-6.4			
Optimal Accuracy Criteria	Designation	S: Use Clinical Judgment				
	# of Paired Readings	0	6			
	Mean Abs. Diff. [MAD%]	n/a	6.7			
	Correlation Coeff. [R]	n/a	n/a			
Excursions High > 11.1mmol/L Low < 7.8mmol/L	# of Excursions*	0	3			
	# of High Excursions*	0	0			
	# of Low Excursions*	0	3			
	Duration Above High Limit	00:00 (0%)	00:00 (0%)			
	Duration Within Limits	00:00 (0%)	01:15 (1%)			
	Duration Below Low Limit	10:00 (100%)	145:05 (99%)			
	Pie Chart Red: Above Limits Green: Within Limits Blue: Below Limits					
	Glucose Area Above High Limit (mmol/L*Day)	0.0	0.0			
	Glucose Area Below Low Limit (mmol/L*Day)	3.7	2.4			
	Breakfast	Pre-Meal (mmol/L) Avg (1 hour) Min/Max				
Post-Meal (mmol/L) Avg (3 hour) Min/Max						
Lunch	Pre-Meal (mmol/L) Avg (1 hour) Min/Max					
	Post-Meal (mmol/L) Avg (3 hour) Min/Max					
Dinner	Pre-Meal (mmol/L) Avg (1 hour) Min/Max					
	Post-Meal (mmol/L) Avg (3 hour) Min/Max					
Time Period 06:00-05:59	A (mmol/L) Avg	3.6	5.4			
	Min/Max	2.2-5.9	2.2-8.8			
Time Period 06:00-23:00	B (mmol/L) Avg	3.6	5.2			
	Min/Max	2.2-5.9	2.2-7.3			
Time Period 20:00-06:00	C (mmol/L) Avg		5.8			
	Min/Max		3.7-8.8			

X: Please use your clinical judgment - this day does not satisfy the optimal accuracy criteria according to set thresholds:  
N=>3, R>=0.79 and MAD<=28% [or <=18% if the range (Min-Max) of meter values is less than 100mg/dL (5.6 mmol/L) - see Criteria Note below].  
C: This day does not have any paired sensor/meter data and no sensor plot is provided. As a result, 'Meter Only' data is available.  
S: Please use your clinical judgment - this day does not have any meter data. As a result, 'Sensor Only' data is available.  
Criteria Note: If the range (Min-Max) of Meter Values is less than 100 mg/dL (5.6 mmol/L) then 'R' will be reported as 'N/A'.  
In this case the optimal accuracy threshold set for MAD is <=18%.  
Excursion Note: Excursions are counted in the day that the excursion event started.

Date Printed: 07-Sep-08 06:41

Figure 11: Example Sensor Summary report (ii) for test subject 'SAP'

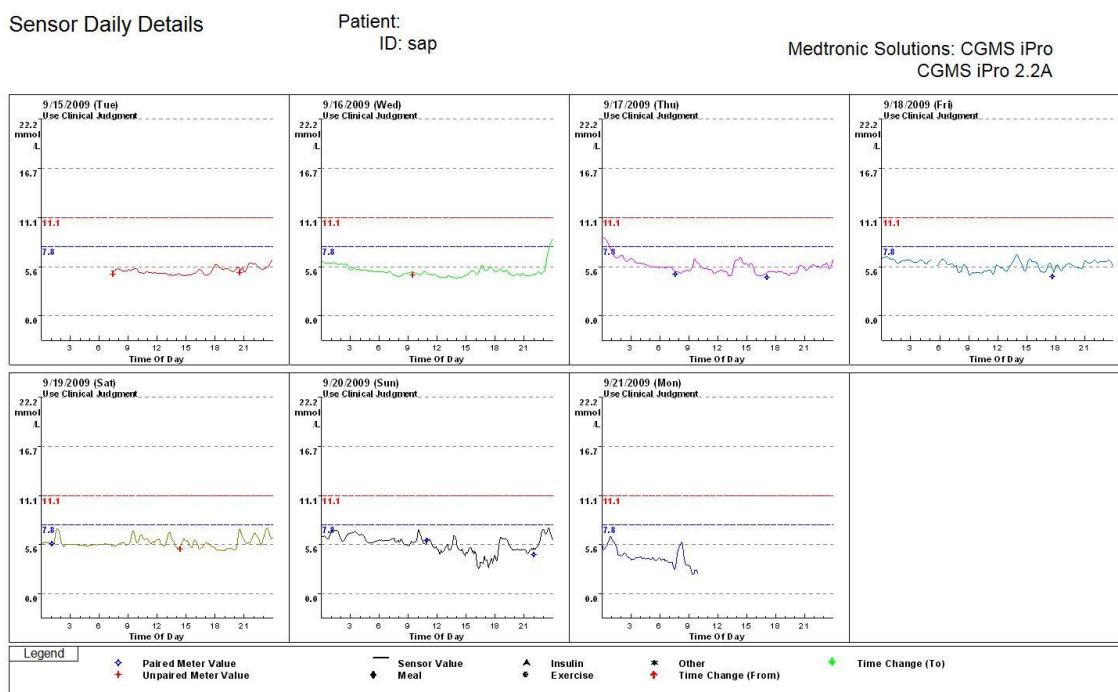
The Sensor Summary report displayed a tabular summary of statistical data from the CGMS iPro™ Recorder as well as the Meter Glucose data, with each column containing one 24 hour period of data.

The Sensor Summary report displayed a tabular summary of statistical data from the CGMS iPro™ Recorder as well as the Meter Glucose data, with each column containing one 24 hour period of data. An example of this, generated for test subject 'SAP' is demonstrated in Figure 10 and Figure 11. The Sensor Daily Details is another type of report generated and this provided up to fourteen days of individual data plots. Graphs



were defined with glucose concentrations on the vertical axis and time on the horizontal axis. The plots showed the sensor data, meter values and user events during each 24 hour period, with the target range set by the investigator; this was indicated by the blue dashed line (lower limit) and the red dashed line (upper limit). Individual glucose sensor values were used to draw a profile line on each sensor detail graph. A gap in the profile indicated an interruption in glucose monitoring. Each day was plotted with a different colour:

**Sunday – Black; Monday – Blue; Tuesday – Red; Wednesday – Green; Thursday – Magenta; Friday – Cyan; Saturday - Olive Green**



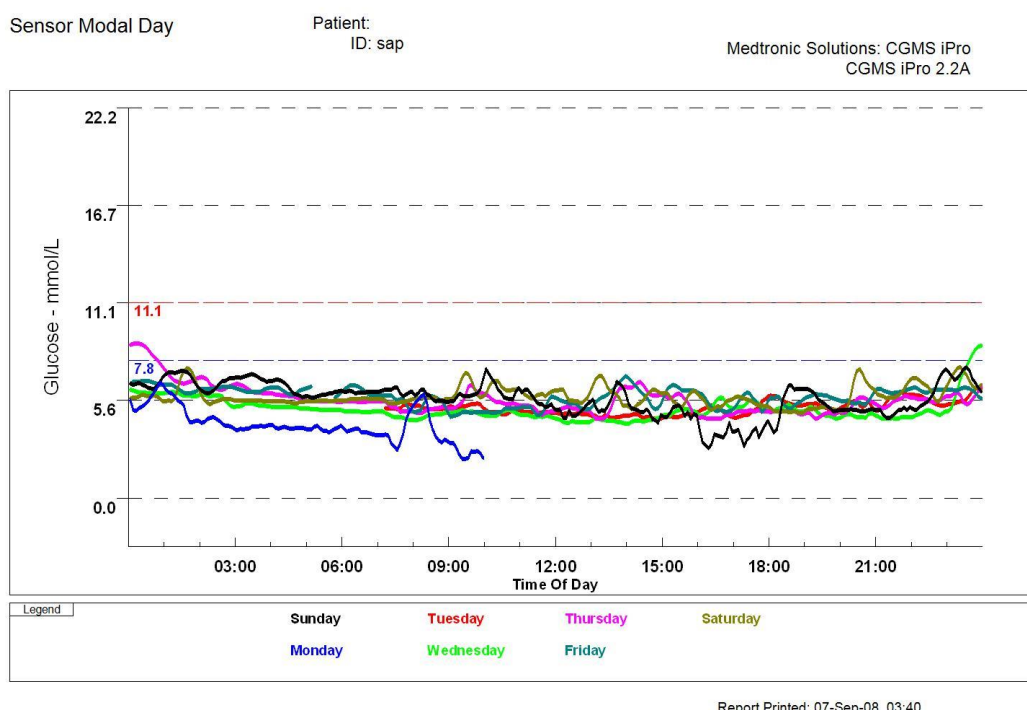
Report Printed: 07-Sep-08 06:38

**Figure 12: Example: Sensor Daily Details for test subject 'SAP'**

-The Sensor Daily Details provided up to fourteen days of individual data plots. Graphs were defined with glucose concentrations on the vertical axis and time on the horizontal axis. The plots showed the sensor data, paired meter values (blue diamond), unpaired meter values (red diamond) and user events during each 24 hour period, with target ranges indicated by the blue dashed line (lower limit) and the red dashed line (upper limit). Individual glucose sensor values were used to draw a profile line on each sensor detail graph. A gap in the profile indicated an interruption in glucose monitoring. Each day was plotted with a different colour.

An example of this, generated for test subject 'SAP' is demonstrated in Figure 12. Each graph also plotted Meter BG values, which were displayed as a blue diamond for paired

meter values and red crosses for unpaired meter values. The maximum glucose value reportable from the CGMS was 22.2 mmol/l and the minimum was 2.2 mmol/l and any glucose values outside this range were displayed as a flat line. The Sensor Modal Day report was another type of report generated by the CGMS and an example of this, generated for test subject 'SAP' is demonstrated in Figure 13. The Sensor Modal Day presented all of the glucose data over a 24 hour period, with each day represented as separate plot line in a different colour. Labelling of axes, reportable glucose values and day plot colours were the same as those in Sensor Daily details.



**Figure 13: Example: Sensor Modal Day for test subject 'SAP'**

-This demonstrated all of the glucose data over a 24 hour period, with each day being represented by a plot line of a different colour (7 days in total).

A further report generated was the Sensor Data report, which is a computerised logbook that presented all of the data entered in the patient file and downloaded from the meter and CGMS *iPro*<sup>TM</sup> recorder memory for a patient download. The Sensor Data report obtained from test subject 'SAP' can be seen in the example below (Figure 14).



Sensor Data		Patient:		Medtronic Solutions: CGMS iPro							
		ID: sap		CGMS iPro 2.2A							
Date/Time	Sample#	ISIG	VCTR	Meter Value*	Slope	Offset	Valid ISIG	Sensor Value*	User Events	System Events	Page 7 Alarms
15-Sep-09 06:38	193	12.77	-0.475								
15-Sep-09 06:43	194	12.69	-0.475								
15-Sep-09 06:48	195	12.62	-0.475								
15-Sep-09 06:53	196	12.58	-0.475								
15-Sep-09 06:58	197	12.59	-0.475								
15-Sep-09 07:03	198	12.63	-0.475								
15-Sep-09 07:08	199	12.68	-0.475								
15-Sep-09 07:13	200	12.70	-0.475		9.9	3	12.30	5.1			
15-Sep-09 07:18	201	12.62	-0.475		9.9	3	12.31	5.1			
15-Sep-09 07:23	202	12.30	-0.475	5.1	9.9	3	12.32	5.1			
15-Sep-09 07:28	203	12.31	-0.475		9.9	3	12.33	5.1			
15-Sep-09 07:33	204	12.32	-0.475		9.9	3	12.22	5.1			
15-Sep-09 07:38	205	12.33	-0.475		9.9	3	12.34	5.1			
15-Sep-09 07:43	206	12.22	-0.475		9.9	3	12.55	5.3			
15-Sep-09 07:48	207	12.34	-0.475		9.9	3	12.71	5.3			
15-Sep-09 07:53	208	12.55	-0.475		9.9	3	12.67	5.3			
15-Sep-09 07:58	209	12.71	-0.475		9.9	3	12.55	5.3			
15-Sep-09 08:03	210	12.67	-0.475		10.0	3	12.45	5.2			
15-Sep-09 08:08	211	12.55	-0.475		10.0	3	12.50	5.3			
15-Sep-09 08:13	212	12.45	-0.475		10.0	3	12.40	5.2			
15-Sep-09 08:18	213	12.50	-0.475		10.0	3	12.23	5.1			
15-Sep-09 08:23	214	12.40	-0.475		10.0	3	12.16	5.1			
15-Sep-09 08:28	215	12.23	-0.475		10.0	3	12.12	5.1			
15-Sep-09 08:33	216	12.16	-0.475		10.0	3	12.07	5.1			
15-Sep-09 08:38	217	12.12	-0.475		10.0	3	12.07	5.1			
15-Sep-09 08:43	218	12.07	-0.475		10.1	3	12.07	5.1			
15-Sep-09 08:48	219	12.07	-0.475		10.1	3	12.06	5.1			
15-Sep-09 08:53	220	12.07	-0.475		10.1	3	12.11	5.1			
15-Sep-09 08:58	221	12.06	-0.475		10.1	3	12.22	5.2			
15-Sep-09 09:03	222	12.11	-0.475		10.1	3	12.30	5.2			
15-Sep-09 09:08	223	12.22	-0.475		10.1	3	12.17	5.2			
15-Sep-09 09:13	224	12.30	-0.525		10.1	3	12.12	5.1			

\* mmol/L

Date Printed: 12-Sep-08 01:58

**Figure 14: Example: Sensor Data report for test subject 'SAP'**

The above section of sensor data was taken from the computerised logbook for the test subject. Two readings of interest can be seen, (i) the meter values, i.e. the SMBG readings taken by the subject and (ii) the sensor values in mmol/L, calculated from the sensor glucose electronic signal and the calibration constants. \*ISIG = CGMS monitor signal value in nano amp units; \*\*VCTR = CGMS monitor signal value in Voltage units; \*\*\*Valid ISIG = Validated sensor signals reported in nano amp units.

In Figure 14, two readings of interest can be seen, (i) the meter values, i.e. the SMBG readings taken by the subject and (ii) the sensor values in mmol/L, calculated from the sensor glucose electronic signal and the calibration constants. The sensor values for each study subject formed the raw data, on which the statistical analysis of the study was based.

## 2.9 Glucose Biomarkers

An attempt was made to identify any biomarkers that predict progression from abnormal glucose tolerance to T2DM. Therefore, for each study subject that attended one year follow up, glycaemic control and beta cell function were further assessed in addition to the repeat OGTT. Glucose (fasting indicator of glucose homeostasis), HbA1c (intermediate term glycaemic control) and biomarkers of beta cell function, C-

peptide and Insulin were assayed (Varvel *et al* 2014) (40); GAD Antibody was also tested. At one year follow up, an additional 10ml of peripheral venous blood was obtained for each study subject at 0 minutes (fasting) for this analysis. Ideally, the analysis of GAD Antibody, C-peptide and Insulin would have been conducted at baseline, however the original ethically agreed study protocol did not have this analysis incorporated. An amendment to the original study protocol was submitted and agreed by the Ethics committee in order for this analysis to occur at Year 1. For this particular procedure, peripheral venous blood was taken into BD Vacutainers® and the sample was inverted five times. It was left to stand for 20 minutes at room temperature and then spun on a swing bucket centrifuge for 10 minutes at 1000 G. The serum was extracted using a pipette and stored in a labelled eppendorf tube at -80<sup>0</sup>c. At the end of the 1 year follow up testing for all subjects, the stored frozen samples were batched and sent to the Diabetes Research Unit, Swansea University on dry ice for analysis of GAD Antibody, C-peptide and Insulin.

### **2.9.1 GAD Antibody**

A glutamic acid decarboxylase autoantibody test (GAD antibody test) was also conducted to look for type 1 diabetes mellitus or latent autoimmune diabetes of adults (LADA) in any of study subjects. The antigens recognised by these antibodies include insulin, glutamic acid decarboxylase (GAD65 kDa isoform) and an islet cell antigen IA-2 or ICA-512. In this study, Glutamic Acid Decarboxylase (GAD) was assayed by Dr Gareth Dunseath at the Diabetes Research Unit, Swansea University, using a kit (GDE/96) obtained from RSR Ltd. The assay sensitivity was 0.06 U/ml, with an assay range of 0 - 2000 U/ml and a reference range of <5 U/ml = negative; ≥5 U/ml = positive (69).

### **2.9.2 C-peptide**

C-peptide is a peptide composed of 31 amino acids and is produced from the pancreatic beta cells during enzymatic cleavage of proinsulin. Proinsulin is the precursor of C-peptide and insulin, which are produced in equal amounts during enzymatic cleavage. C-peptide has negligible extraction by the liver and constant peripheral clearance. It is mainly excreted by the kidney, and its half-life is 3-4 times longer (20-30 v 3-5 minutes) than that of insulin. It therefore circulates at concentrations approximately five times higher than insulin in the systemic circulation and can therefore be used to assess endogenous insulin secretion (70).

The C-Peptide Assay kit (IV2-004/104) was obtained from Invitron and assayed by Dr Gareth Dunseath at the Diabetes Research Unit, Swansea University. The assay had a sensitivity of 5 pmol/ml and a 100% specificity for human C-peptide; the assay range was 0-5.00 pmol/ml, with a reference range of 0.17-0.96 pmol/ml (71).

### **2.9.3 Insulin**

Insulin is an anabolic hormone that promotes glucose uptake, glycogenesis, lipogenesis, and protein synthesis of skeletal muscle and fat tissue through the tyrosine kinase receptor pathway. Insulin is the most important factor in the regulation of plasma glucose homeostasis, as it counteracts glucagon and other catabolic hormones—epinephrine, glucocorticoid, and growth hormone, as described by FJ (1995) (70). Insulin resistance is a condition in which the body produces insulin but does not use it effectively. When people have insulin resistance, glucose builds up in the blood instead of being absorbed by the cells, leading to T2DM or pre-diabetes. Insulin testing can be used to assist in diagnosing early T2DM, where there is a relatively increased production of insulin with a concurrent increase in blood glucose levels (43).

The insulin assay kit was obtained from Invitron (IV2-001/101) Invitron Ltd and assayed by Dr Gareth Dunseath at the Diabetes Research Unit, Swansea University. The assay had a sensitivity of 0.25 mU/L and a 100% specificity for human insulin, with an assay range of 0 - 1200 pmol/L (0 - 200 mU/L) and a reference range of fasting plasma of 6 - 100 pmol/L (1 - 16 mU/L) (72).

### **2.10 Statistical Analysis**

In order to perform the statistical analysis, the CGM subject data was exported from Solutions 2.2A® Software for CGMS® *iPro*™ into Microsoft Office Excel 2007. Initial data review and construction of specific data selectors and tables occurred here. The cleaned data was then imported into a statistical package, IBM SPSS Statistics 20, for the final data analysis to occur.

As the aim of this study was to attempt to predict progression from abnormal OGTT to T2DM, the progression of each subject following a repeat OGTT/ FPG, HbA1c +/- CGM was compared to analogous baseline data. This occurred for study subjects at one and three year follow up respectively. With regard to glucose variability, the CGM subject outcome data (which was analysed in SPSS) was derived from the CGM sensor

data (as described in 2.8.3, Figure 14 ) at baseline and one year follow up, respectively. The raw CGM sensor data for each subject at both baseline and one year follow up was exported into Microsoft Office Excel 2007. As an attempt was being made to identify any biomarkers that predict progression from abnormal glucose tolerance, a marker of deteriorating carbohydrate homeostasis would be increased fluctuations in blood glucose levels. In this study, continuous glucose sensing was used to quantify the glucose fluctuations, to assess whether CGMS could help identify people with abnormal glucose tolerance who progress to T2DM. Glycaemic excursion variables were constructed and applied in Excel 2007 to the raw CGM sensor data which had been imported; this was done with the assistance of Professor Richard Ollerton (Sydney Australia). This generated outcome data for each study subject at baseline and year one follow up. This outcome data for each study subject at baseline and year one follow up was then imported into SPSS Statistics 20 for further analysis. The glycaemic excursion parameters devised were as follows:

- (i): Average Glucose (Av Gl): The average glucose over a day for each subject.
- (ii): Average Glucose Excursion from Average (Av Gl excursion from Av): This calculated how far the data varied from the average over the day.
- (iii): Average above Normal Glucose (Av above Norm Gl): This calculated how far the average daily values above a representative "normal" glucose value.
- (iv): Average Glucose Excursion from Normal Glucose (Av Gl excursion from norm Gl): This calculated a similar variability as Av Gl excursion from Av but used the "normal" glucose value rather than the daily average as the baseline.

For each study subject, the glucose variability subject outcome data from CGM based on the chosen parameter of glycaemic excursion noted above (i-iv) underwent statistical analysis in SPSS Statistics 20, with regard to correlation with both OGTT and HbA1c results, for both baseline and follow up data. This was in order to compare glycaemic variability and monitor any change with time. It was also to identify any markers that predicted progression from abnormal glucose tolerance to T2DM based on glycaemic variability results detected by CGM. This process was conducted based on raw sensor data that reflected a complete 24 hours.

## **2.11 SPSS Results Information**

While working in SPSS Statistics 20, for the purpose of this study, a number of individual variables were grouped and labelled within their individual variable group.

### **2.11.1 Age**

The age of the study subjects was noted as part of the demographic data collected at baseline. Subjects were labelled in SPSS Statistics 20 for the analysis as follows (73):

- |   |                      |                     |
|---|----------------------|---------------------|
| 1 | Young Adulthood      | 15 years - 24 years |
| 2 | Middle Adulthood     | 25 years - 44 years |
| 3 | Older Adulthood      | 45 years - 64 years |
| 4 | Adult Retirement Age | > 65 years          |

### **2.11.2 BMI**

The BMI of the study subjects (weight [kg]/height [m<sup>2</sup>]) was noted as part of the demographic data collected at baseline, Year 1 and Year 3 follow - up. Subjects were labelled in SPSS Statistics 20 for the analysis, as follows (74):

- |   |   |
|---|---|
| 1 | Underweight (BMI < 18.5 kg/m <sup>2</sup> )       |
| 2 | Normal weight (BMI 18.5 to 25 kg/m <sup>2</sup> ) |
| 3 | Overweight (BMI 25 to 30 kg/m <sup>2</sup> )      |
| 4 | Obese (BMI > 30 kg/m <sup>2</sup> )               |

### **2.11.3 Smoking Status**

The smoking status of the study subjects was noted as part of the social history collected. Subjects were labelled in SPSS Statistics 20 for the analysis, as follows:

- |   |                                      |
|---|--------------------------------------|
| 0 | non smoker (non smoker + ex- smoker) |
| 1 | smoker                               |

### **2.11.4 CGM Profiles**

The CGM profiles of the study subjects at baseline and Year 1 follow up were visually inspected and divided into three categories according to normal glucose variability. The categories were labelled least variability (APPENDIX 2), medium variability (APPENDIX 3) and most variability (APPENDIX 4). This was repeated for the Year 1

subjects CGM profiles. Subjects were labelled in SPSS Statistics 20 for the analysis as follows:

- 0 least variability
- 1 medium variability
- 2 most variability

The CGM profiles of the study subjects at baseline and Year 1 follow up were inspected blindly by the PI [CGM profiles labelled (SAP)] and by an independent observer, a Lead Diabetic Specialist Nurse [CGM Profiles labelled (PUMP)].

#### **2.11.5 Study Subject Identification**

At the start of the study, each of the subjects that constituted the study sample were given a unique identification code i.e. 'IPRO' plus a number 01 to 045, which they kept throughout the study. At baseline the suffix 'a' was added to the unique identifier; at Year 1, the suffix 'b' was added to the unique identifier and at Year 3, the suffix 'c' was added to the unique identifier. This was in order to collect and hold the data confidentially.

## **3.0 Results: Screening Data**

### **3.1 Analysis of Screening Data**

The recruitment period for the study occurred between 26<sup>th</sup> October 2009 and 20<sup>th</sup> June 2011. In total, 486 subjects were screened over this 20 month period. All subjects made an informed decision as to whether they wished to participate in the study or not, when they attended for their oral glucose tolerance test (OGTT). Those subjects who agreed to participate were then contacted if they met the study inclusion criteria and on the basis of their OGTT result i.e. were found to have either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). They were invited to attend for continuous glucose monitoring (CGM). If subjects did not meet the study inclusion criteria i.e. were normal glucose tolerant (NGT) or had Type 2 diabetes (DM) they were excluded at this point and were not invited to attend for CGM. This process resulted in a total of 486 subjects being screened.

Out of the 486 subjects screened 72.4 % (n = 352) agreed to participate in the study and were not discounted based on initial exclusion criteria. 27.6 % (n = 134) either declined outright to participate or were excluded based on initial exclusion criteria.

#### **3.1.1 Gender**

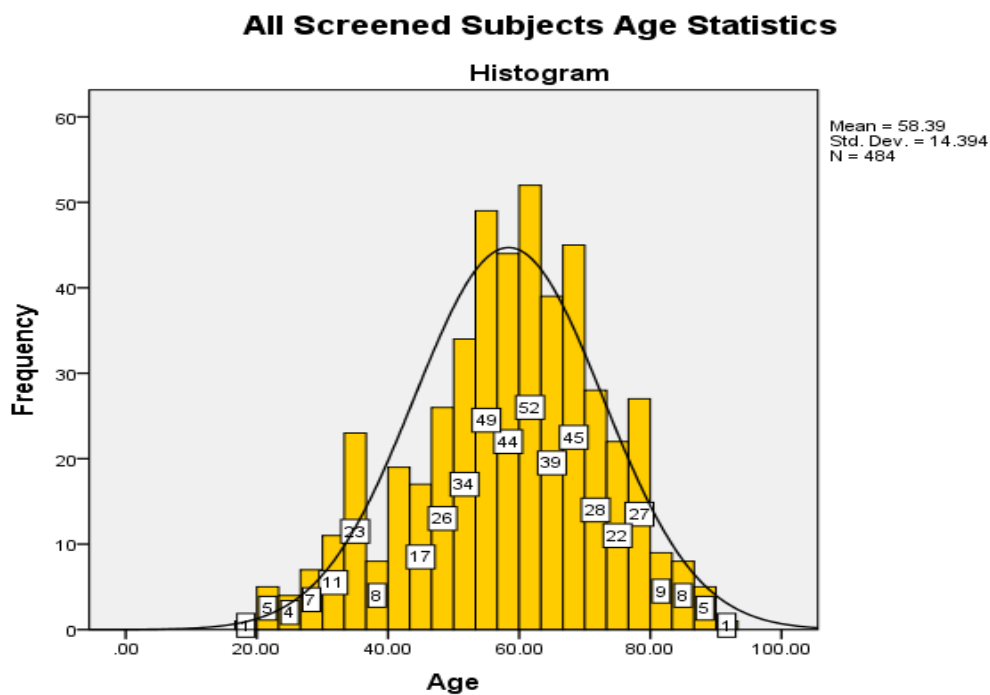
Out of the 486 subjects screened, 42.2% (n = 205) were female and 57.8% (n = 281) were male.

#### **3.1.2 Age (years)**

Out of the 486 subjects eligible for screening, 2 did not give their date of birth and so were classed as missing data. The mean age was 58.39 years and the median age was 59.50 years. The minimum age was 19 years and the maximum age was 90 years, a range in age of 71.5 years (Table 1) (Figure 15).

**Table 1: Total Subjects Screened: Age**

N	Valid	484
	Missing	2
Mean		58.39
Median		59.50
Std. Deviation		14.41
Range		71.50
Minimum		19.00
Maximum		90.50



**Figure 15: Total Subjects Screened: Age**

-484 subjects were screened in the study. The mean age of the subjects screened was 58.39 years and the median age of the subjects screened was 59.50 years, with a range of 71.5 years.

### 3.1.3 Consent

As already discussed, out of 486 subjects initially screened for the study, 352 subjects agreed to participate and 134 subjects did not give consent to participate in the study or were discounted based on study exclusion criteria, respectively. If one looked at the 134 subjects who did not give consent to participate in the study or discounted at the outset based on study exclusion criteria, n = 58 (11.9 %) did not actually attend for the initial screening appointment (DNA); n = 29 (6%) did not give consent to participate in the



study at the outset; n = 22 (4.5%) were aged over 80 years (and therefore were excluded based on study criteria), n = 7 (1.4%) samples / results were lost in processing; n= 5 (1%) were excluded on medical grounds (as discussed in Section 2.5); n = 4 (0.8%) cancelled OGTT appointment; n = 4 (0.8%) declined at the outset due to language difficulties; n = 2 (0.4%) had labelling/request form errors; n = 1 (0.2%) was excluded due to pregnancy; n = 1 (0.2%) had eaten when they should have been fasted and n = 1 (0.2%) did not return for the OGTT 120 minute blood test (Table 2).

**Table 2: Screened Subjects Outcome**

Screened Subject Outcome	Frequency	%	Cumulative Percent
<b>CONSENTED (NGT/DM)</b>	<b>208</b>	<b>42.8</b>	<b>42.8</b>
ABSCONDED MID TEST	1	.2	43.0
CANCELLED	4	.8	43.8
<b>CHANGED MIND</b>	<b>99</b>	<b>20.4</b>	<b>64.2</b>
DECLINED AT OUTSET	29	6.0	70.2
DECLINED AT OUTSET- LANGUAGE	4	.8	71.0
DNA	58	11.9	82.9
EATEN	1	.2	83.1
<b>ENTERED (IFG / IGT)</b>	<b>45</b>	<b>9.3</b>	<b>92.4</b>
EXCLUDED AS PREGNANT	1	.2	92.6
EXCLUDED MEDICAL GROUND	5	1.0	93.6
EXCLUDED OVER 80	22	4.5	98.1
LOST SAMPLES	7	1.4	99.6
NO FORM	1	.2	99.8
WRONG LABEL	1	.2	100.0
Total	486	100.0	

### 3.1.3.1 Consent and Gender

If consent to participate in the study is looked at with regard to gender, out of initial 352 subjects who agreed to participate, 206 (58.52%) were male and 146 (41.48%) were female. If one looked at the initial 134 subjects who did not give agree to participate in the study at the outset or were discounted based on study exclusion criteria, 75 (55.97%) were male and 59 (44.03%) were female (Figure 16).

### Total Subjects Screened: Consent according to Gender

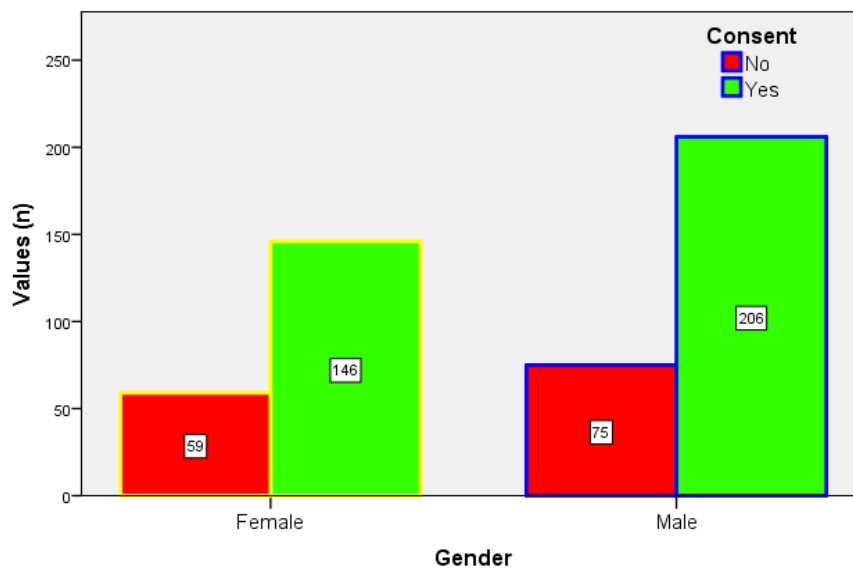


Figure 16: Total Subjects Screened: Consent according to Gender

-Consented subjects consisted of 206 (58.52%) males and 146 (41.48%) females. This compared to 134 non consenting subjects, consisting of 75 (55.97%) males and 59 (44.03%) females.

#### 3.1.3.2 Consent and Age

If consent to participate in the study is looked at with regard to age, out of initial 352 subjects who agreed to participate, there were no missing values. The mean age was 58.99 years and the median age was 60.47 years. The minimum age was 20.52 years and the maximum age was 79.93 years, with a range of 59.41 years (Figure 17).

If one looked at the initial subjects who did not agree to participate in the study at the outset or were discounted based on study exclusion criteria, (Non Consented Group), the mean age was 56.77 years and the median age was 55.80 years. The minimum age was 18.98 years and the maximum age was 90.47 years, with a range of 71.50 years (Figure 18).

Therefore, as the mean age of the consented population was 58.99 years and the mean age of the non-consented population was 56.77 years, the consented population was similar to the non-consented population with regard to age. As 58.52% males gave consent as opposed to 55.97% males who did not give consent and 41.48% females gave consent as opposed to 44.03% who did not give consent, the consented population was also similar to the non-consented population with regard to gender.

### Consented Subjects Age Statistics

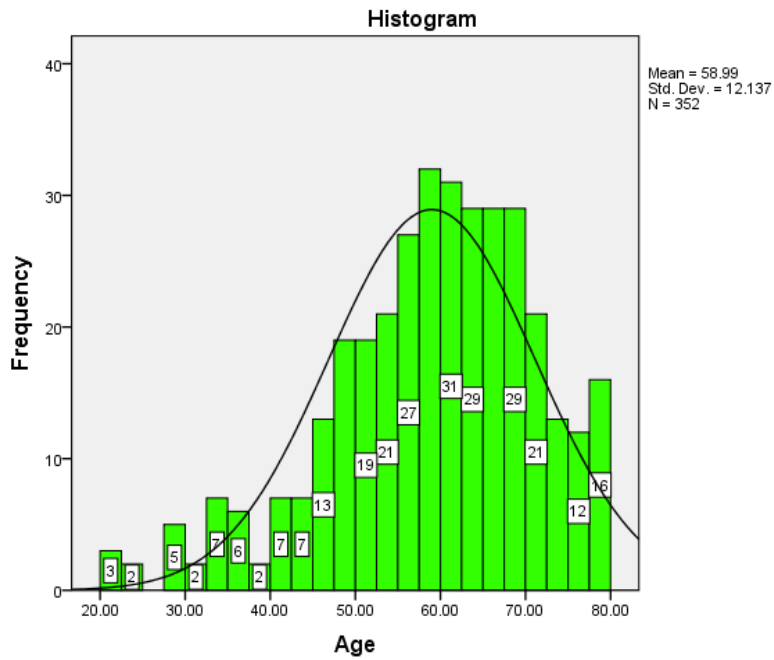


Figure 17: Consented Subjects Age Statistics

-The mean age of consented subjects was 58.99 and the median age was 60.47 years, respectively. The minimum age was 20.52 and the maximum age was 79.93 years, respectively, with a range of 59.41 years. Std Dev was 12.13.

### Non Consented Subjects Age Statistics

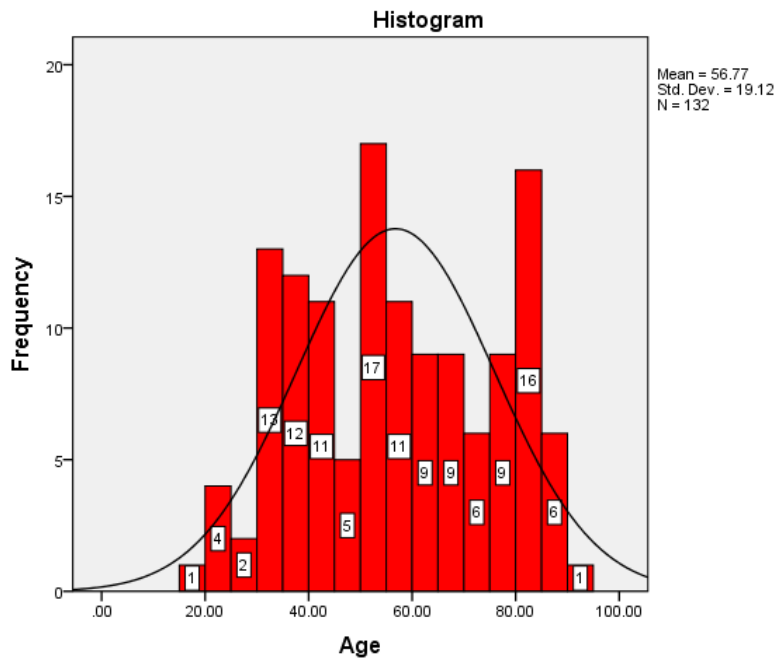


Figure 18: Non Consented Subjects Age Statistics

-The mean age of non consented subjects was 56.77 and the median age was 55.80 years, respectively. The minimum age was 18.98 and the maximum age was 90.47 years, respectively, with a range of 71.50 years. Std Dev was 19.12.

### 3.2 Analysis of OGTT Data

There were 352 subjects who agreed to participate in the study and fitted the study inclusion criteria. All underwent OGTT, which demonstrated n = 208 (42.8%) subjects had either NGT (n = 65) or DM (n = 143) and n = 144 (29.7%) subjects had either IFG/IGT or both. This group of 144 subjects were the key group of subjects suitable to be invited for CGM studies. The 144 subjects in this group were all contacted to attend for CGM studies, however, n = 99 (20.4%) subjects changed their mind about participating in the study despite initially agreeing and being eligible and therefore were lost at this point. This left a total of n = 45 (9.3%) subjects, with either IFG/IGT or both to be entered into the study (Table 3; Figure 19; Figure 20).

**Table 3: OGTT Results of Consented Subjects (n = 352)**

			OGTT				Total
			DM	IFG	IGT	NGT	
DECISION	CHANGED MIND	Count	0	49	50	0	99
		% within DECISION	0.0%	49.5%	50.5%	0.0%	100.0%
		% within OGTT	0.0%	71.0%	66.7%	0.0%	28.1%
	ENTERED	Count	0	20	25	0	45
		% within DECISION	0.0%	44.4%	55.6%	0.0%	100.0%
		% within OGTT	0.0%	29.0%	33.3%	0.0%	12.8%
	UNSUITABLE	Count	143	0	0	65	208
		% within DECISION	68.8%	0.0%	0.0%	31.2%	100.0%
		% within OGTT	100.0%	0.0%	0.0%	100.0%	59.1%
Total		Count	143	69	75	65	352
		% within DECISION	40.6%	19.6%	21.3%	18.5%	100.0%
		% within OGTT	100.0%	100.0%	100.0%	100.0%	100.0%

### OGTT Results of Consented Subjects

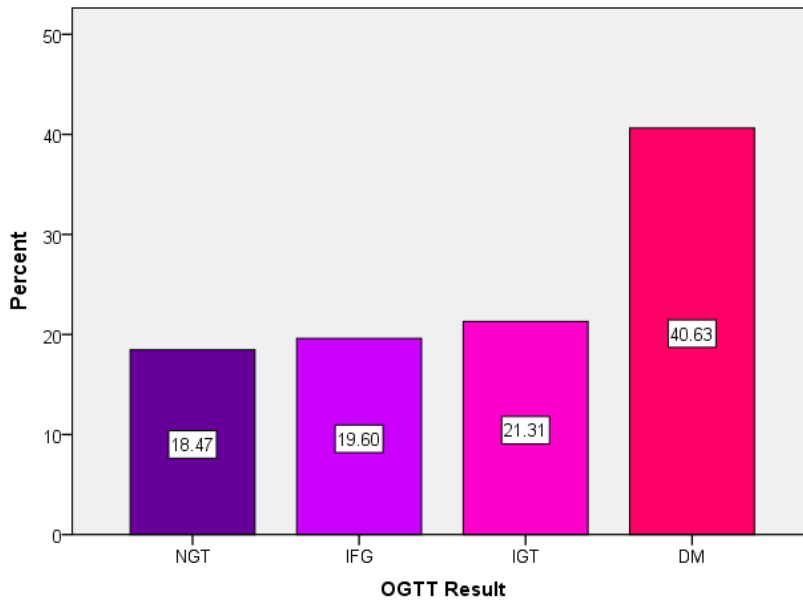


Figure 19: OGTT Results of Consented Subjects (n = 352)

-OGTT demonstrated n = 208 subjects had either NGT (n = 65) or DM (n = 143) and n = 144 subjects had either IFG/IGT or both.

### OGTT Result of Consented Subjects

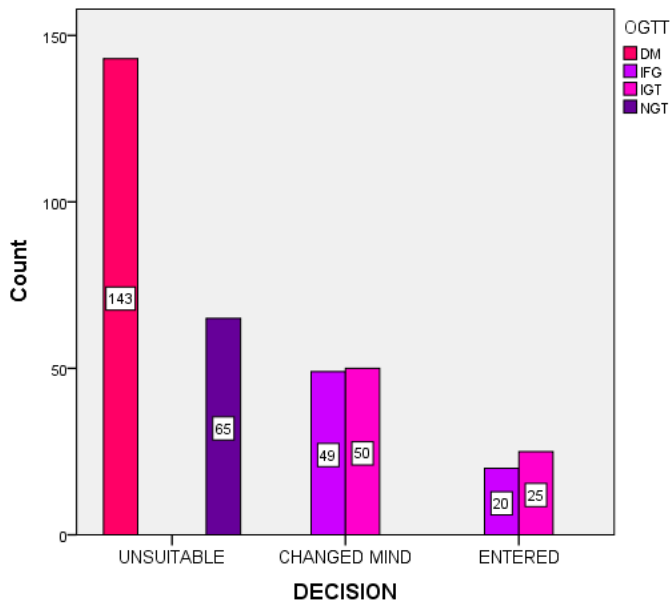


Figure 20: OGTT Results of Consented Subjects

-OGTT testing on consented subjects revealed n = 65 subjects had NGT and n = 143 subjects had DM and were unsuitable. Of the suitable subjects, n = 99 changed their mind, leaving n = 45 subjects, with either IFG/IGT or both entered into the study.

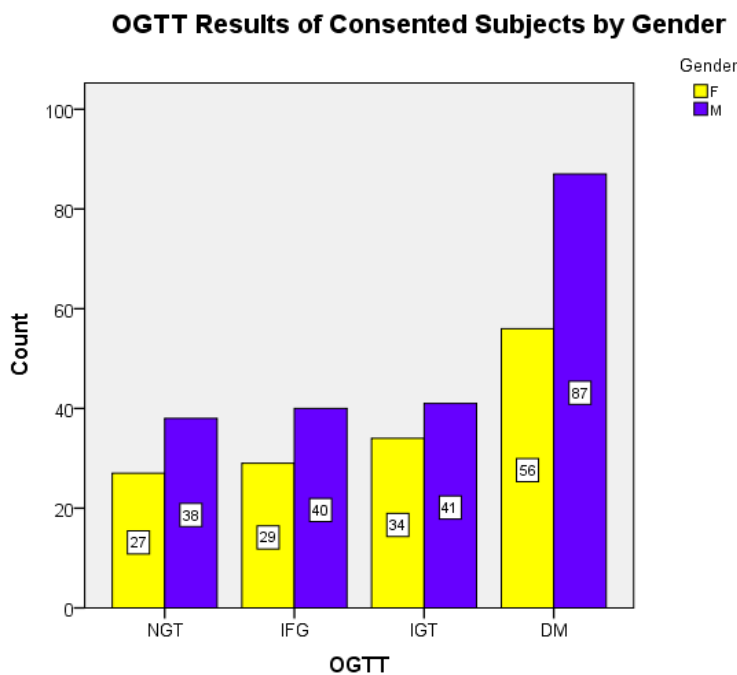
### 3.2.1 Gender

If one looks at consented subjects by gender, out of the 352 subjects with OGTT results, 41.48% (n = 146) were female and 58.52% (n = 206) were male (Table 4; Figure 21).

**Table 4: OGTT Results of Consented Subjects by Gender**

		Gender		Total
		Female	Male	
OGTT	NGT	27	38	65
	IFG	29	40	69
	IGT	34	41	75
	DM	56	87	143
Total		146	206	352

\*Looking specifically at the 146 females, 38.36% had DM, 19.86% had IFG, 23.29% had IGT and 18.49% had NGT; 43.15% having IFG/IGT or both. Looking specifically at the 206 males, 42.23% had DM, 19.42% had IFG, 19.90% had IGT and 18.45% had NGT; 39.32% having IFG/IGT or both.



**Figure 21: OGTT Results of Consented Subjects by Gender.**

-Regarding females, 38.36% (n=56) had DM, 19.86% (n = 29) had IFG, 23.29% (n = 34) had IGT and 18.49% (n = 27) had NGT; 43.15% (n = 63) having IFG/IGT or both at OGTT. Regarding males, 42.23% (n = 87) had DM, 19.42% (n = 40) had IFG, 19.90%, (n = 41) had IGT and 18.45% (n = 38) had NGT; 39.32% (n = 81) having IFG/IGT or both at OGTT.

### 3.2.2 Age

If one looks at consented subjects by age, the mean age of the NGT was 57.42 years, with a range of 58.93 years (minimum 20.52 years; maximum 79.45 years). The mean age of the IFG was 58.55 years, with a range of 49.79 years (minimum 28.85 years; maximum 78.64 years). The mean age of the IGT was 59.86 years, with a range of 56.20 years (minimum 21.74 years; maximum 77.94 years). The mean age of the DM was 59.46 years, with a range of 57.57 years (minimum 22.36 years; maximum 79.93 years); (Table 5).

**Table 5: OGTT Results of Consented Subjects by Age Statistics**

OGTT	Mean	N	Std. Deviation	Minimum	Maximum	Range	Median	% of Total Sum
<b>NGT</b>	<b>57.42</b>	65	12.99	20.52	79.45	58.93	59.79	18.0%
<b>IFG</b>	<b>58.55</b>	69	11.28	28.85	78.64	49.79	59.95	19.5%
<b>IGT</b>	<b>59.86</b>	75	11.81	21.74	77.94	56.20	60.42	21.6%
<b>DM</b>	<b>59.46</b>	143	12.34	22.36	79.93	57.57	60.54	40.9%
Total	58.9938	352	12.13	20.52	79.93	59.41	60.47	100.0%

### 3.3 Consort Diagram of Screening Data

The recruitment outcome for each subject approached to take part in the study can be found summarised in the consort diagram below (Figure 22).

### 3.4 Summary: Chapter 3

In summary, the analysis of screening data and recruitment of study subjects occurred over a 20 month period, between 26<sup>th</sup> October 2009 and 20<sup>th</sup> June 2011. In total, of the 486 subjects that were screened 352 subjects agreed to participate in the study and 134 subjects did not agree to participate or were excluded based on study criteria. With regard to the (n = 352) subjects that agreed to participate and were suitable for study participation, the breakdown of the OGTT result demonstrated 40% had DM (T2DM), 40% had IFG/IGT and 20% were NGT. Out of the 352 that did agree to participate in the study, a further 208 were lost to the study at this point, as they were unsuitable based on OGTT result (NGT or DM). This left 144 subjects suitable for the study (IFG/IGT or both). However, 99 of these subjects changed their mind when contacted to attend for the study. This resulted in 45 suitable subjects (9.3%) being entered into the study at baseline (year 0).

With regard to the (n = 352) subjects that agreed to participate and were suitable for study participation, the breakdown of the OGTT result demonstrated 40% had DM (T2DM), 40% had IFG/IGT and 20% were NGT. These percentages are in keeping with what is reported in the general literature (75)(76). When one looked at 'drop - out' rate for this study, (n = 99) of (n = 144) suitable subjects changed their mind when they were contacted to attend. This gave a 'drop out' rate (at this point in proceedings) of 68.75%. This resulted in an increased amount of time being allocated to recruitment, in order to obtain a sufficient number of subjects for the study (as described in section 2.2).



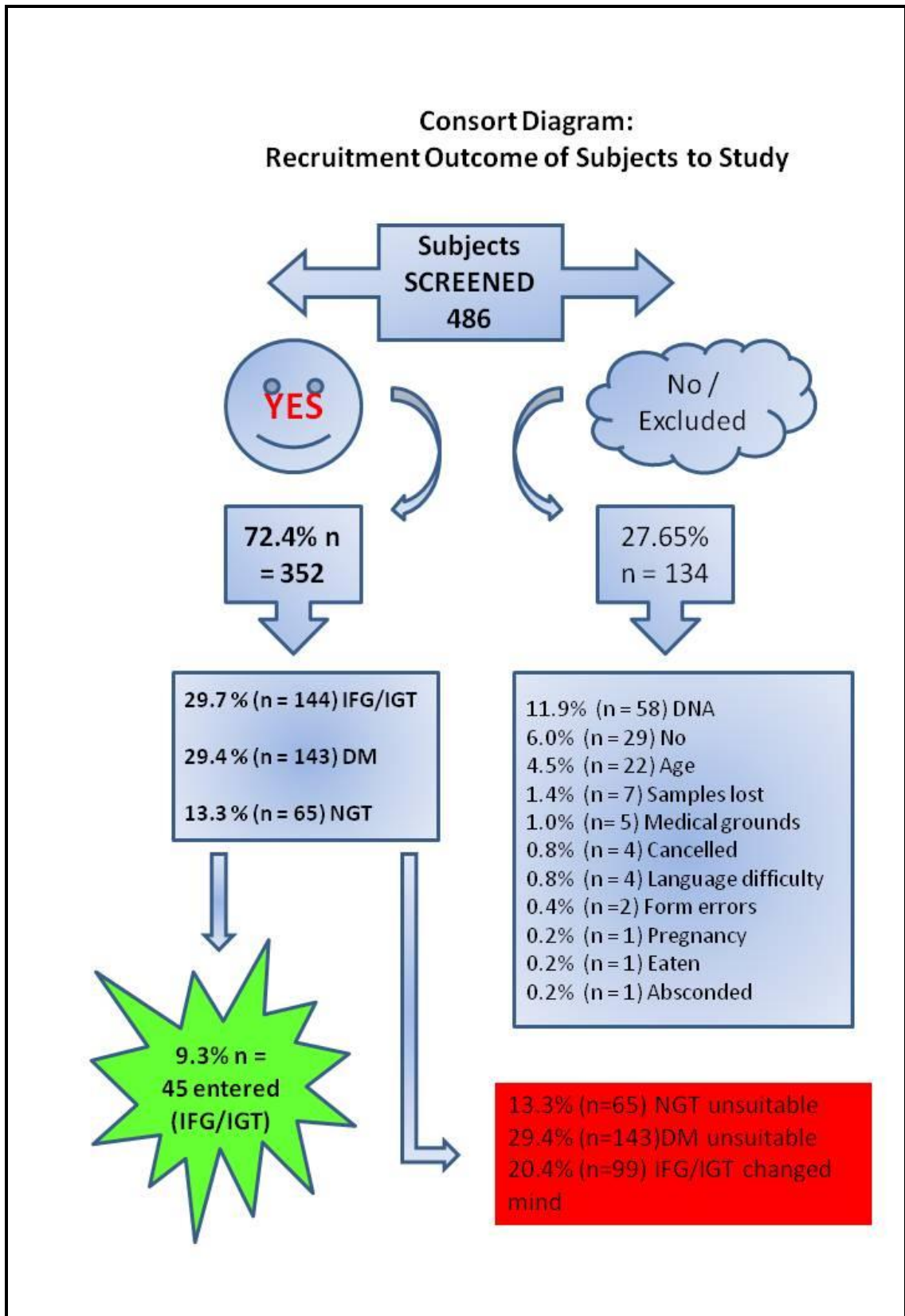


Figure 22: Consort Diagram Summarising Screening Outcome for all Subjects

-n = 352 subjects agreed to participate in the study and 134 did not. 208 were unsuitable, leaving 144 suitable study subjects (IFG/IGT or both), of which 99 of these changed their mind. This resulted in 45 suitable subjects being entered into the study at baseline (year 0).

## 4.0 Results: Study Data

### 4.1 Analysis of Baseline Data

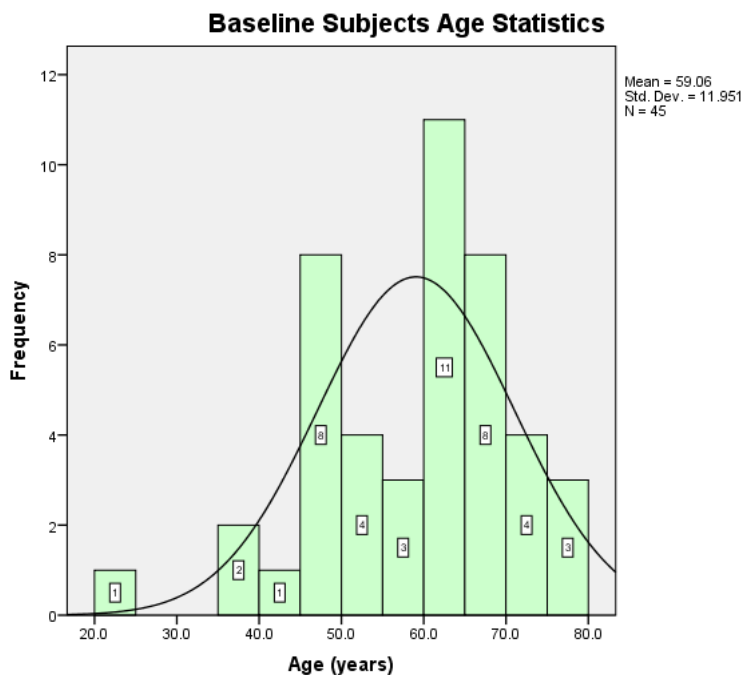
Out of the original screening population, a total of  $n = 45$  (9.3%) subjects, with either IFG/IGT or both to be entered into the study at baseline (Figure 22).

#### 4.1.1 Gender

Out of the 45 baseline subjects ( $n = 45$ ), 42.2% ( $n = 19$ ) were female and 57.8% ( $n = 26$ ) were male.

#### 4.1.2 Age (years)

The mean age of the baseline subjects ( $n = 45$ ) was 59.05 years, with a range in age of 57.4 years (minimum age 21.4 and maximum age 78.8 years) (Figure 23).



**Figure 23: Baseline Subjects Age Statistics (years).**

-The mean age of the baseline subjects screened was 59.06 years, with a range of 57.4 years and a standard deviation of 11.95.

When age was broken down into categories as described in Section 2.11.1, 2.2 % of the subjects were in young adulthood, 6.7% of the subjects were in middle adulthood, 57.8% of the subjects were in older adulthood and 33.3% were of adult retirement age (Table 6).

**Table 6: Baseline Subjects per Age Categories**

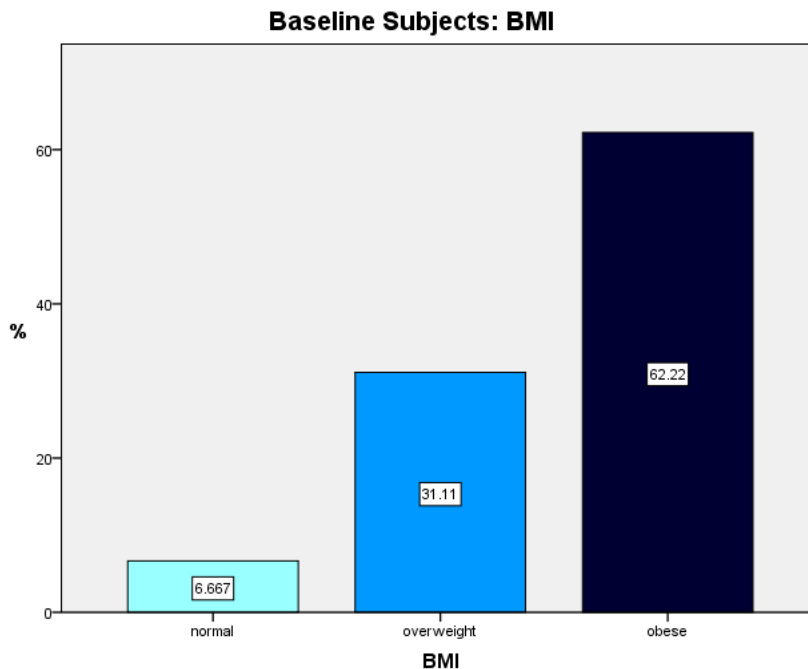
		Frequency	%
	young adulthood	1	2.2
	middle adulthood	3	6.7
	older adulthood	26	57.8
	adult retirement age	15	33.3
	Total	45	100.0

**4.1.3 Ethnicity**

When ethnicity was considered, 91.1% (n = 41) baseline subjects (n = 45) were Caucasian and 8.9% (n = 4) baseline study subjects were non Caucasian.

**4.1.4 Body Mass Index (BMI)**

With regard to BMI, as per the categories stated in Section 2.11.2, 6.7% (n = 3) of the subjects at baseline (n = 45) were classed as having normal weight, 31.1% (n = 14) were classes as being overweight and 62.2% (n = 28) fell into the obese category. No underweight subjects were present (Figure 24).



**Figure 24: Baseline Subjects: BMI**

-With regard to BMI, the majority of the baseline subjects were overweight (31.1%) or obese (62.2%).

#### 4.1.5 Family History of Diabetes

The baseline subjects (n = 45) were questioned regarding family history of diabetes and 44.4% (n = 20) of them had a positive family history of DM and 55.6% (n = 25) did not.

#### 4.1.6 Smoking Status

The baseline subjects (n = 45) were asked about their smoking status. Results demonstrated that 68.9% (n = 31) were non smokers, 13.3% (n=6) were smokers and 17.8% (n = 8) were ex-smokers (Figure 25).

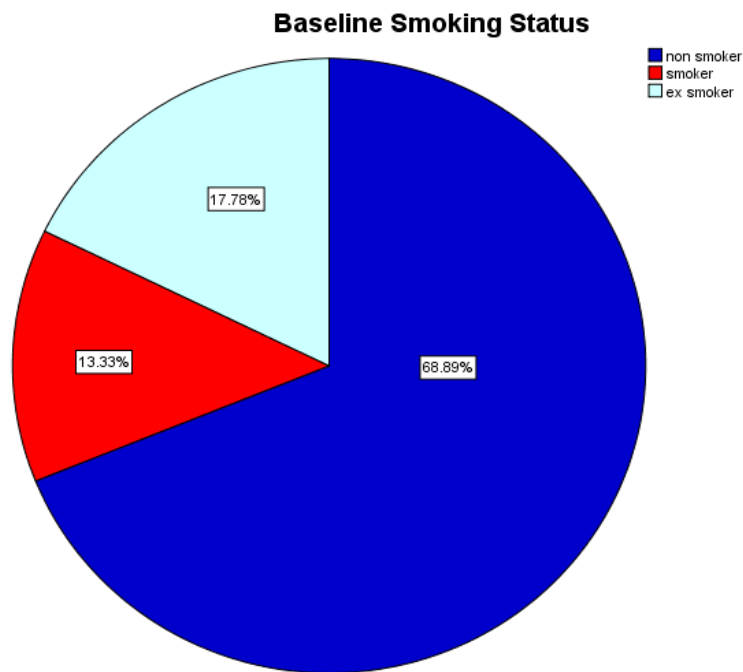


Figure 25: Baseline Subjects: Smoking Status

-68.89% of the baseline subjects were non smokers, 17.78% were ex-smokers and 13.33% were smokers.

#### 4.1.7 Blood Pressure (BP) Status

The baseline subjects (n = 45) were asked if they had a history of hypertension. 68.9% (n = 31) did have hypertension and were on medication for this and 31.1% (n = 14) did not have hypertension.

#### 4.1.8 Lipid Status

The baseline subjects (n = 45) were asked whether they were being prescribed lipid lowering therapy. 46.7% (n = 21) were on lipid lowering medication and 53.3% (n = 24) reported that they were not.

#### 4.1.9 OGTT 0 minutes Glucose (mmol/l)

The mean fasting plasma glucose (FPG) in the baseline subjects (n = 45) was 6.25 mmol/L, with a maximum value of 6.9 mmol/l and a minimum value of 4.8 mmol/l. This demonstrated a range of FPG of 2.1 mmol/l, with a standard deviation (Std Dev) of 0.45 (Figure 26a). IFG in baseline subjects (n = 45) was seen when FPG > 6.1 mmol/l and was indicated by the vertical red line on the X axis, as illustrated in Figure b.

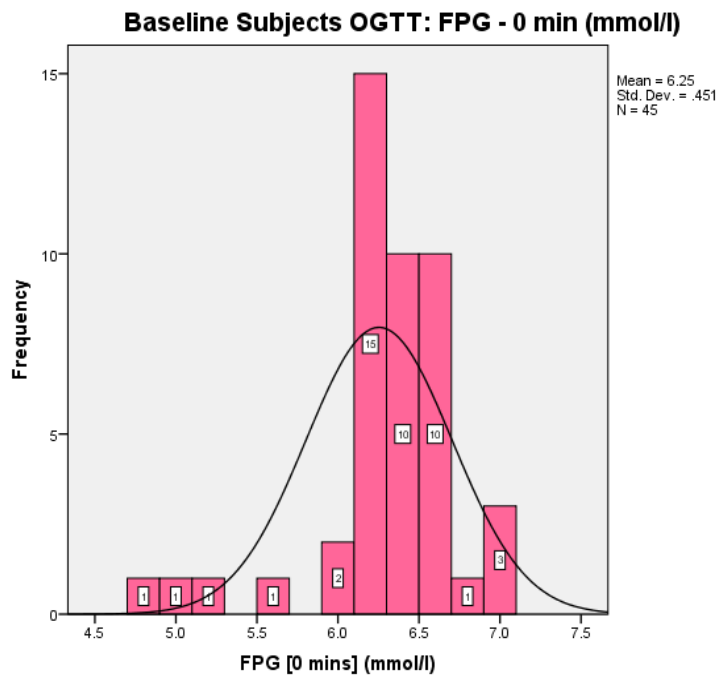
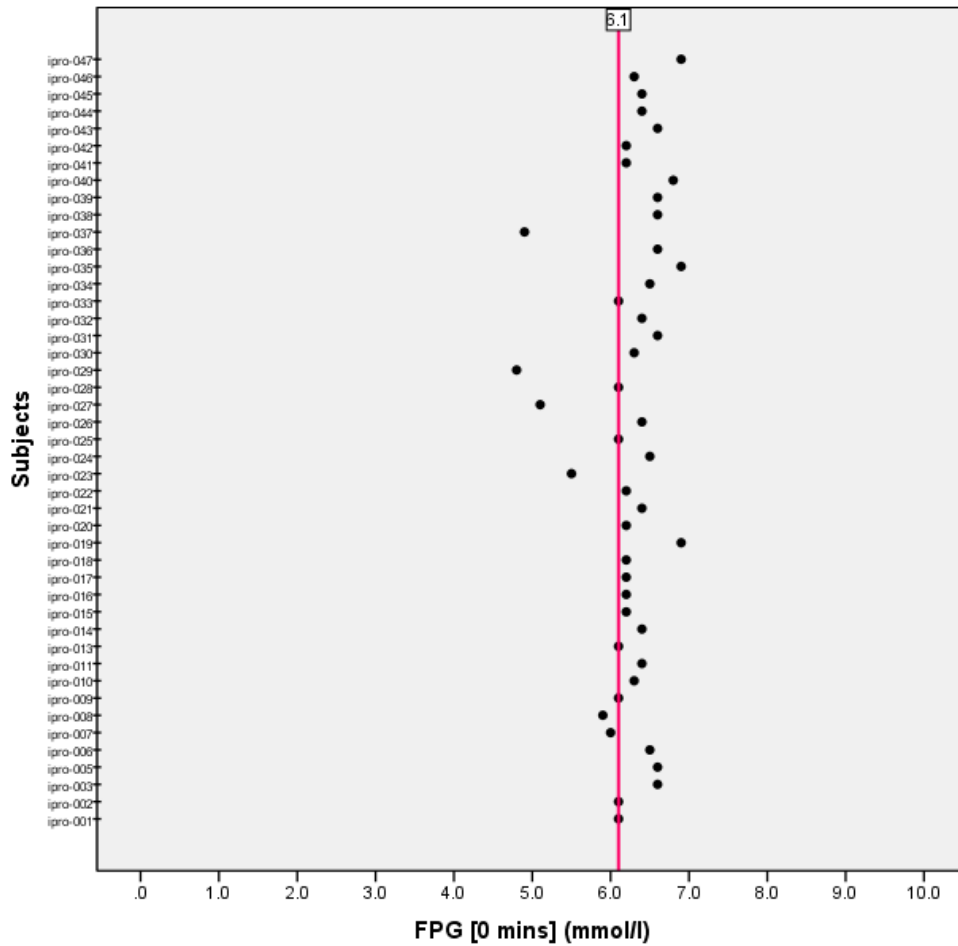


Figure 26a: Baseline Subjects: OGTT 0 minutes Glucose (mmol/l)

-FPG for baseline subjects (n = 45) demonstrated a mean of 6.25 mmol/l and a Std Dev of 0.45.



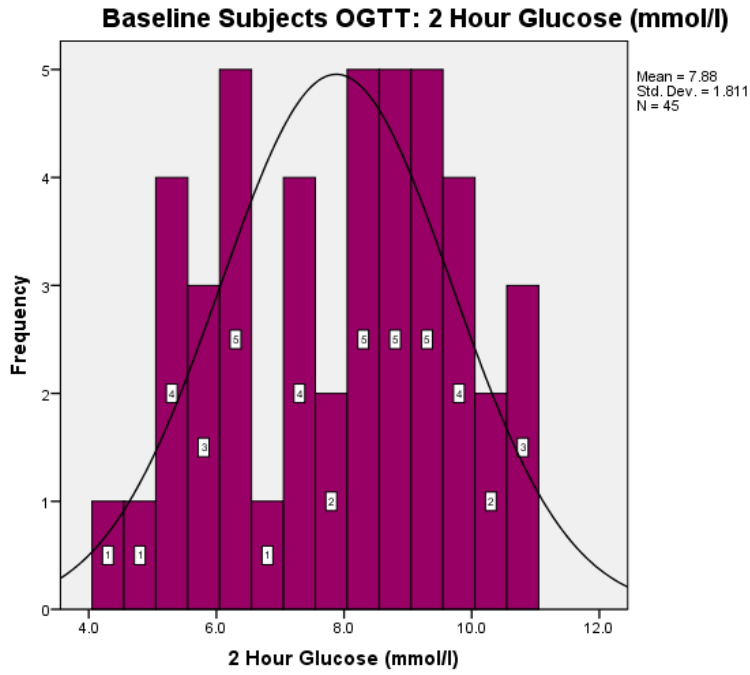
**Figure 26b: Baseline Subjects: OGTT 0 minutes Glucose (mmol/l)**

-Baseline subjects (n = 45) are labelled IPRO-01 to IPRO-47, as can be seen from the Y axis (IPRO-04 and IPRO-12 withdrew from the study prior to its initiation). The X axis represents FPG (mmol/l) and a FPG > 6.1mmol/l for baseline subjects reflected IFG; this point on the X axis was indicated by the vertical red line.

#### **4.1.10 OGTT 2 Hour Glucose (mmol/l)**

The mean OGTT 2 hour Glucose in the baseline subjects (n = 45) was 7.87 mmol/l, with a maximum value of 11.0 mmol/l and a minimum value of 4.3 mmol/l. This gave a range of OGTT 2 hour range of OGTT 2 hour Glucose values of 6.7 mmol/l and a Std Dev of 1.81 (Figure 27: Baseline Subjects OGTT 2 hour Glucose (mmol/l)

).



**Figure 27: Baseline Subjects OGTT 2 hour Glucose (mmol/l)**

-The OGTT 2 Hour Glucose for baseline subjects (n = 45) demonstrated a mean of 7.88 mmol/l and a Std Dev of 1.81.

**4.1.11 HbA1c (mmol/mol)**

The mean HbA1c in the baseline subjects (n = 40) was 43.92 mmol/mol, with a maximum of 53 mmol/mol and minimum of 37 mmol/mol (range 16 mmol/mol) (Figure 28: Baseline Subjects: HbA1c (mmol/mol)

).

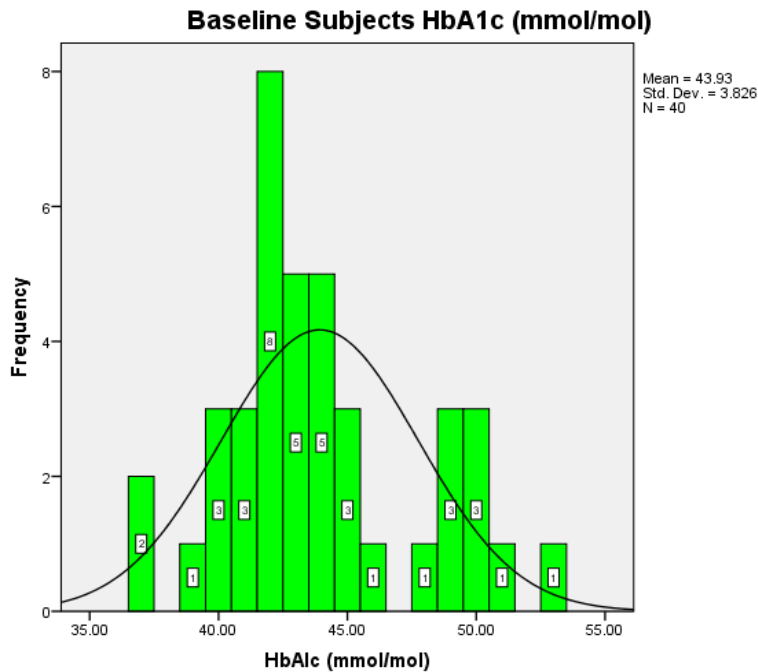


Figure 28: Baseline Subjects: HbA1c (mmol/mol)

-The HbA1c for baseline subjects (n = 40) had a mean of 43.83 mmol/mol and a Std Dev of 3.82, with a maximum of 53 mmol/mol and a minimum of 37 mmol/mol (range 16 mmol/mol).

#### 4.1.12 CGM Glucose Excursion Parameters

As described in Section 2.10, four CGM based glucose excursion parameters were devised and the results at baseline for each subject (n = 45) are seen in Table 7.

Table 7: Summary of CGM Glucose Parameters - Baseline

		MEAN Baseline Av GI (mmol/l)	MEAN Baseline AvGI excursion from Av (mmol/l)	MEAN Baseline Av above norm GI (mmol/l)	MEAN Baseline Av GI excursion from norm GI (mmol/l)
N	Valid	45	45	45	45
	Missing	0	0	0	0
Mean		6.73	.99	2.23	2.48
Std. Deviation		.66	.34	.66	.65
Range		3.13	1.86	3.13	3.02
Minimum		5.27	.36	.77	1.03
Maximum		8.40	2.22	3.90	4.05

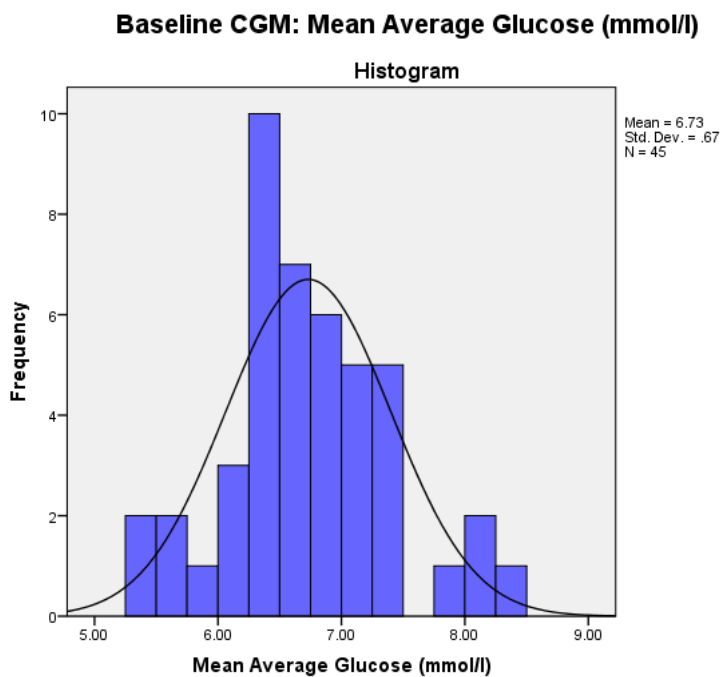
The CGM Mean Average Glucose for the baseline data (n = 45) was 6.73 mmol/l, with a maximum and a minimum CGM Mean Average Glucose of 8.40 and 5.27 mmol/l, respectively (Table 7; Figure respectively (Table 7; Figure 29: Baseline Subjects: CGM Mean Average Glucose (mmol/l)



). The CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l) for the baseline data (n = 45) was 1.0 mmol/l, with a maximum and minimum CGM Mean Average Excursion from the Average Glucose of 2.22 and 0.36 mmol/l, respectively (Table 7, Error! Reference source not found.). The CGM Mean Average Glucose above Normal Glucose (mmol/l) for the baseline data (n = 45) was 2.23 mmol/l, with a maximum and minimum CGM Mean Average Glucose above Normal Glucose of 3.90 and 0.77 mmol/l, respectively (Table 7; Figure 31: Baseline Subjects CGM: Mean Average Glucose above Normal Glucose (mmol/l))

). The CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l) for the baseline data (n = 45) was 2.49 mmol/l, with a maximum and minimum CGM Mean Average Glucose Excursion from Normal Glucose of 4.05 and 1.03 mmol/l, respectively (Table 7, Figure 32: Baseline Subjects CGM: Mean Average Glucose Excursion from Normal Glucose (mmol/l))

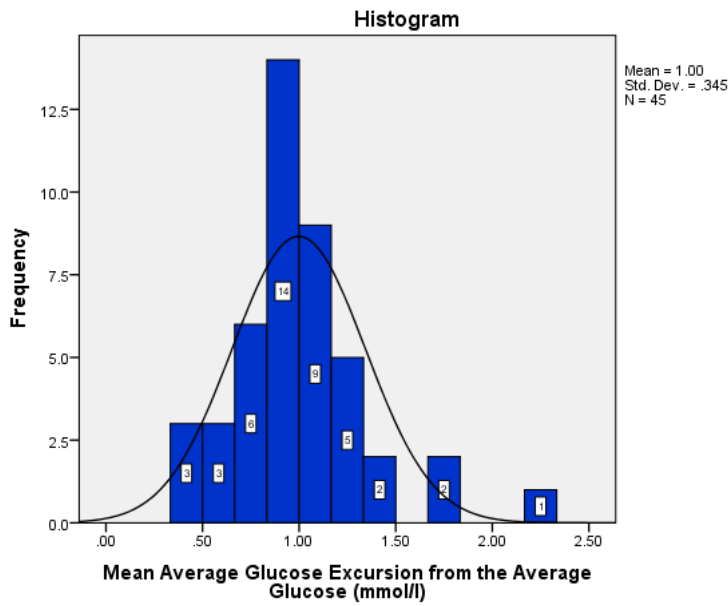
).



**Figure 29: Baseline Subjects: CGM Mean Average Glucose (mmol/l)**

-The CGM Mean Average Glucose for the baseline data (n = 45) was 6.73 mmol/l, with a maximum and minimum CGM Mean Average Glucose of 8.40 and 5.27 mmol/l, respectively.

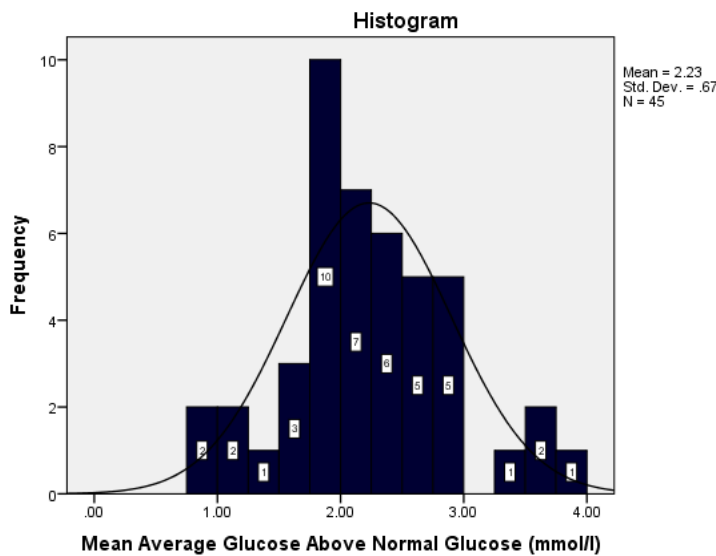
**Baseline CGM: Mean Average Glucose Excursion from the Average Glucose (mmol/l)**



**Figure 30: Baseline Subjects: CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l)**

-The CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l) for the baseline data (n = 45) was 1.0 mmol/l, with a maximum and minimum CGM Mean Average Excursion from the Average Glucose of 2.22 and 0.36 mmol/l, respectively

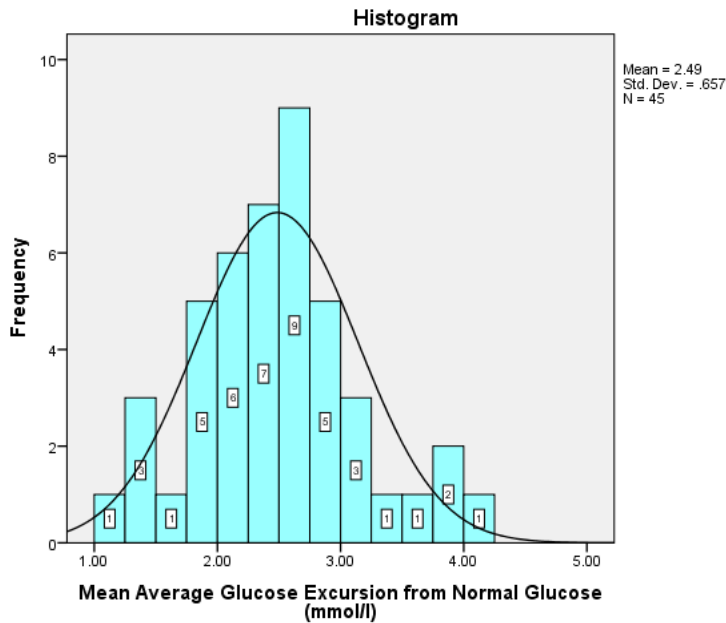
**Baseline CGM: Mean Average Glucose Above Normal Glucose (mmol/l)**



**Figure 31: Baseline Subjects CGM: Mean Average Glucose above Normal Glucose (mmol/l)**

-The CGM Mean Average Glucose above Normal Glucose (mmol/l) for the baseline data (n = 45) was 2.23 mmol/l, with a maximum and minimum CGM Mean Average Glucose above Normal Glucose of 3.90 and 0.77 mmol/l, respectively.

**Bseline CGM: Mean Average Glucose Excursion from Normal Glucose (mmol/l)**



**Figure 32: Baseline Subjects CGM: Mean Average Glucose Excursion from Normal Glucose (mmol/l)**

-The CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l) for the baseline data (n = 45) was 2.49 mmol/l, with a maximum and minimum CGM Mean Average Glucose Excursion from Normal Glucose of 4.05 and 1.03 mmol/l, respectively.

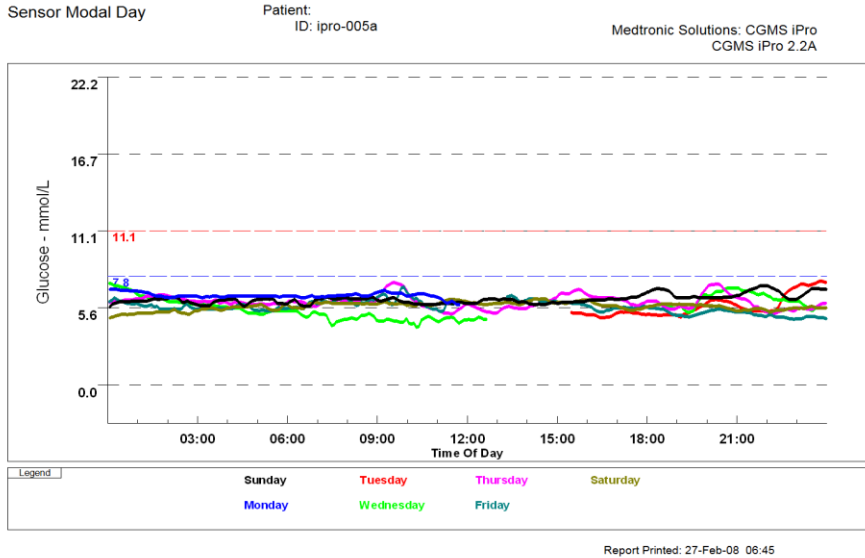
**4.1.13 CGM Profiles**

CGM was conducted on all the subjects at baseline (n= 45). Each CGM profile (Sensor Modal Day) was then inspected by eye and the profiles were placed in one of three groups, according to the groups, according to the “flatness” or “peakiness” of the profile. The three groups were labelled least variability, as illustrated in Figure 33: CGM Profile (SAP) - Least Variability: (a subject illustration)

, medium variability as illustrated in Figure 34: CGM Profile (SAP) - Medium Variability: (a subject illustration)

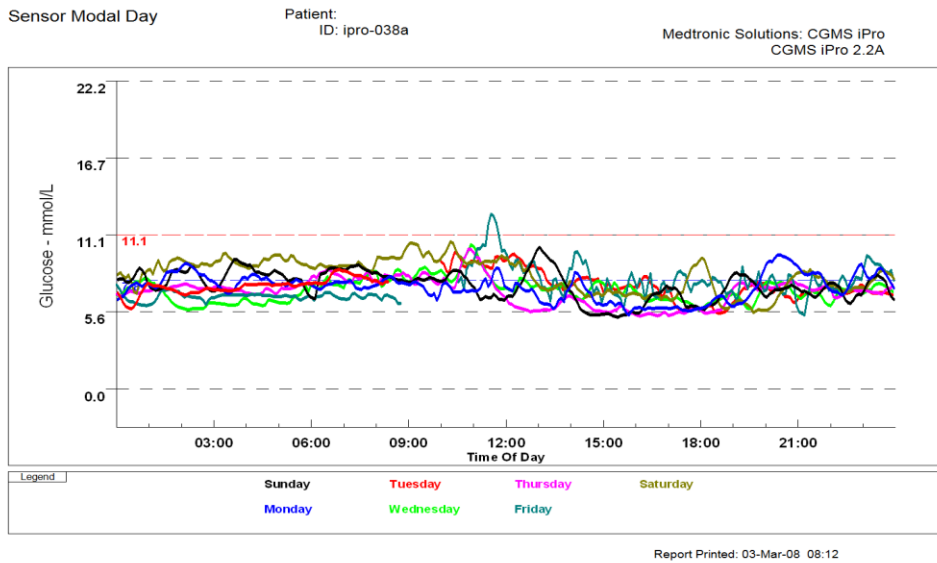
and most variability, as illustrated in Figure 35: CGM Profiles (SAP) - Most Variability: (a subject illustration)

A representative example of each of these three different profiles described above can be seen in the illustrations below. The CGM profiles were inspected blindly by the PI (CGM profiles SAP) and by an independent observer (CGM Profiles PUMP); the independent observer was a Lead Diabetic Specialist Nurse. CGM profiles for each subject at baseline can be seen in the APPENDIX, as APPENDIX 2: Least Variability; APPENDIX 3 – medium variability and APPENDIX 4 – most variability.



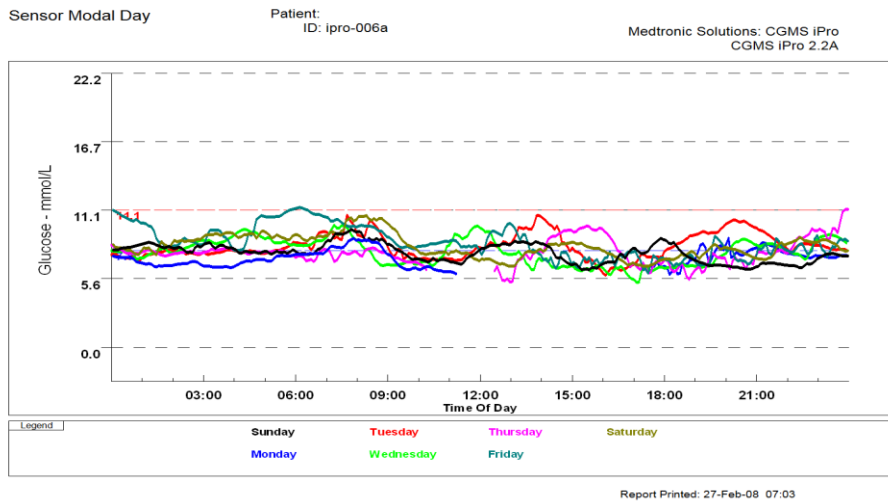
**Figure 33: CGM Profile (SAP) - Least Variability: (a subject illustration)**

-Sensor Modal Day demonstrated all of the glucose data over a 24 hour period from subject IPRO-05a, with each day represented as separate plot line in a different colour.



**Figure 34: CGM Profile (SAP) - Medium Variability: (a subject illustration)**

-Sensor Modal Day demonstrated all of the glucose data over a 24 hour period from subject IPRO-38a, with each day represented as separate plot line in a different colour.



**Figure 35: CGM Profiles (SAP) - Most Variability: (a subject illustration)**

-Sensor Modal Day demonstrated all of the glucose data over a 24 hour period, from subject ipro-006a, with each day represented as separate plot line in a different colour.

One can see that when the CGM (SAP) profiles were inspected (Table 8), 20% (n = 9) of them were observed to demonstrate least variability, 48.9% (n = 22) were observed to demonstrate medium variability and 31.1% (n = 14) were observed to demonstrate most variability. These results closely mirrored the independent observers assessment of variability (

Table 9), which demonstrated 22.2% (n = 10) of the CGM (PUMP) profiles were observed to demonstrate least variability, 46.7% (n = 21) were observed to demonstrate medium variability and 31.1% (n = 14) were observed to demonstrate most variability. When this was looked at in more detail, there was only a single disagreement between assessors. The investigator placed one less CGM profile in the least variability category, compared to the independent observer (PUMP); the remainder were identical.

**Table 8: Baseline Subjects CGM Profiles (SAP)**

	Frequency	%
least variability	9	20.0
medium variability	22	48.9
most variability	14	31.1
Total	45	100.0

**Table 9: Baseline Subjects CGM Profiles (PUMP)**

		Frequency	%
	least variability	10	22.2
	medium variability	21	46.7
	most variability	14	31.1
	Total	45	100.0

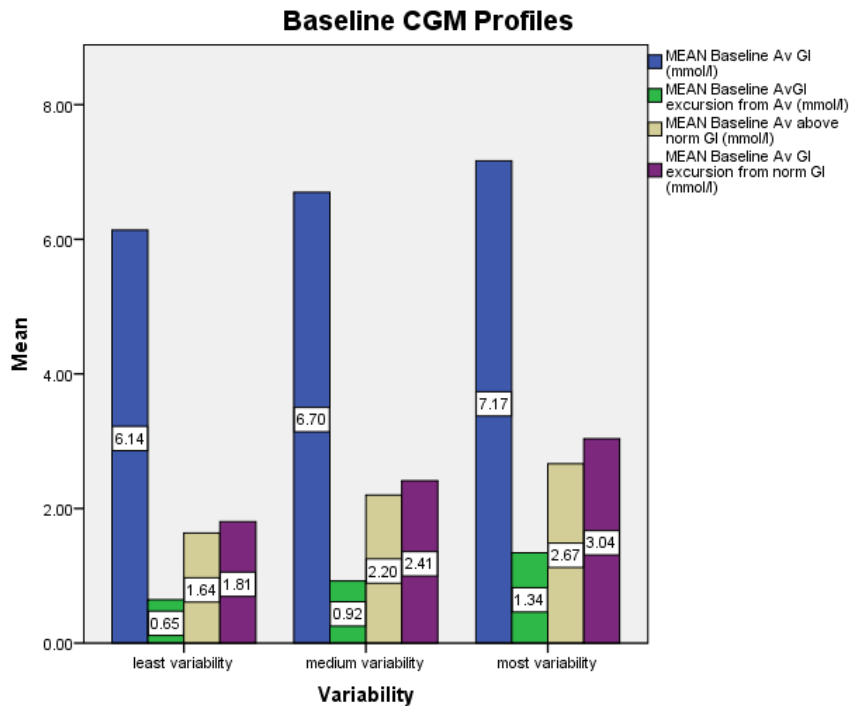
At baseline, each of the four CGM parameters described in Section 2.10, were looked at with regard to their relationship with the CGM profile groups. As both the PI (SAP) and the independent observer (PUMP) findings were very similar, with regard to the degree of observed glucose of observed glucose variation in the subjects CGM profiles, for the purpose of this study, the investigators observations of CGM Profiles (SAP) were used in the remaining analysis. The CGM analysis. The CGM parameter Mean Baseline Average Glucose was 6.14 mmol/l for least variability CGM profile (SAP), rising to 6.70 mmol/l for medium variability CGM profile (SAP) up to 7.17 mmol/l for most variability CGM profile (SAP) (Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Baseline Average Glucose Excursion from Average (mmol/l) was 0.65 mmol/l for least variability CGM profile (SAP), rising to 0.92 mmol/l for medium variability CGM profile (SAP) up to 1.34 mmol/l for most variability CGM profile (SAP) (Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Baseline Average Glucose above Normal Glucose (mmol/l) was 1.64 mmol/l for least variability CGM profile (SAP), rising to 2.20 mmol/l for medium variability CGM profile (SAP), up to 2.67 mmol/l for most variability CGM profile (SAP) (Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l) was 1.81 mmol/l for least variability CGM profile (SAP), rising to 2.41 mmol/l for medium variability CGM profile (SAP), up to 3.04 mmol/l for most variability CGM profile (SAP) (Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP))

).



**Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP)**

-At baseline, for CGM profile (SAP): the CGM parameter Mean Baseline Average Glucose was 6.14 mmol/l for least variability, rising to 6.70 mmol/l for medium variability and up to 7.17 mmol/l for most variability; the CGM parameter Mean Baseline Average Glucose Excursion from Average (mmol/l) was 0.65 mmol/l for least variability, rising to 0.92 mmol/l for medium variability and up to 1.34 mmol/l for most variability; the CGM parameter Mean Baseline Average Glucose above Normal Glucose (mmol/l) was 1.64 mmol/l for least variability, rising to 2.20 mmol/l for medium variability and up to 2.67 mmol/l for most variability; the CGM parameter Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l) was 1.81 mmol/l for least variability, rising to 2.41 mmol/l for medium variability and up to 3.04 mmol/l for most variability.

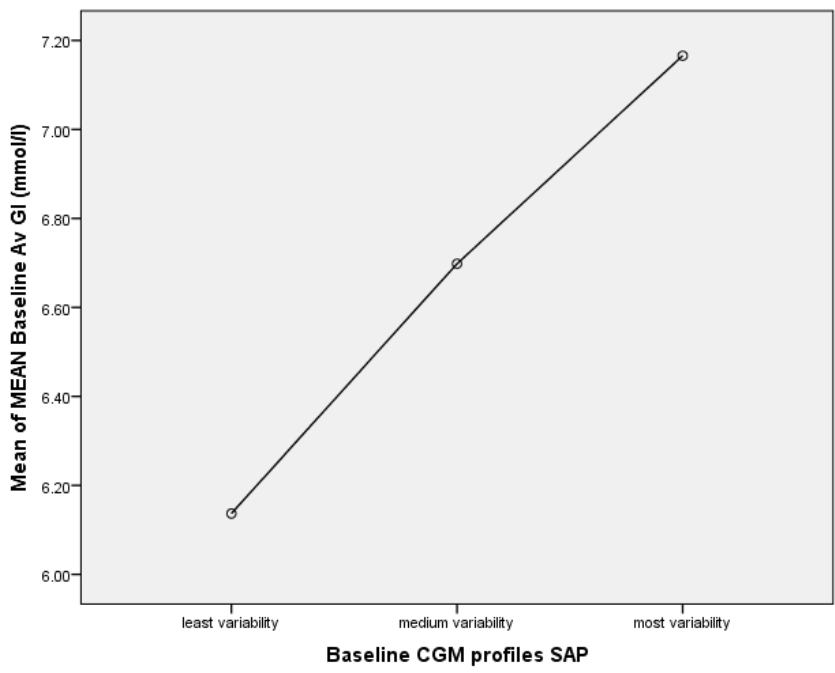
At baseline, each of the four CGM parameters described in Section 2.10, were looked at with regard to their relationship with the CGM profile groupings. As the degree of variability of the subject variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability to most variability, the mean of each of the four CGM parameters was observed to increase also, as observed to increase also, as discussed above, which can be seen from the mean plots (Figure 37: Summary Baseline Subjects CGM Parameters v CGM Profile (SAP) - Mean Plots

) (Figure 38: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

) (Figure 39: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

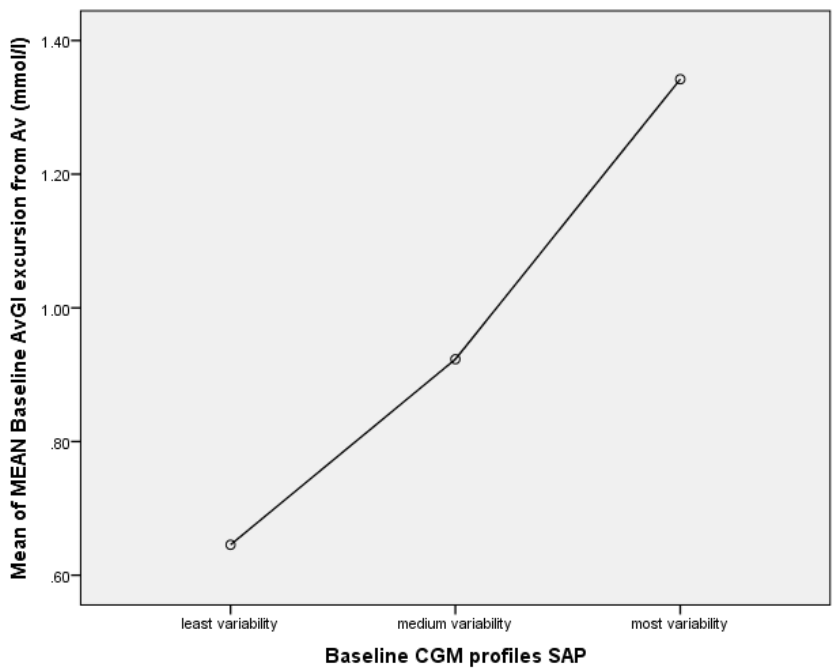
) (Figure 40: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

) respectively.



**Figure 37: Summary Baseline Subjects CGM Parameters v CGM Profile (SAP) - Mean Plots**

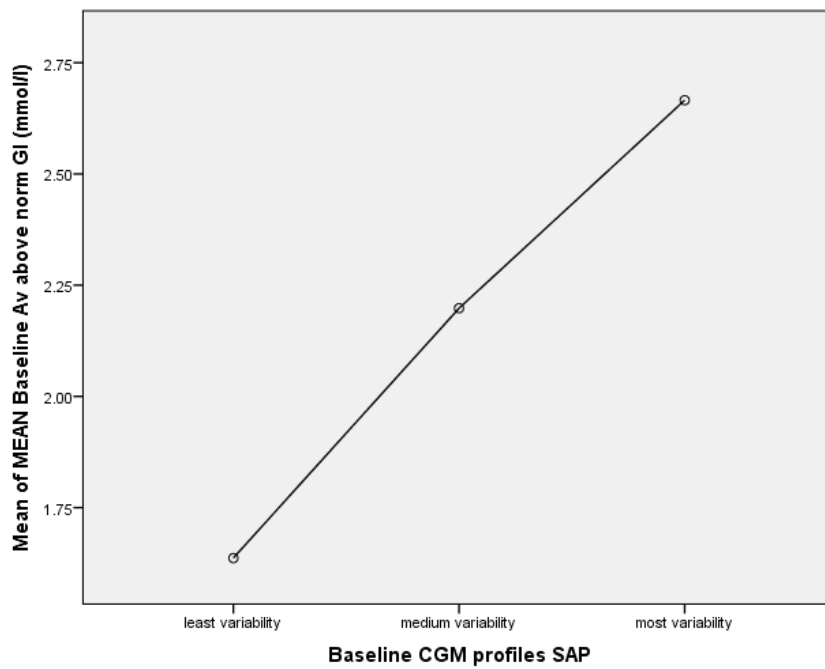
-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose (mmol/l) was also observed to increase.



**Figure 38: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

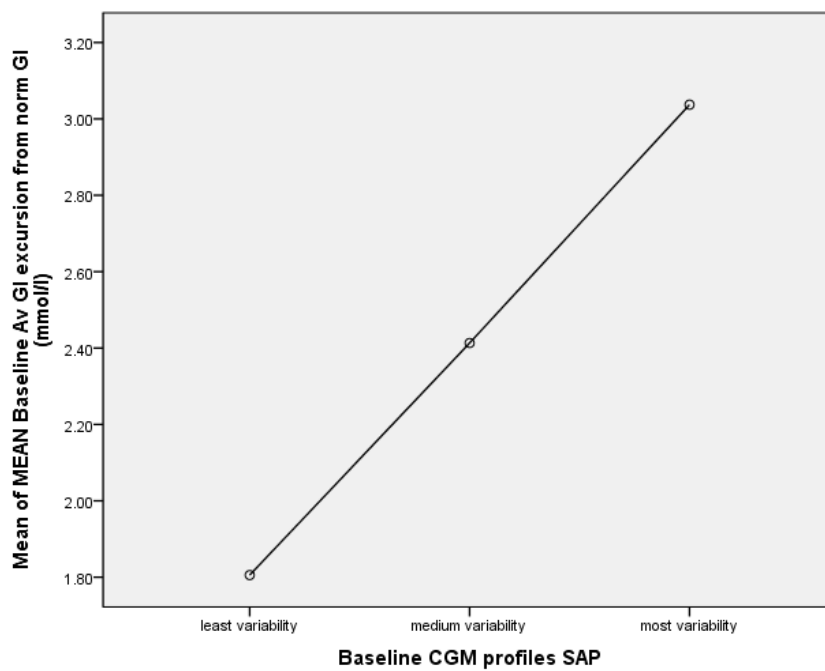
-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from Average (mmol/l) was also observed to increase.





**Figure 39: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average above normal Glucose (mmol/l) was also observed to increase.



**Figure 40: Summary Baseline Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from normal Glucose (mmol/l) was also observed to increase.

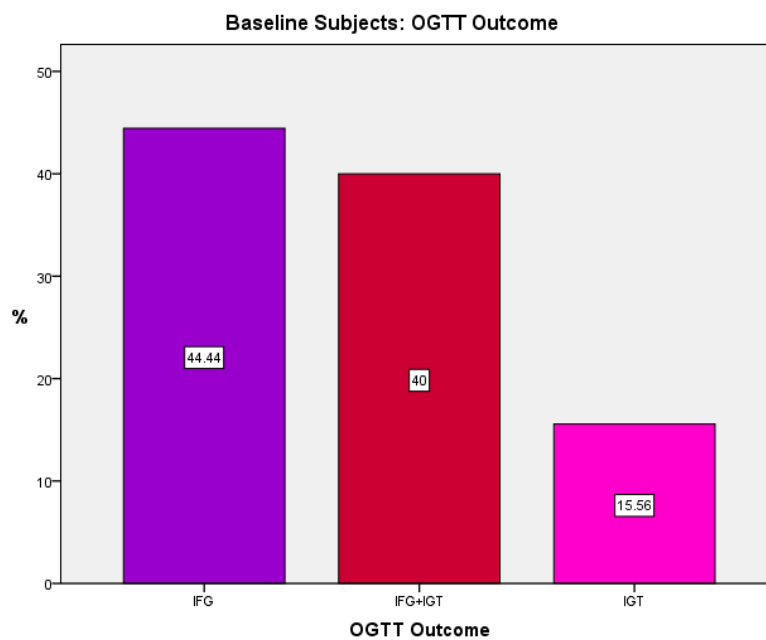
#### 4.1.14 OGTT Results

The OGTT outcome at baseline for all subjects (n = 45) demonstrated 44.4% (n= 20) subjects with IFG, 40% 9 (n = 18) subjects with both IFG and IGT and 15.6% (n = 7) subjects as having IGT (Table 10, Figure 41: Baseline OGTT Outcome

).

**Table 10: Baseline OGTT Outcome**

	Frequency	Percent
IFG	20	44.4
IFG+IGT	18	40.0
IGT	7	15.6
Total	45	100.0



**Figure 41: Baseline OGTT Outcome**

-The OGTT outcome at baseline for all subjects (n = 45) demonstrated 44.4% (n= 20) subjects with IFG, 40% (n = 18) subjects with both IFG and IGT and 15.6% (n = 7) subjects as having IGT.

If one looked at age at baseline with regard to OGTT outcome, one can see that there were very few young adults with IFG, IGT or both, while those who were in middle adulthood appeared to have a fairly even distribution of subjects in all OGTT categories. IFG was demonstrated to be more prominent in older subjects, while IGT was demonstrated to be more prominent in subjects of adult retirement age (Table 11, Figure 42: Baseline OGTT Outcome: Age)

). If one looked at gender at baseline with regard to OGTT, there appeared to be more male subjects with IFG and IFG+IGT than females, who themselves appeared to have more IGT (Table 12, Figure 43: Baseline OGTT Outcome: Gender)

). If one looked at OGTT outcome with regard to ethnicity, it is very difficult to make any real informative comments in this instance, given over 90% of the study subjects were Caucasian (Table 13, Figure 44: Baseline OGTT Outcome: Ethnicity)

). Out of the 20 subjects that had a positive family history (FHx) of DM, 60% of them at baseline had IFG+IGT, while this outcome was far less prominent in those who didn't have a FHx of DM (Table 14, Figure DM (Table 14, Figure 45: Baseline OGTT Outcome: FHx DM

). If one looked at BMI at baseline with regard to OGTT, it was demonstrated that 62% of the subjects were classed as obese while only approximately 7% of subjects fell into the normal category. Of those subjects that were obese, over 50% of them had IFG (Table 15, Figure 46: Baseline OGTT Outcome: BMI

). If one looked at OGTT outcome at baseline with regard to smoking status, 68% of the subjects were non smokers and almost 20% of subjects were ex-smokers, leaving 13% of study subjects that smoked. If one that smoked. If one looked at the subjects that smoked, 50% had IFG at baseline OGTT (Table 16, Figure 47: (Table 16, Figure 47: Baseline OGTT Outcome: Smoking

). If one looked at OGTT outcome at baseline with regard to hypertension, approximately 70% of the subjects had hypertension and approximately 30% of subjects did not have hypertension. Of those subjects that had hypertension, more that 80% of them had IFG or IFG+IGT (Table 17, Figure 48: Baseline OGTT Outcome: Hypertension

). If one looked at OGTT outcome at baseline, approximately the same number of subjects had dyslipidemia to those who didn't. Of those subjects who didn't have dyslipidemia, 50% of them had IFG rather than IFT+IGT and IGT (Table 18, Figure 49: Baseline OGTT Outcome: Dyslipidemia

).

**Table 11: Baseline OGTT Outcome: Age**

		MIX Baseline OGTT result			Total	
		IFG	IFG+IGT	IGT		
baseline age groups	young adulthood	Count	0	0	1	1
		% within baseline age groups	0.0%	0.0%	100.0%	100.0%

	middle adulthood	% of Total	0.0%	0.0%	2.2%	2.2%
		Count	1	1	1	3
		% within baseline age groups	33.3%	33.3%	33.3%	100.0%
		% of Total	2.2%	2.2%	2.2%	6.7%
	older adulthood	Count	14	10	2	26
		% within baseline age groups	53.8%	38.5%	7.7%	100.0%
		% of Total	31.1%	22.2%	4.4%	57.8%
	adult retirement age	Count	5	7	3	15
		% within baseline age groups	33.3%	46.7%	20.0%	100.0%
		% of Total	11.1%	15.6%	6.7%	33.3%
	Total	Count	20	18	7	45
		% within baseline age groups	44.4%	40.0%	15.6%	100.0%
% of Total		44.4%	40.0%	15.6%	100.0%	

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.

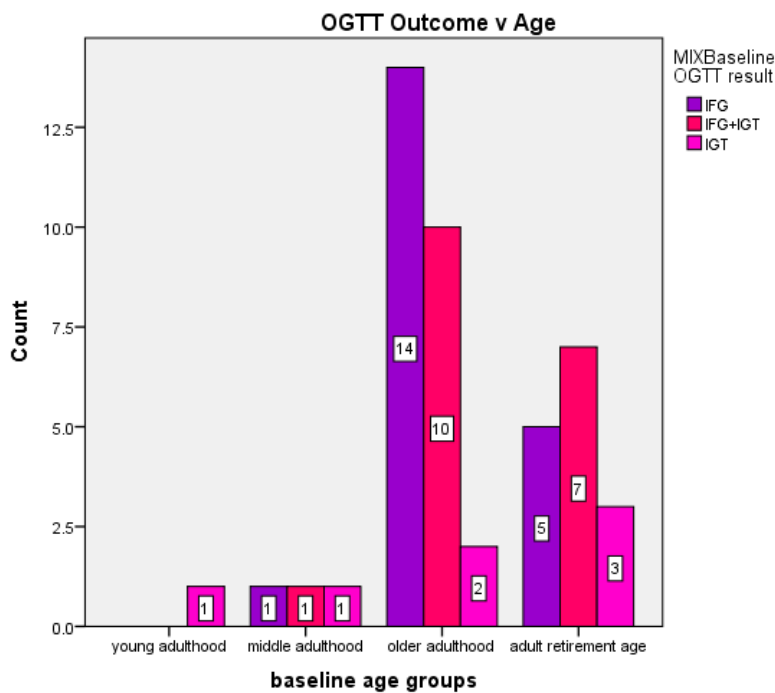


Figure 42: Baseline OGTT Outcome: Age

-If one looked at age at baseline with regard to OGTT outcome, one can see that there were very few young adults with IFG, IGT or both, while those who were in middle adulthood appeared to have a fairly even distribution of subjects in all OGTT categories. IFG was demonstrated to be more prominent in older subjects, while IGT was demonstrated to be more prominent in subjects of adult retirement age.

Table 12: Baseline OGTT Outcome: Gender

			MIXBaseline OGTT result			Total
			IFG	IFG+IGT	IGT	
Gender	F	Count	9	6	4	19
		% within Gender	47.4%	31.6%	21.1%	100.0%

		% within MIXBaseline OGTT result	45.0%	33.3%	57.1%	42.2%
		% of Total	20.0%	13.3%	8.9%	42.2%
	M	Count	11	12	3	26
		% within Gender	42.3%	46.2%	11.5%	100.0%
		% within MIXBaseline OGTT result	55.0%	66.7%	42.9%	57.8%
		% of Total	24.4%	26.7%	6.7%	57.8%
Total	Count	20	18	7	45	
	% within Gender	44.4%	40.0%	15.6%	100.0%	
	% within MIXBaseline OGTT result	100.0%	100.0%	100.0%	100.0%	
	% of Total	44.4%	40.0%	15.6%	100.0%	

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.

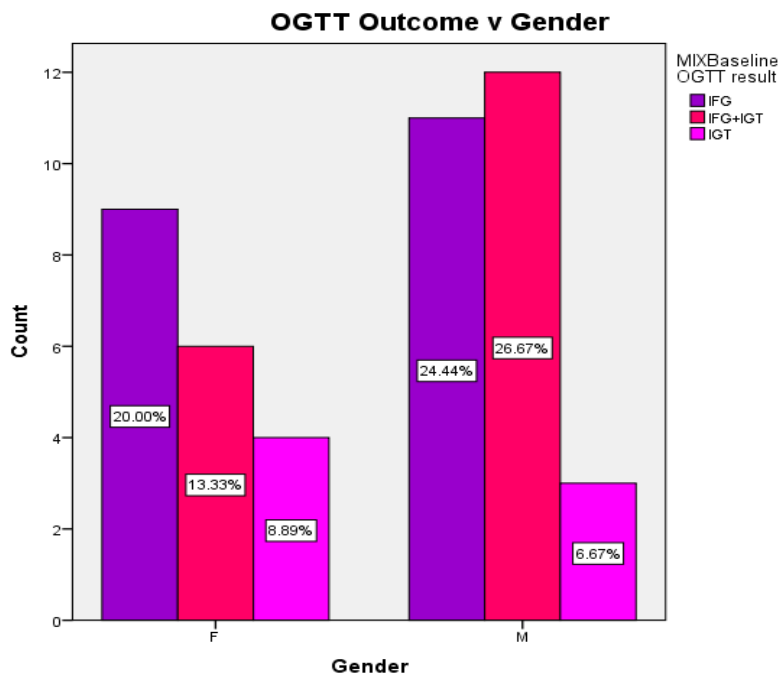


Figure 43: Baseline OGTT Outcome: Gender

-If one looked at gender at baseline with regard to OGTT, there appeared to be more male subjects with IFG and IFG+IGT than females, who themselves appeared to have more IGT; F = female; M = male.

Table 13: Baseline OGTT Outcome: Ethnicity

		MIXBaseline OGTT result			Total	
		IFG	IFG+IGT	IGT		
Ethnicity	Caucasian	Count	19	16	6	41
		% within Ethnicity	46.3%	39.0%	14.6%	100.0%

		% within MIXBaseline OGTT result	95.0%	88.9%	85.7%	91.1%
		% of Total	42.2%	35.6%	13.3%	91.1%
	n.Caucasian	Count	1	2	1	4
		% within Ethnicity	25.0%	50.0%	25.0%	100.0%
		% within MIXBaseline OGTT result	5.0%	11.1%	14.3%	8.9%
Total			2.2%	4.4%	2.2%	8.9%
	Count		20	18	7	45
	% within Ethnicity		44.4%	40.0%	15.6%	100.0%
	% within MIXBaseline OGTT result		100.0%	100.0%	100.0%	100.0%
		44.4%	40.0%	15.6%	100.0%	

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.

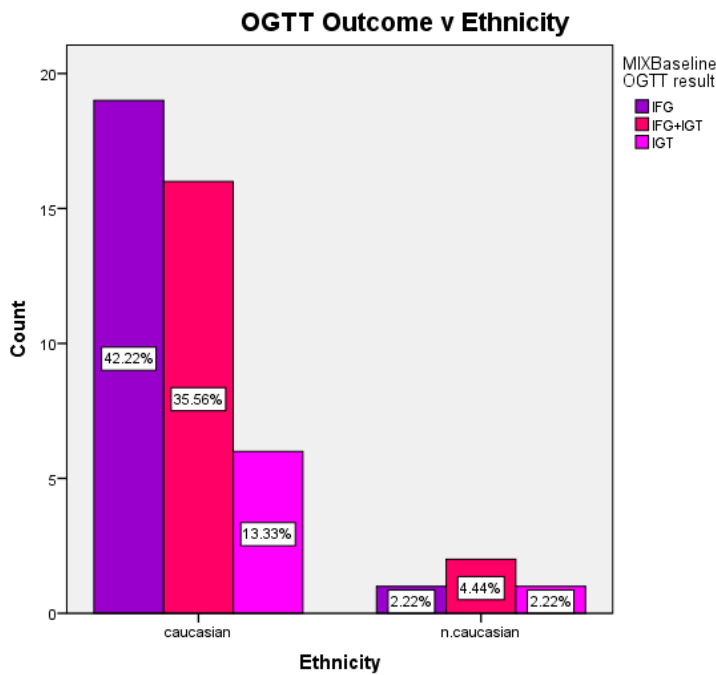


Figure 44: Baseline OGTT Outcome: Ethnicity

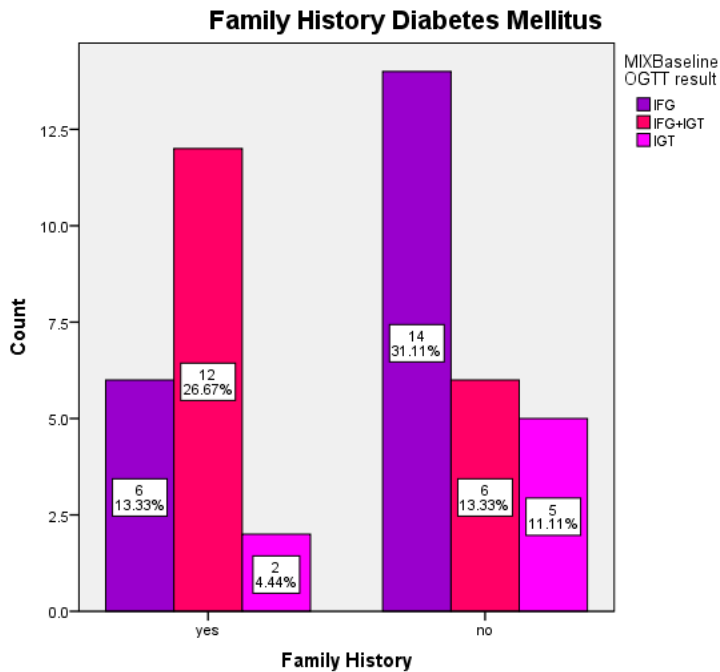
-If one looked at OGTT outcome with regard to ethnicity, it is very difficult to make any real informative comments in this instance, given over 90% of the study subjects were Caucasian.

Table 14: Baseline OGTT outcome: FHx DM

		MIXBaseline OGTT result			Total	
		IFG	IFG+IGT	IGT		
FHx DM	Yes	Count	6	12	2	20
		% within Family History DM	30.0%	60.0%	10.0%	100.0%

		% within MIXBaseline OGTT result	30.0%	66.7%	28.6%	44.4%
		% of Total	13.3%	26.7%	4.4%	44.4%
	No	Count	14	6	5	25
		% within Family History DM	56.0%	24.0%	20.0%	100.0%
		% within MIXBaseline OGTT result	70.0%	33.3%	71.4%	55.6%
		% of Total	31.1%	13.3%	11.1%	55.6%
Total	Count	20	18	7	45	
	% within Family History DM	44.4%	40.0%	15.6%	100.0%	
	% within MIXBaseline OGTT result	100.0%	100.0%	100.0%	100.0%	
	% of Total	44.4%	40.0%	15.6%	100.0%	

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.



**Figure 45: Baseline OGTT Outcome: FHx DM**

-Out of the 20 subjects that had a positive family history (FHx) of DM, 60% of them at baseline had IFG+IGT, while this outcome was far less prominent in those who didn't have a FHx of DM.

**Table 15: Baseline OGTT Outcome: BMI**

			MIXBaseline OGTT result			Total
			IFG	IFG+IGT	IGT	
BMI	Normal	Count	1	1	1	3

baseline		% within BMI baseline	33.3%	33.3%	33.3%	100.0%
		% within MIXBaseline OGTT result	5.0%	5.6%	14.3%	6.7%
		% of Total	2.2%	2.2%	2.2%	6.7%
	Overweight	Count	4	8	2	14
		% within BMI baseline	28.6%	57.1%	14.3%	100.0%
		% within MIXBaseline OGTT result	20.0%	44.4%	28.6%	31.1%
	obese	Count	15	9	4	28
		% within BMI baseline	53.6%	32.1%	14.3%	100.0%
		% within MIXBaseline OGTT result	75.0%	50.0%	57.1%	62.2%
Total	Count	20	18	7	45	
	% within BMI baseline	44.4%	40.0%	15.6%	100.0%	
	% within MIXBaseline OGTT result	100.0%	100.0%	100.0%	100.0%	
	% of Total	44.4%	40.0%	15.6%	100.0%	

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.

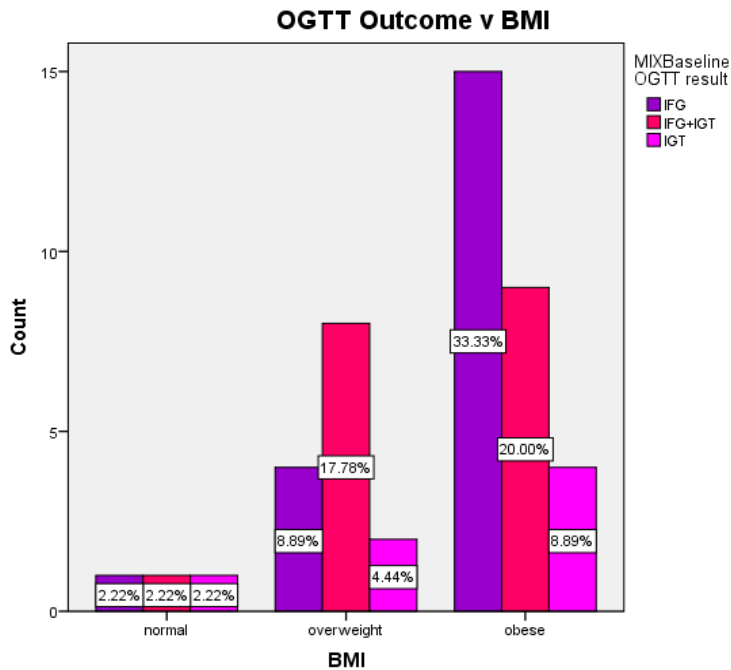


Figure 46: Baseline OGTT Outcome: BMI

-If one looked at BMI at baseline with regard to OGTT, it was demonstrated that 62% of the subjects were classed as obese while only approximately 7% of subjects fell into the normal category. Of those subjects that were obese, over 50% of them had IFG.

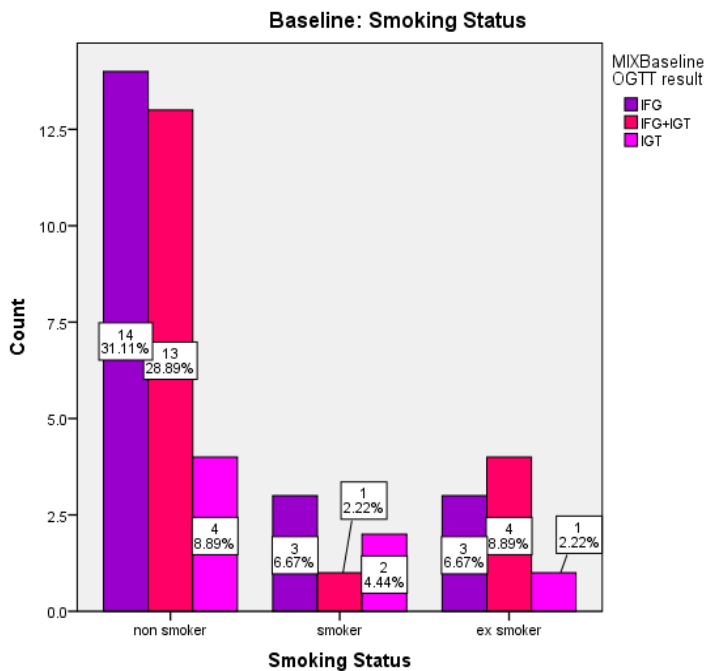
Table 16: Baseline OGTT Outcome: Smoking

			MIXBaseline OGTT result			Total
			IFG	IFG+IGT	IGT	
Smoking	non	Count	14	13	4	31



Status	smoker	% within Baseline Smoking Status	45.2%	41.9%	12.9%	100.0%	
		% within MIXBaseline OGTT result	70.0%	72.2%	57.1%	68.9%	
		% of Total	31.1%	28.9%	8.9%	68.9%	
	Smoker	Count	3	1	2	6	
		% within Baseline Smoking Status	50.0%	16.7%	33.3%	100.0%	
		% within MIXBaseline OGTT result	15.0%	5.6%	28.6%	13.3%	
		% of Total	6.7%	2.2%	4.4%	13.3%	
		ex smoker	Count	3	4	1	8
			% within Baseline Smoking Status	37.5%	50.0%	12.5%	100.0%
	% within MIXBaseline OGTT result		15.0%	22.2%	14.3%	17.8%	
	% of Total		6.7%	8.9%	2.2%	17.8%	
	Total	Count	20	18	7	45	
% within Baseline Smoking Status		44.4%	40.0%	15.6%	100.0%		
% within MIXBaseline OGTT result		100.0%	100.0%	100.0%	100.0%		
% of Total		44.4%	40.0%	15.6%	100.0%		

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.



**Figure 47: Baseline OGTT Outcome: Smoking**

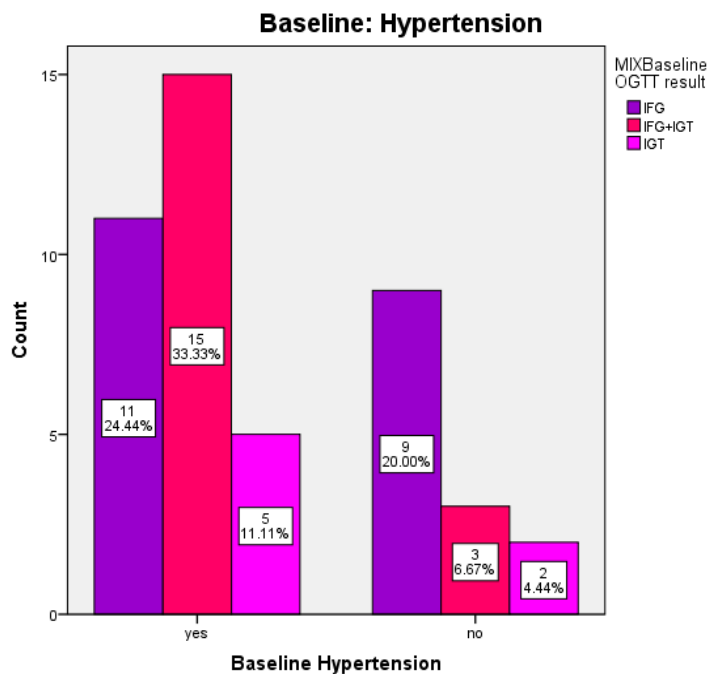
-If one looked at OGTT outcome at baseline with regard to smoking status, 68% of the subjects were non smokers and almost 20% of subjects were ex-smokers, leaving 13% of study subjects that smoked. If one looked at the subjects that smoked, 50% had IFG at baseline OGTT.

**Table 17: Baseline OGTT Outcome: Hypertension**

	MIXBaseline OGTT result			TOTAL
	IFG	IFG+IGT	IGT	

Baseline Hypertension	yes	Count	11	15	5	31
		% within Baseline Hypertension	35.5%	48.4%	16.1%	100%
		% within MIXBaseline OGTT result	55.0%	83.3%	71.4%	68.9%
		% of Total	24.4%	33.3%	11.1%	68.9%
	no	Count	9	3	2	14
		% within Baseline Hypertension	64.3%	21.4%	14.3%	100%
		% within MIXBaseline OGTT result	45.0%	16.7%	28.6%	31.1
Total			20	18	7	45
			44.4%	40.0%	15.6%	100%
			100.0%	100.0%	100.0%	100%
			44.4%	40.0%	15.6%	100%

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.



**Figure 48: Baseline OGTT Outcome: Hypertension**

-If one looked at OGTT outcome at baseline with regard to hypertension, approximately 70% of the subjects had hypertension and approximately 30% of subjects did not have hypertension. Of those subjects that had hypertension, more than 80% of them had IFG or IFG+IGT.

**Table 18 Baseline OGTT Outcome: Dyslipidemia**

	MIXBaseline OGTT result	TOTALS
--	-------------------------	--------

			IFG	IFG+IGT	IGT	
Baseline Dyslipidemia	yes	Count	8	9	4	21
		% within Baseline Dyslipidemia	38.1%	42.9%	19.0%	100%
		% within MIXBaseline OGTT result	40.0%	50.0%	57.1%	46.7%
		% of Total	17.8%	20.0%	8.9%	46.7%
	no	Count	12	9	3	24
		% within Baseline Dyslipidemia	50.0%	37.5%	12.5%	100%
		% within MIXBaseline OGTT result	60.0%	50.0%	42.9%	53.3%
		% of Total	26.7%	20.0%	6.7%	53.3%
Total	Count		20	18	7	45
	% within Baseline Dyslipidemia		44.4%	40.0%	15.6%	100%
	% within MIXBaseline OGTT result		100.0%	100.0%	100.0%	100%
	% of Total		44.4%	40.0%	15.6%	100%

\*MIXBaseline OGTT Result = Baseline OGTT result outcome: IFG, IFG+IGT or IGT.

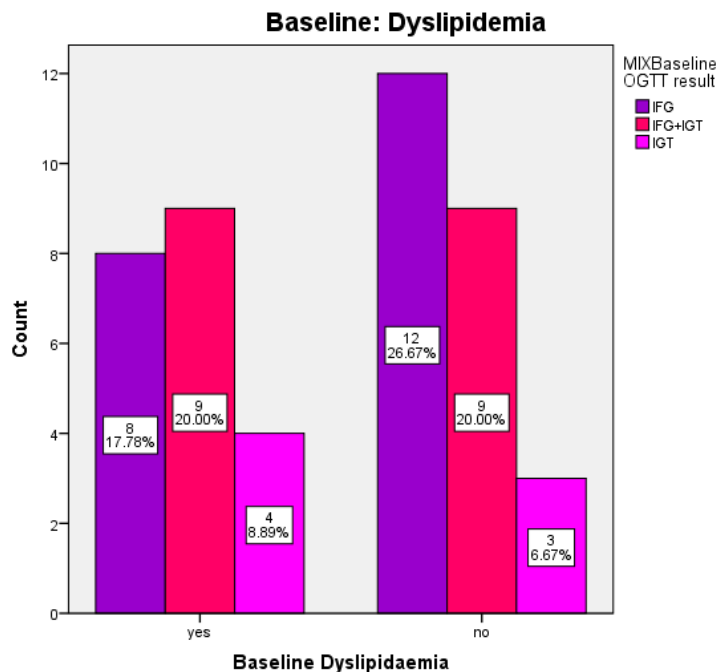


Figure 49: Baseline OGTT Outcome: Dyslipidemia

-If one looked at OGTT outcome at baseline, approximately the same number of subjects had dyslipidemia to those who didn't. Of those subjects who didn't have dyslipidemia, 50% of them had IFG rather than IFT+IGT and IGT

#### 4.1.15 Summary: Chapter 4

In summary, at baseline more men than women participated in study (60:40 split) and over 90% of the total over 90% of the total baseline subjects fell into the older adulthood or retirement age group. The majority of group. The majority of subjects at baseline were Caucasian (> 90%) and obese (63%). It was demonstrated that approximately half of the baseline subjects had a positive FHx of DM and 10% smoked. Interestingly, 70% of baseline subjects were on prescribed medication for hypertension and 50% of them were on prescribed medication for dyslipidemia. At baseline, the mean subject FPG was 6.25 mmol/l, the mean subject 2 hr OGTT result was 7.87 mmol/l and the mean subject HbA1c was 43.92 mmol/mol. In general,

when the CGM profiles (SAP) were inspected by eye, approximately 20% of them were thought to demonstrate least variability, 50% medium variability and 30% most variability. As the degree of variability of the subject CGM profiles (SAP) increased from least variability to most variability, the mean of each of the four CGM parameters was observed to increase also (Figure 36: Summary: Baseline Subjects CGM Parameters v CGM Profiles (SAP))

), which is what you would expect to observe. From the OGTT at baseline for study subjects, one can suggest that IFG is most prominent in older, obese males, who smoke and have hypertension.

## **5.0 Results: Study Data: Analysis of Year 1 and Year 3 Data**

### **5.1 Analysis Variables**

At Year 1, all subjects from the baseline study were invited back for re-analysis. At Year 1, a number of parameters were analysed, including age and study follow up interval, BMI, OGTT 0 hrs (FPG), OGTT 2 hours, HbA1c, CGM Glucose Excursion Parameters and CGM Profiles. At Year 3, variables which were analysed included age and follow up interval, BMI, OGTT 0 hrs (FPG) and HbA1c. The aim of this was to identify any parameter that demonstrated trends associated with progression to DM (T2DM).

At baseline there were 45 subjects (n = 45) and out of these 37 (n = 37) returned for re-analysis. Therefore, 82.2% of subjects re-attended at Year 1. At Year 3, 15.6% of the original baseline subjects (n = 7) did not return for re-analysis and 84.4% (n = 38) did, a response rate of 84.4%. This was an increase of 2.2% in response rate compared to Year 1. The subjects that developed DM (T2DM) at Year 1 were also included in the subjects invited for re-analysis at Year 3.

#### **5.1.1 Age (years)**

At Year 1 and Year 3, approximately 80% of subjects returned for re-analysis. Approximately 80% of these were either older adults or adults of retirement age. When baseline age categories were compared to Year 1 and Year 3 subjects, one can see that the majority of subjects were still in older adulthood or of adult retirement age. When we looked back to see the OGTT status at baseline for the non returners at both Year 1 and Year 3 respectively, 50% of them had IFG. When we looked at the follow up time interval, between subjects attending for re-analysis we can see from Table 19 that at Year 1, the mean time interval for re-attendance from baseline was 1.3 years (maximum 2.37 – minimum 0.85 years). At Year 3, the mean time interval for re-attendance from baseline was 3.64 years (maximum 4.75 – minimum 2.74). The interval between Year 1 and Year 3 attendances was 2.32 years.

**Table 19: Baseline, Year 1 and Year 3: Re-attendance Intervals**

		interval (0 to 1) years	interval (0 to 3) years	interval (1 to 3) years
N	Valid	36	38	34
	Missing	9	7	11
Mean		1.31	3.64	2.32
Std. Deviation		.35	.48	.32
Range		1.52	2.01	1.49
Minimum		.85	2.74	1.65
Maximum		2.37	4.75	3.14

### 5.1.2 Body Mass Index (BMI)

At baseline 31.1% (n = 14) were classed as being overweight and 62.2% (n = 28) fell into the obese category; mean BMI at baseline was 33.26. At Year 1, the n = 37 subjects were re-assessed for BMI and if one takes into account an 82.2% return rate, almost 60% were still classed as obese, with a mean BMI of 33.48. Similar findings were also seen at Year 3, where BMI mean was 32.54. If one takes into account an 84.4% return rate, over 50% were classed as obese, albeit with a slight improvement in BMI from previous years. In general however, the majority of subjects in this study were either overweight or obese (Table 20). Interestingly, all of the subjects that were obese at baseline were still obese at Year 3.

**Table 20: BMI: Summary - Baseline, Year 1 and Year 3**

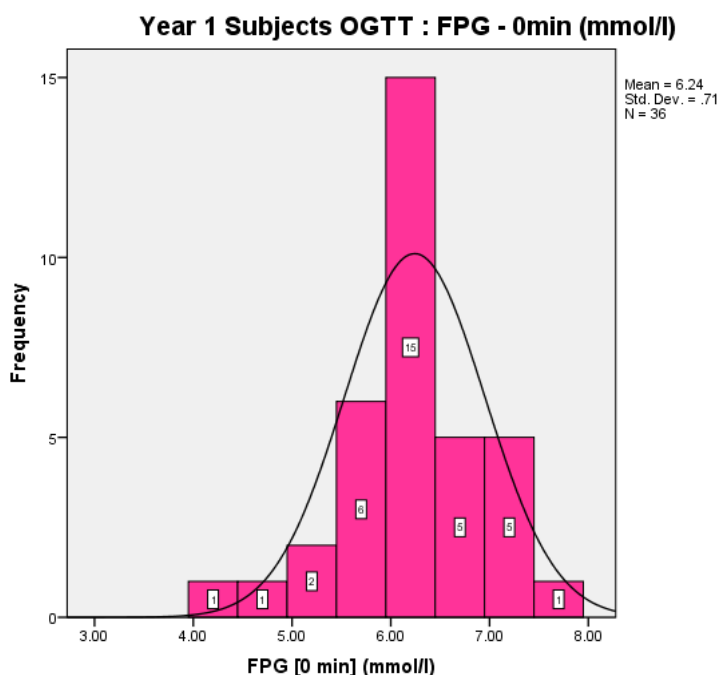
	N	Minimum	Maximum	Mean	Std. Deviation
Baseline BMI (kg/m <sup>2</sup> )	45	21.59	48.65	33.26	6.50
Year 1 BMI (kg/m <sup>2</sup> )	37	20.44	47.37	33.48	6.49
Year 3 BMI (kg/m <sup>2</sup> )	38	19.38	49.15	32.54	6.93
Valid N	34				

### 5.1.3 OGTT 0 minutes Glucose (mmol/l)

At baseline, the mean subject FPG was 6.25 mmol/l, which increased in a stepwise fashion as the study progressed with time. At Year 1, the mean subject OGTT at 0 minutes Glucose (mmol/l) i.e. the FPG was 6.24 mmol/l. The minimum FPG was 4.20 mmol/l and the maximum was 7.70 mmol/l, with a range of 3.50 mmol/l (Table 21, Figure 50: Year 1 Subjects OGTT: FPG - 0 min (mmol/l)).

**Table 21: OGTT 0 minutes Glucose (FPG): Summary: - Baseline, Year 1 and Year 3**

	N	Minimum	Maximum	Mean	Std. Deviation
Baseline OGTT 0 mins (mmol/l)	45	4.8	6.9	6.25	.45
Year 1 OGTT 0 mins (mmol/l)	36	4.20	7.70	6.24	.71
Year 3 FPG (mmol/L)	37	4.60	16.30	6.96	1.83
Valid N	32				



**Figure 50: Year 1 Subjects OGTT: FPG - 0 min (mmol/l)**

-At Year 1 (n = 36), the mean FPG was 6.24 mmol/l and the Std Dev was 0.71.

At Year 3, FPG was performed and used as outcome indicator, instead of OGTT. Although ethical approval was obtained for a OGTT at Year 3, when the study subjects contacted and invited contacted and invited back, they were not keen to have OGTT; however, they were happy to attend for a FPG. Therefore, in order to gain a Year 3 outcome FPG was used. The mean fasting plasma glucose (FPG) at Year 3 (n = 37) was 6.96 mmol/l, which was an increase of 0.71 mmol/l from baseline. At Year 3, the maximum FPG demonstrated was up to 16.30 mmol/l, with a corresponding minimum FPG of 4.60 mmol/l. The range at Year 3 was 11.70 mmol/l compared to the baseline range of 2.1 mmol/l (Figure 51: Year 3 Subjects OGTT: FPG - 0 min (mmol/l))

). This demonstrated a degree of FPG instability, as at Year 1, (n = 8) subjects had progressed and had crossed the threshold to DM (T2DM) (a subject ratio of 6: 1: 1 - IFG+IGT: IFG: IGT). At Year 3, a further (n = 13) subjects had progressed and demonstrated DM (T2DM) based on FPG, (over 50% of which were IFG+IGT at Year 1), which was almost 50% of the study subjects. If one looked at the baseline OGTT of the subjects (n = 21) who had progressed to DM by Year 3 (based on FPG), a subject ratio of 9:3:9 – IFG+IGT: IGT: IFG was demonstrated; i.e. 86% of the subjects (divided equally) were either a mix of IFG+IGT or purely IFG had progressed to DM (TDM) by Year 3. If one looked at the FPG at Year 3 of the (n = 8) subjects found to be DM at Year 1, all of them continued to have elevated FPG  $\geq 6.5$  mmol/l except one (this subject was taking oral anti-diabetic medication).

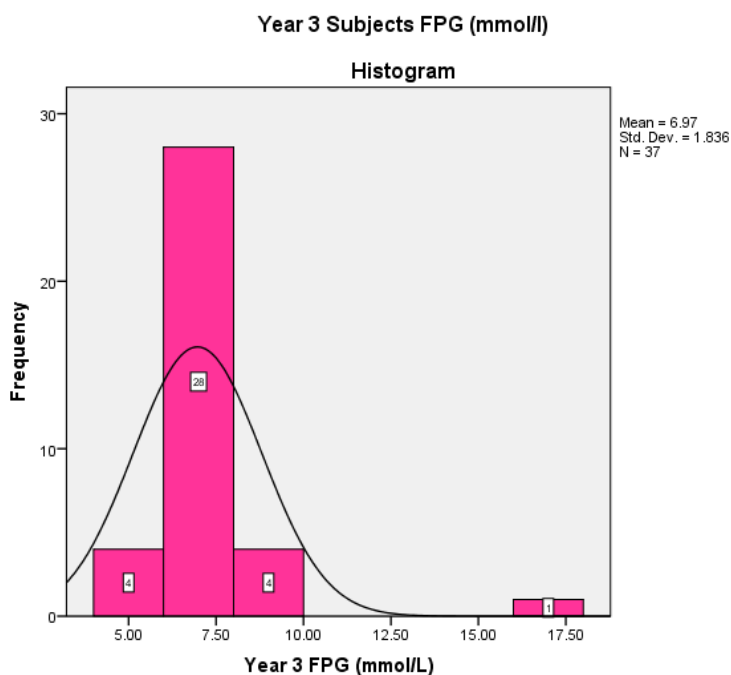


Figure 51: Year 3 Subjects OGTT: FPG - 0 min (mmol/l)

-FPG for Year 3 subjects demonstrated a mean of 6.96 mmol/l and a Std Dev of 1.83.

#### 5.1.4 OGTT 2 Hour Glucose (mmol/l)

At baseline, the mean 2 Hour Glucose (mmol/l) was 7.87 mmol/l, with a range of 6.7 mmol/l. The OGTT 2 mmol/l. The OGTT 2 Hour Glucose at Year 1 was 8.04 mmol/l, an increase of 0.17 mmol/l. At Year 1, the mmol/l. At Year 1, the maximum value was 12.70 mmol/l and the minimum value was 4.4 mmol/l, with an 4.4 mmol/l, with an increased range of 8.30 mmol/l (Table 22, Figure 52: Year 1 Subjects OGTT [2 hours] (mmol/l)

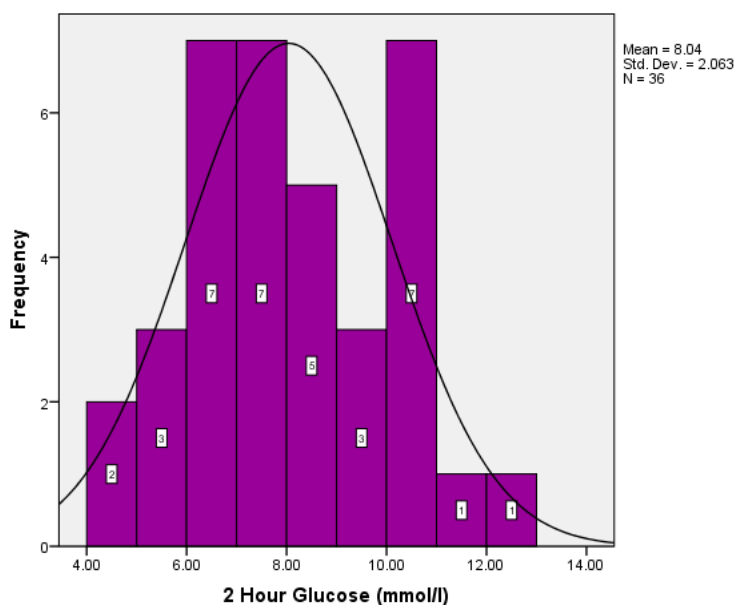


). At Year 1, (n = 8) subjects had progressed to T2DM and of these only one of these had pure IGT at baseline, the majority of them (75%) had IFG+IGT. In this study, an OGTT was not conducted at Year 3, as when the study subjects were contacted and invited back for OGTT, they were not keen to have it; they did however, consent for FPG, which was used as an indicator of outcome.

**Table 22: OGTT 2 hours (mmol/l): Summary - Baseline and Year 1**

	N	Minimum	Maximum	Mean	Std. Deviation
Baseline OGTT 120 mins (mmol/l)	45	4.3	11.0	7.87	1.81
Year 1 OGTT 120mins ( mmol/l)	36	4.40	12.70	8.04	2.06
Valid N	36				

**Year 1 Subjects OGTT : 2 Hour Glucose (mmol/l)**



**Figure 52: Year 1 Subjects OGTT [2 hours] (mmol/l)**

-At Year 1 (n = 36), the 2 hour subject OGTT mean was 8.04 mmol/l and the Std Dev was 2.06.

### 5.1.5 HbA1c (mmol/mol)

At 1 Year, the mean subject HbA1c (n = 37) was 45.08 mmol/mol (with a maximum of 62.0 mmol/mol and a minimum of 37 mmol/mol), rising from 43.92 mmol/mol at baseline to 48.84 mmol/mol in subjects at Year 3. The minimum HbA1c at Year 3 was 38 mmol/mol and the

38 mmol/mol and the maximum was 105 mmol/mol, giving a range of 67 mmol/mol, which was increased compared to the baseline range of 16 mmol/mol (Table 23, Figure 53: Year 1 Subjects HbA1c (mmol/mol)

and Figure 54: Year 3 Subjects HbA1c (mmol/mol)

).

Table 23: HbA1c: Summary - Baseline, Year 1 and Year 3

	N	Minimum	Maximum	Mean	Std. Deviation
Baseline HbA1c (mmol/l)	40	37.00	53.00	43.92	3.82
Year 1 HbA1c (mmol/l)	37	37.00	62.00	45.08	4.76
Year 3 HbA1c (nmol/mol)	38	38.00	105.00	48.84	12.28
Valid N	31				

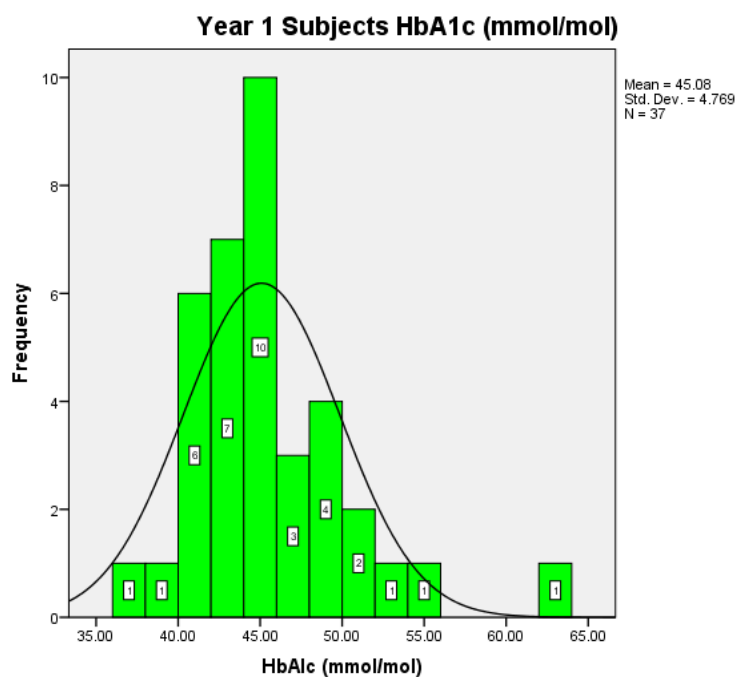


Figure 53: Year 1 Subjects HbA1c (mmol/mol)

-At Year 1 (n = 37), the mean HbA1c was 45.08 mmol/mol and the Std Dev was 4.76.

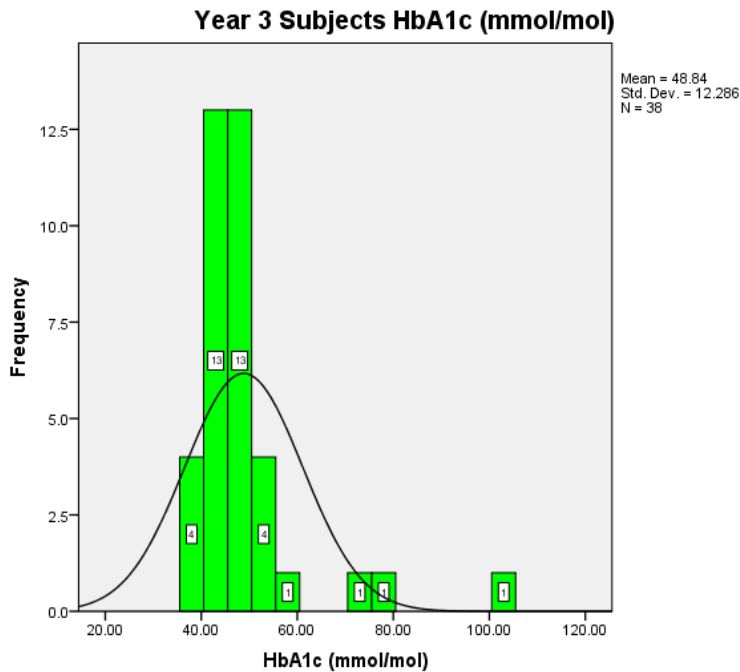


Figure 54: Year 3 Subjects HbA1c (mmol/mol)

-At Year 3, (n = 38), the mean HbA1c was 48.84 mmol/mol and the St Dev was 12.26.

### 5.1.6 CGM Glucose Excursion Parameters

As described in Section 2.10, four CGM based glucose excursion parameters were devised and the results at Year 1 for each subject (n = 37) are seen in ( In this study, CGM was not conducted at Year 3, as when the study subjects were contacted and invited back, they were not keen to have it; in addition, PI/funding availability at this time was also limiting factor.

Table 24). In this study, CGM was not conducted at Year 3, as when the study subjects were contacted and invited back, they were not keen to have it; in addition, PI/funding availability at this time was also limiting factor.

Table 24: Summary of CGM Glucose Parameters - Year 1

		MEAN Year1 AvGI (mmol/l)	MEAN Year1 AvGI excursion from Av (mmol/l)	MEAN Year1 Av above norm GI (mmol/l)	MEAN Year1 AvGI excursion from norm GI (mmol/l)
N	Valid	37	37	37	37
	Missing	8	8	8	8
Mean		6.89	1.15	2.39	2.70
Std. Deviation		1.04	.58	1.04	1.10

Range	5.44	3.00	5.44	5.16
Minimum	5.03	.41	.53	1.03
Maximum	10.47	3.41	5.97	6.19

Table 24 is a summary of the CGM glucose parameters at Year 1. Each of these 4 parameters was then looked compared at baseline to year 1.

CGM Mean Average Glucose for the Year 1 data (n = 37) was 6.89 mmol/l at Year 1, compared to a lower compared to a lower baseline value of 6.73 mmol/l. The maximum and minimum CGM Mean Average Glucose Mean Average Glucose at Year 1 was 10.47 and 5.03 mmol/l (a range of 5.44 mmol/l), compared to range of compared to range of 3.13 mmol/l at baseline (Table 25, Figure 55: Year 1 Subjects CGM Mean Average Glucose (mmol/l)

).

Table 25: Comparison Table: CGM Mean Average Glucose (mmol/l)

	N	Minimum	Maximum	Mean	Std. Deviation
MEAN Baseline Av GI (mmol/l)	45	5.27	8.40	6.73	.66
MEAN Year1 AvGI (mmol/l)	37	5.03	10.47	6.89	1.04
Valid N	37				

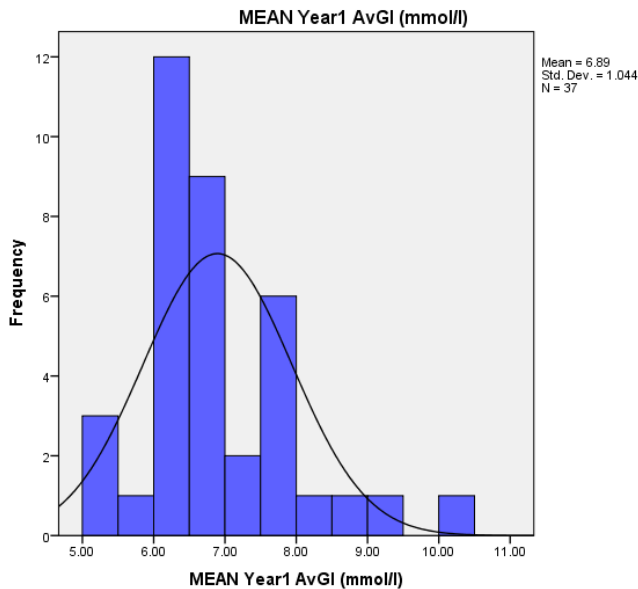


Figure 55: Year 1 Subjects CGM Mean Average Glucose (mmol/l)

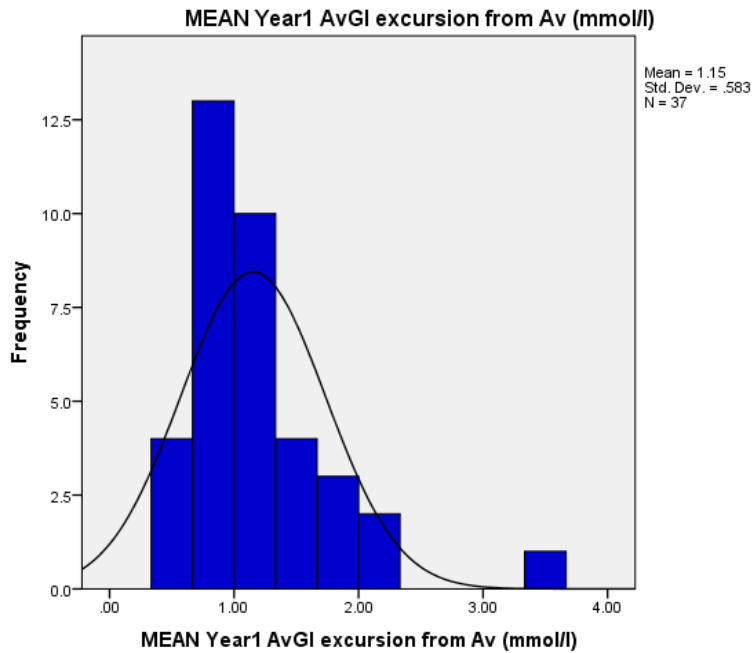
-CGM Mean Average Glucose for the Year 1 data (n = 37) was 6.89 mmol/l, with a maximum and minimum CGM Mean Average Glucose of 10.47 and 5.03 mmol/l (a range of 5.44 mmol/l).

The CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l) for Year 1 data (n = 37) was 1.15 mmol/l, compared to a lower excursion at baseline of 0.99 mmol/l. The maximum and minimum CGM Mean Average Excursion from the Average Glucose was 3.41 and 0.41 mmol/l (a range of 3.0 mmol/l), compared to a smaller range of 1.86 mmol/l at baseline (Table 26, Figure 56: Year 1 Subjects CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l)

).

**Table 26: Comparison Table: CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l)**

	N	Minimum	Maximum	Mean	Std. Deviation
MEAN Baseline AvGI excursion from Av (mmol/l)	45	.36	2.22	.99	.34
MEAN Year1 AvGI excursion from Av (mmol/l)	37	.41	3.41	1.15	.58
Valid N	37				



**Figure 56: Year 1 Subjects CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l)**

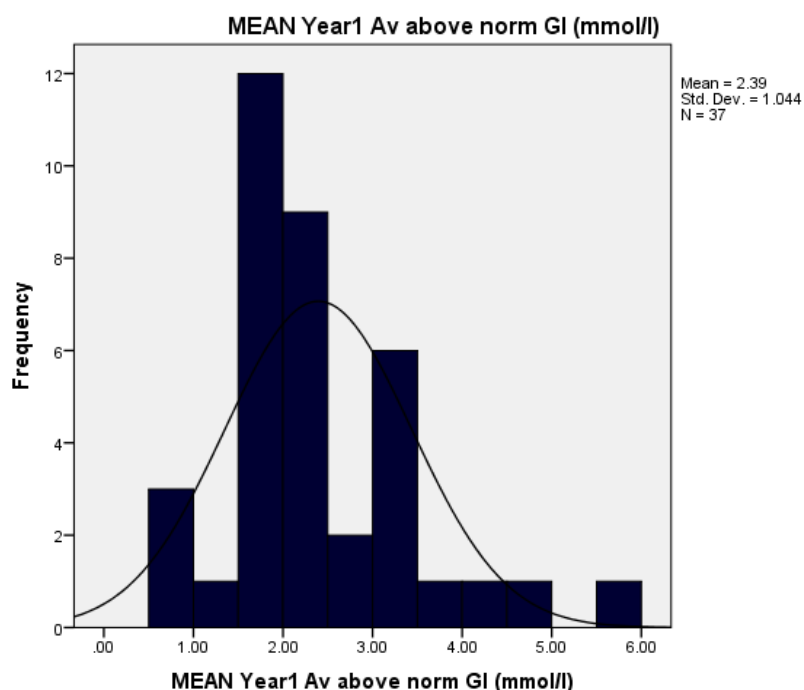
-The CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l) for the Year 1 data (n = 37) was 1.15 mmol/l, with a maximum and minimum CGM Mean Average Excursion from the Average Glucose of 3.41 and 0.41 mmol/l (a range of 3.0 mmol/l).

The CGM Mean Average Glucose above Normal Glucose (mmol/l) for the Year 1 data (n = 37) was 2.39 mmol/l, compared to a mean at baseline of 2.23 mmol/l. The maximum and minimum CGM Mean Average Glucose above Normal Glucose at Year 1 was 5.97 and 0.53 mmol/l (range of 5.44 mmol/l) compared to a reduced range at baseline of 3.13 mmol/l, respectively (Table 27, Figure 57: Year 1 Subjects CGM: Mean Average Glucose above Normal Glucose (mmol/l)

).

**Table 27: Comparison Table: CGM Mean Glucose above Normal Glucose (mmol/l)**

	N	Minimum	Maximum	Mean	Std. Deviation
MEAN Baseline Av above norm GI (mmol/l)	45	.77	3.90	2.23	.66
MEAN Year1 Av above norm GI (mmol/l)	37	.53	5.97	2.39	1.04
Valid N	37				



**Figure 57: Year 1 Subjects CGM: Mean Average Glucose above Normal Glucose (mmol/l)**

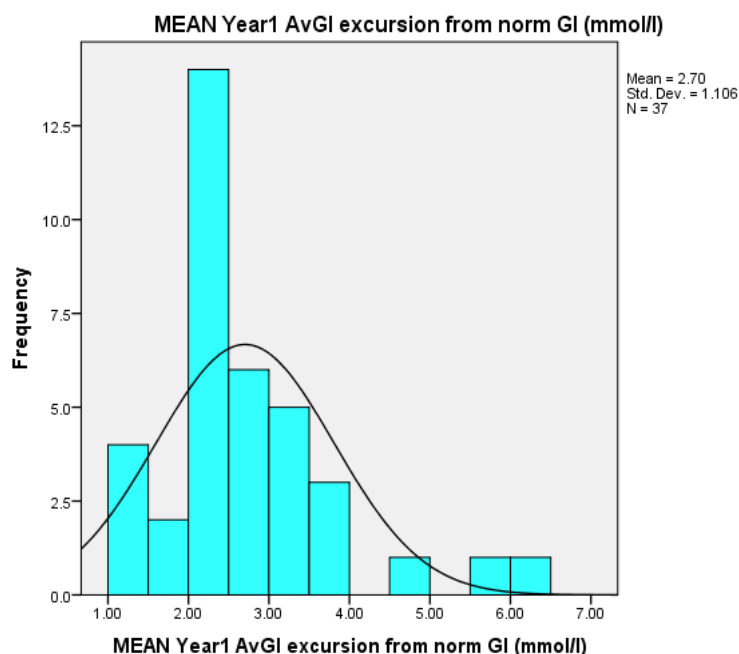
-The CGM Mean Average Glucose above Normal Glucose (mmol/l) for the Year 1 data (n = 37) was 2.39 mmol/l, with a maximum and minimum CGM Mean Average Glucose above Normal Glucose at Year 1 of 5.97 and 0.53 mmol/l (range of 5.44 mmol/l).

The CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l) for the Year 1 data (n = 37) was 2.70 mmol/l, compared to 2.48 mmol/l at baseline. The maximum and minimum CGM Mean Average Glucose Excursion from Normal Glucose was 6.19 and 1.03 mmol/l at Year 1 with a range of 5.16 mmol/l (compared to a reduced range of 3.02 mmol/l at baseline) (Table 28, Figure 58: Year 1 Subject CGM: Mean Average Glucose Excursion from Normal Glucose (mmol/l)

).

**Table 28: Comparison Table: CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l)**

	N	Minimum	Maximum	Mean	Std. Deviation
MEAN Baseline Av GI excursion from norm GI (mmol/l)	45	1.03	4.05	2.48	.656
MEAN Year1 AvGI excursion from norm GI (mmol/l)	37	1.03	6.19	2.70	1.10
Valid N	37				



**Figure 58: Year 1 Subject CGM: Mean Average Glucose Excursion from Normal Glucose (mmol/l)**

-The CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l) for the Year 1 data (n = 37) was 2.70 mmol/l, with a maximum and minimum CGM Mean Average Glucose Excursion from Normal Glucose of 6.19 and 1.03 mmol/l (range of 5.16 mmol/l).

### 5.1.7 CGM Profiles

CGM was done on all the subjects at Year 1 (n= 37). Each CGM profile (Sensor Modal Day) was then inspected by eye and the profiles were placed in one of three groups, according to the “flatness” or “peakiness” of the profile. The three groups were labelled least variability, medium variability and most variability. As at baseline, each subject CGM profile was then placed into one of these groups following inspection. The CGM profiles were inspected blindly by the investigator (CGM profiles SAP) and by an independent observer (CGM Profiles PUMP) (the independent observer was a Lead Diabetic Specialist Nurse). CGM profiles for each subject at Year 1 can be seen in the APPENDIX, as APPENDIX 2: Least Variability; APPENDIX 3 – medium variability and APPENDIX 4 – most variability.

**Table 29: Year 1 Subjects CGM Profiles (SAP)**

		Frequency	%
Valid	'non returners'	8	17.8
	least variability	8	17.8
	medium variability	16	35.6
	most variability	13	28.9
	Total	45	100.0

In Table 29, out of the 45 baseline subjects, n = 8 (17.8%) did not attend at Year 1 and were classed as 'non returners'. One can see that 17.8% (n = 8) of the CGM (SAP) profiles were observed to demonstrate least variability, 35.6% (n = 16) were observed to demonstrate medium variability and 28.9% (n = 13) were observed to demonstrate most variability. These results closely mirrored the independent observer assessment of variability (Table 30).

**Table 30: Year 1 Subjects CGM Profiles (PUMP)**

		Frequency	%
	'non returners'	8	17.8
	least variability	9	20.0
	medium variability	16	35.6
	most variability	12	26.7
	Total	45	100.0



In Table 30, out of the 45 baseline subjects  $n = 8$  (17.8%), did not attend at Year 1 and were classed as ‘non returners’. One can see that 20% ( $n = 9$ ) of the CGM (PUMP) profiles were observed to demonstrate least variability, 35.6% ( $n = 16$ ) were observed to demonstrate medium variability and 26.7% ( $n = 12$ ) were observed to demonstrate most variability. These results closely mirrored the investigators assessment of variability.

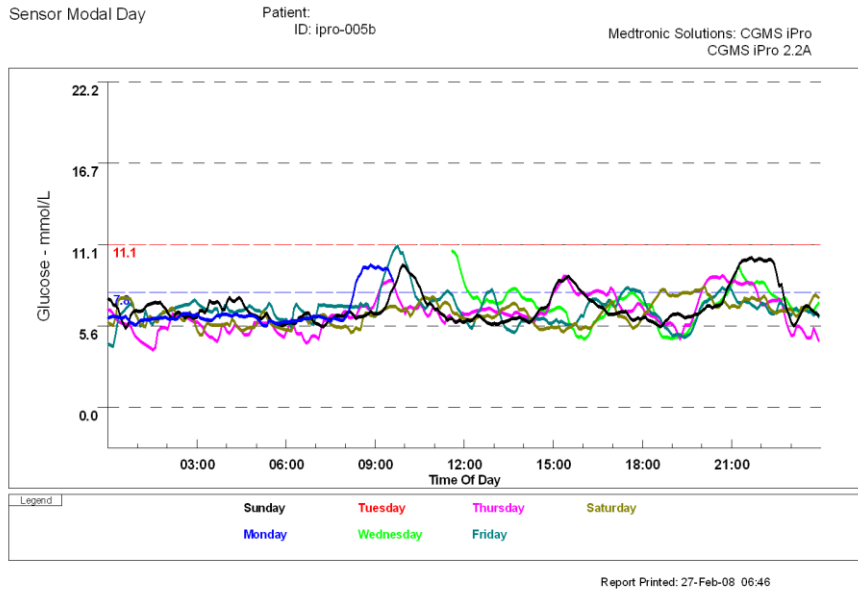
When this was looked at in more detail, there were two disagreements between assessors. The investigator placed one less CGM profile in the least variability category, compared to the independent observer (PUMP), who placed the subject in the medium category; also the investigator placed one more subject in the most variability category compared to the independent observer (PUMP) who placed it in the medium variability category; the remainder were identical.

As one was interested in progression to DM (T2DM), one looked at what happened at Year 1 with regard to Year 1 with regard to the CGM profiles visually and whether the categories the subjects were placed into by eye had any bearing on what the outcome was at year 3. The three subjects that were subjects that were illustrated at baseline were IPRO-05a, IPRO-38a and IPRO-06a. At Year 1, these subjects Year 1, these subjects were labelled IPRO-05b, IPRO-38b and IPRO-06b, respectively and can be seen and can be seen illustrated below (Figure 59: CGM Profile (SAP): Subject IPRO-05b

, Figure 60: CGM Profile (SAP): Subject IPRO-38b

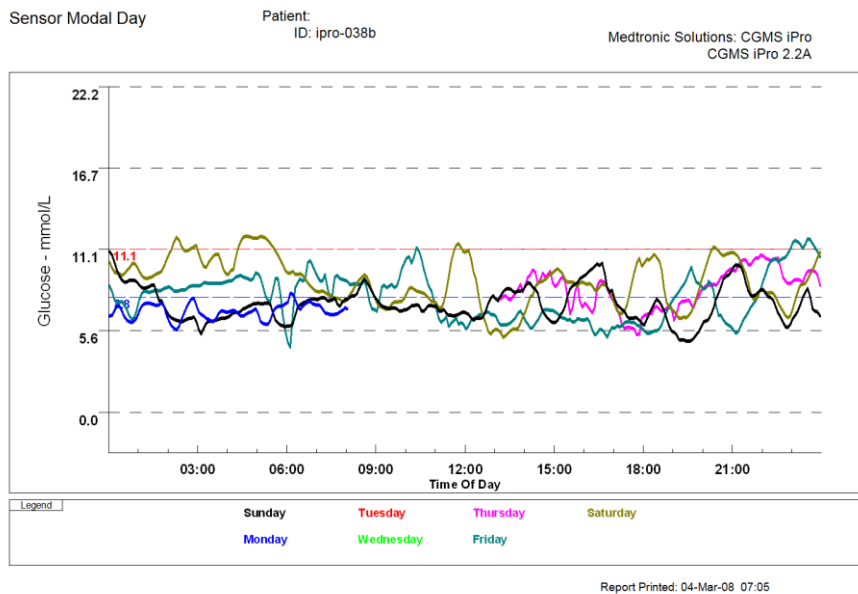
and Figure 61: CGM Profile (SAP): Subject IPRO-06b

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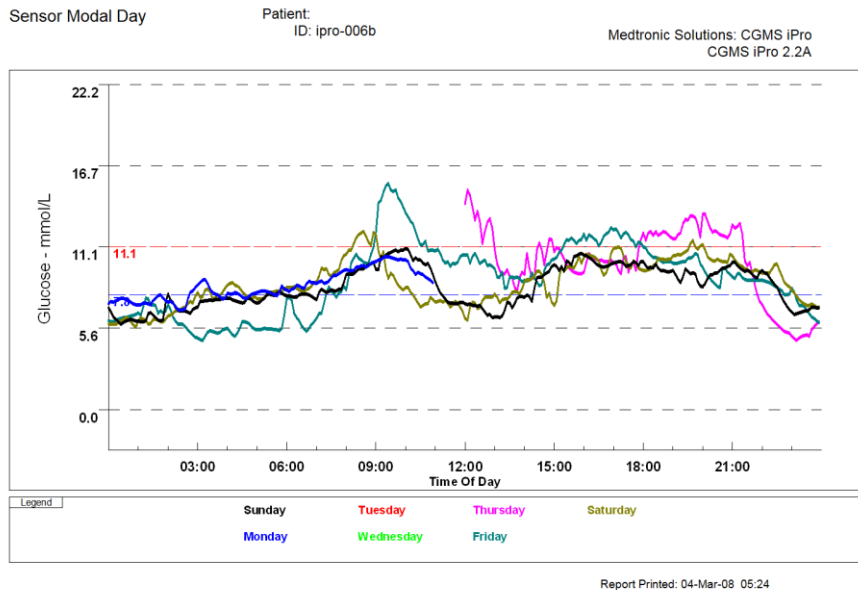
**Figure 59: CGM Profile (SAP): Subject IPRO-05b**

-Sensor Model Day over a 24 hour period, each day represented by as a separate plot line in a different colour. CGM profile (SAP) categorised as having medium variability at Year 1.



**Figure 60: CGM Profile (SAP): Subject IPRO-38b**

-Sensor Model Day over a 24 hour period, each day represented by as a separate plot line in a different colour. CGM profile (SAP) categorised as having most variability at Year 1.



**Figure 61: CGM Profile (SAP): Subject IPRO-06b**

-Sensor Model Day over a 24 hour period, each day represented by as a separate plot line in a different colour. CGM profile (SAP) categorised as having most variability at Year 1.

At baseline, IPRO-05 CGM profile (SAP) was categorised as having least variability and at Year 1 this had changed to medium variability. However, the OGTT result at baseline had not changed and subject IPRO-05 remained IFG+IGT at Year 1. At Year 3, IPRO-05 was not a diabetic on FPG testing. At baseline, IPRO-38 CGM profile (SAP) was categorised as having medium variability and at Year 1, this had changed to having most variability. At baseline and Year 1, subject-38 had IFG and at Year 3 was DM on FPG testing. At baseline, IPRO-06 CGM profile (SAP) was categorised as being most variable and at Year 3, this remained the case. At baseline subject IPRO-06 had IFG+IGT at baseline and was DM (T2DM) at year 1. Just looking at these examples, it may be that the CGM profile category at baseline may have a bearing on the outcome.

At Year 1, each of the four CGM parameters described in Section 2.10, were looked at with regard to their relationship with the CGM profile groups. As both the Investigator (SAP) and the independent observer (PUMP) findings were very similar, with regard to the degree of observed glucose variation in the subjects CGM profiles, for the purpose of this study, the investigators observations of CGM Profiles (SAP) were used in the remaining analysis. As the degree of variability of the subject CGM profiles (SAP)

increased from least variability to most variability, the mean of each of the four CGM parameters was observed to increase also.

The CGM parameter Mean Year 1 Average Glucose was 5.96 mmol/l for least variability CGM profile (SAP), variability CGM profile (SAP), rising to 6.65 mmol/l for medium variability CGM profile (SAP) up to 7.16 profile (SAP) up to 7.16 mmol/l for most variability CGM profile (SAP) (Figure 62: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Year 1 Average Glucose Excursion from Average (mmol/l) was 0.65 mmol/l for least variability CGM profile (SAP), rising to 0.97 mmol/l for medium variability CGM profile (SAP) up to 1.68 mmol/l for most variability CGM profile (SAP) (Figure 62: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Year 1 Average Glucose above Normal Glucose (mmol/l) was 1.48 mmol/l for least variability CGM profile (SAP), rising to 2.15 mmol/l for medium variability CGM profile (SAP), up to 3.26 mmol/l for most variability CGM profile (SAP) (Figure 62: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP))

). The CGM parameter Mean Year 1 Average Glucose Excursion from Normal Glucose (mmol/l) was 1.68 mmol/l for least variability CGM profile (SAP), rising to 2.38 mmol/l for medium variability CGM profile (SAP), up to 3.73 mmol/l for most variability CGM profile (SAP) (Figure 62: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP))

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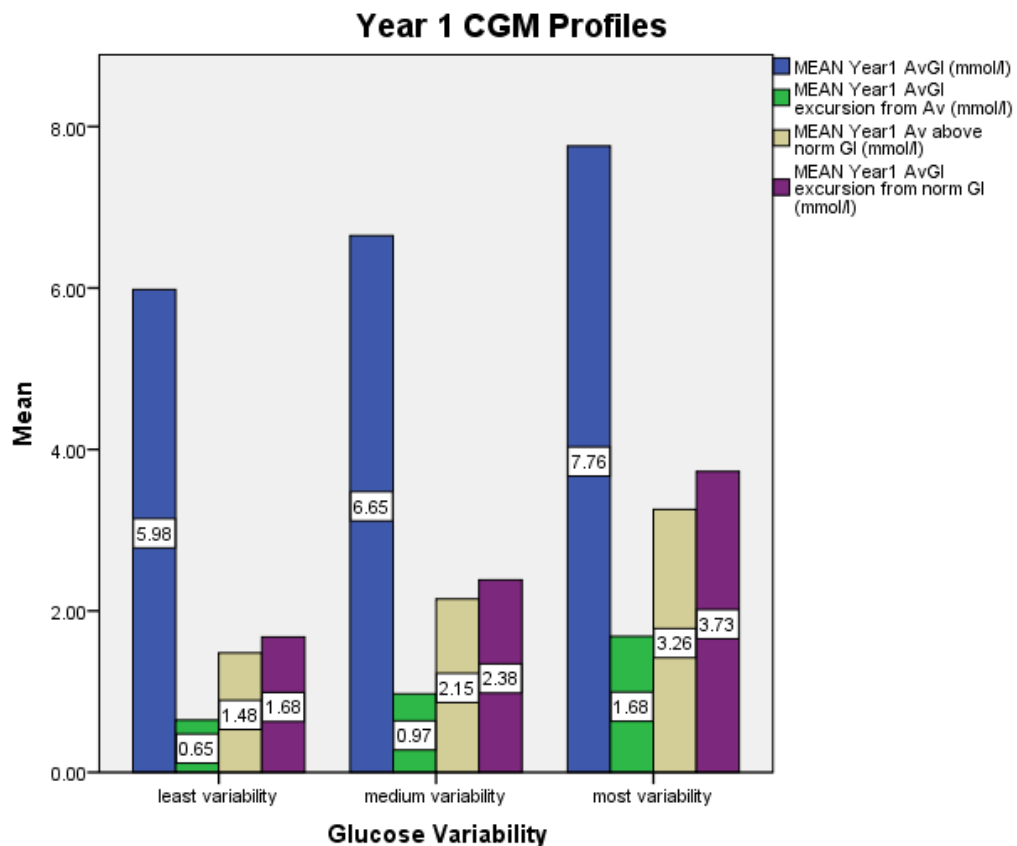


Figure 62: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP)

-The CGM parameter Mean Year 1 Average Glucose was 5.96 mmol/l for least variability CGM profile (SAP), rising to 6.65 mmol/l for medium variability CGM profile (SAP) up to 7.16 mmol/l for most variability CGM profile

(SAP). The CGM parameter Mean Year 1 Average Glucose Excursion from Average (mmol/l) was 0.65 mmol/l for least variability CGM profile (SAP), rising to 0.97 mmol/l for medium variability CGM profile (SAP) up to 1.68 mmol/l for most variability CGM profile (SAP). The CGM parameter Mean Year 1 Average Glucose above Normal Glucose (mmol/l) was 1.48 mmol/l for least variability CGM profile (SAP), rising to 2.15 mmol/l for medium variability CGM profile (SAP), up to 3.26 mmol/l for most variability CGM profile (SAP). The CGM parameter Mean Year 1 Average Glucose Excursion from Normal Glucose (mmol/l) was 1.68 mmol/l for least variability CGM profile (SAP), rising to 2.38 mmol/l for medium variability CGM profile (SAP), up to 3.73 mmol/l for most variability CGM profile (SAP).

At Year 1, each of the four CGM parameters described in Section 2.10, were looked at with regard to their relationship with the CGM profile groupings. As the degree of variability of the subject variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability to most variability, the mean of each of the four CGM parameters was observed to increase also, as observed to increase also, as seen from the mean plots (Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

a-d). The mean plots demonstrated a fairly positive linear correlation for each of these graphically, indicating a likely relationship between these two variables.

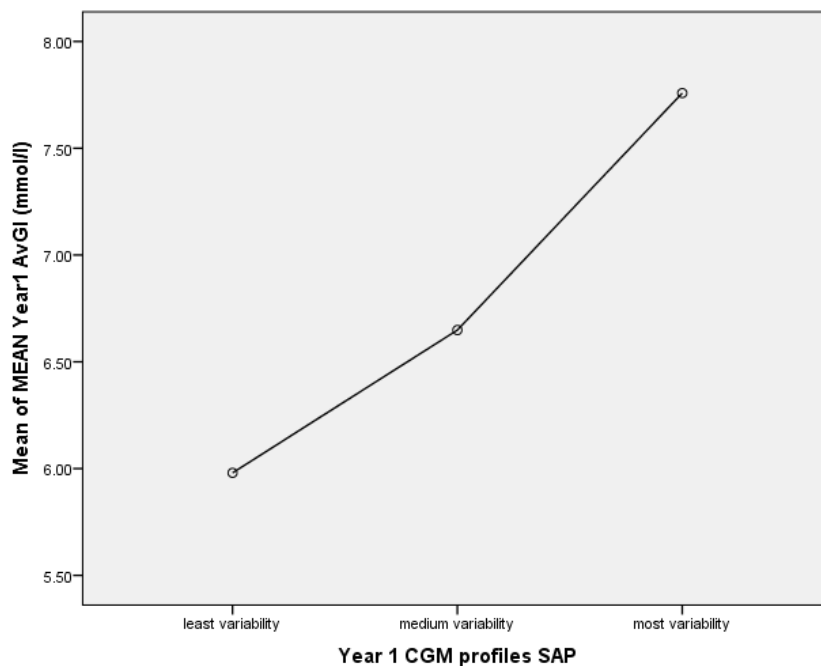
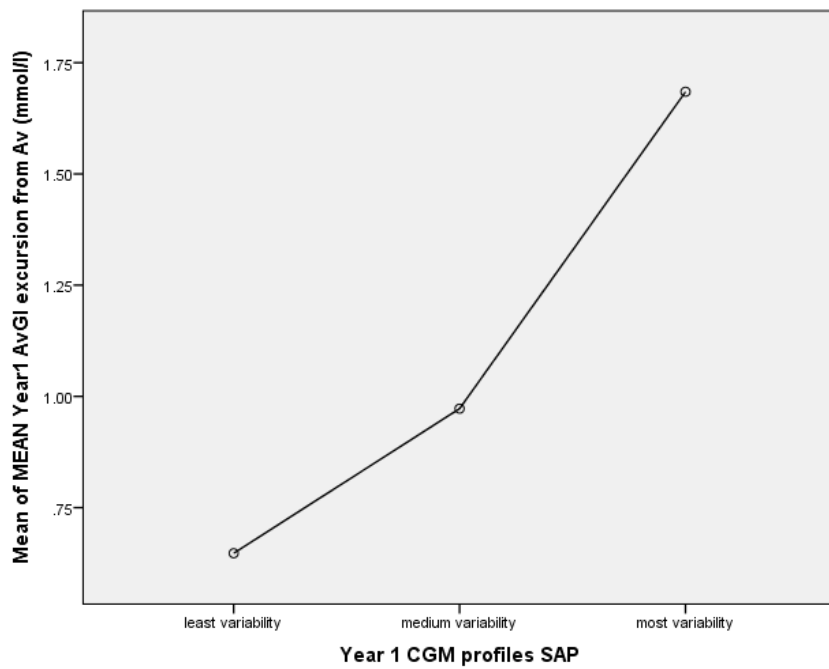


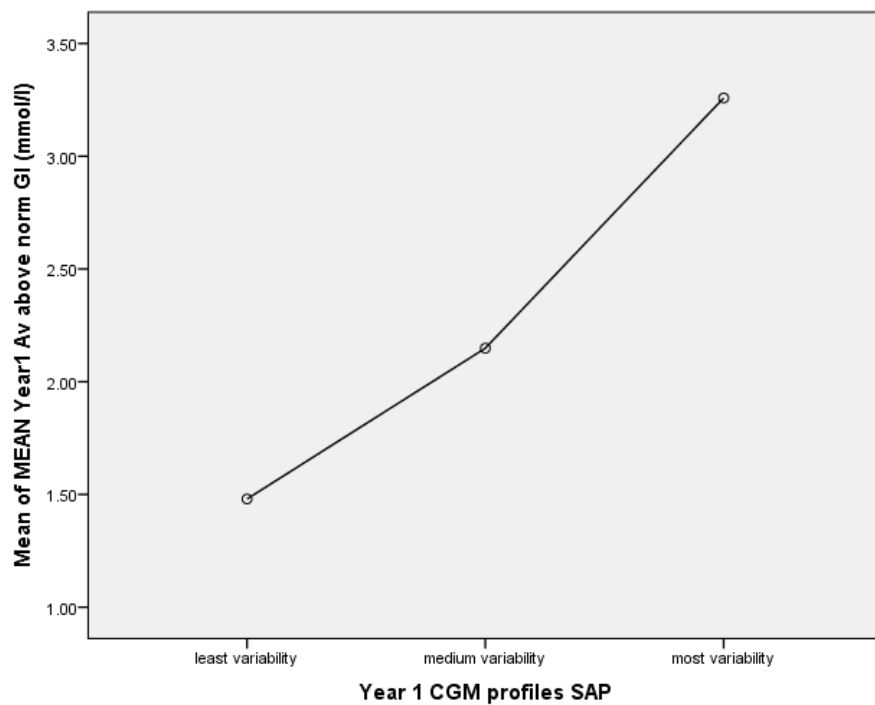
Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from normal Glucose (mmol/l) was also observed to increase.



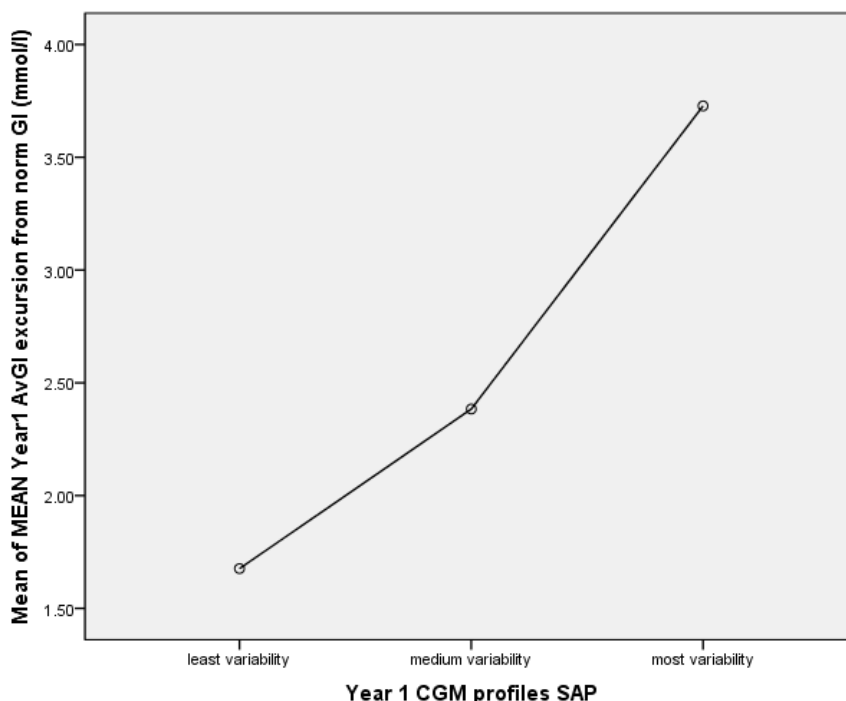
**Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from normal Glucose (mmol/l) was also observed to increase.



**Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from normal Glucose (mmol/l) was also observed to increase.



**Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots**

-As the degree of variability of the subject CGM profiles (SAP) observed by eye, increased from least variability to most variability, the mean of MEAN Baseline Average Glucose excursion from normal Glucose (mmol/l) was also observed to increase.

### 5.1.8 OGTT Results

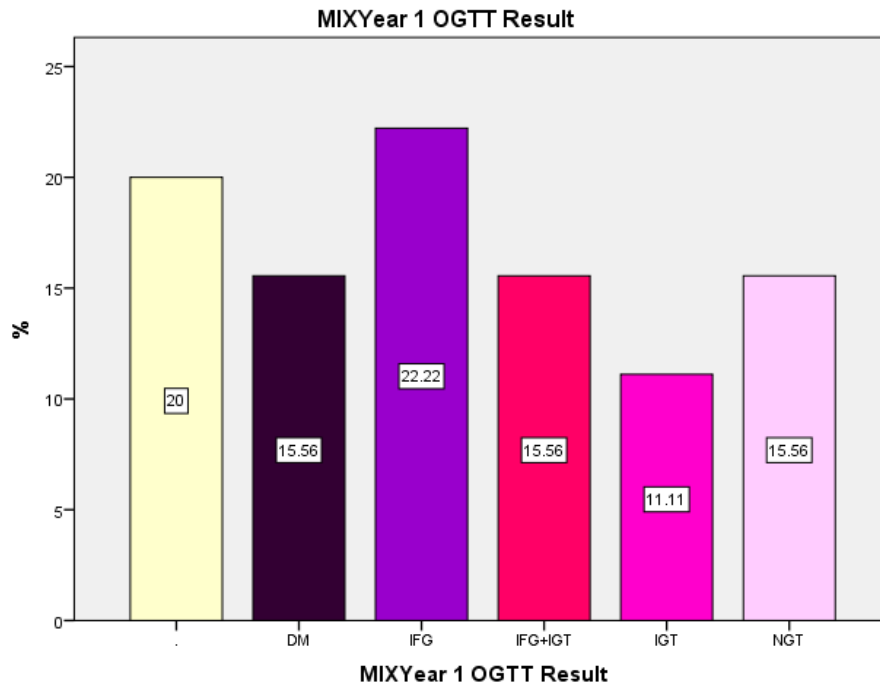
At Year 1, 20% (n = 9) of subjects either did not return or refused an OGTT, respectively. These were labelled respectively. These were labelled as ‘missing’ on the Table below; (n = 8) did not return and (n = 1) refused a and (n = 1) refused a repeat OGTT. The OGTT outcome at Year 1 for all subjects (n = 36), taking into account 36), taking into account those who did not return, demonstrated 22.5% (n = 10) subjects had IFG, 15.6% (n = had IFG, 15.6% (n = 7) subjects had both IFG and IGT, 11.1% (n = 5) subjects had IGT and 15.6% (n = 7) and 15.6% (n = 7) had progressed to DM (T2DM) (Table 31; Figure 64: Year 1 Subjects OGTT Outcome

).

**Table 31: MIX Year 1 OGTT Result**

		Frequency	%
	‘Missing’	9	20.0
	DM	7	15.6
	IFG	10	22.2

	IFG+IGT	7	15.6
	IGT	5	11.1
	NGT	7	15.6
	Total	45	100.0



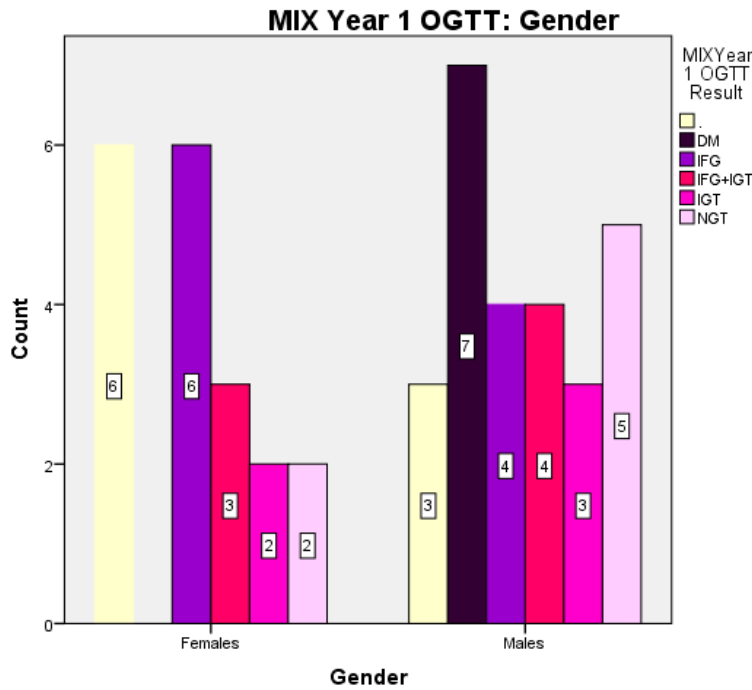
**Figure 64: Year 1 Subjects OGTT Outcome**

- The OGTT outcome at Year 1 for all subjects (n = 36), taking into account those who did not return (the cream bar) demonstrated 22.2% (n = 10) subjects had IFG, 15.6% (n = 7) subjects had both IFG and IGT, 11.1% (n = 5) subjects had IGT and 15.6% (n = 7) had progressed to DM (T2DM).

**If one looked at OGTT outcome at Year 1 (n = 36), for female gender, after taking into account those who did not return, 31.6% (n = 6) had IFG, 15.8% (n = 3) had IFG+IGT, 10.5% (n = 2) had IGT, 0% had DM (T2DM) and 10.5% (n = 2) had NGT (normal glucose tolerance). If OGTT outcome at Year 1 was looked at for male gender, after taking into account those who did not return, 15.4% (n = 4) of the male gender had IFG, 15.4% (n = 4) had IFG+IGT, 11.5% (n = 3) had IGT and 4.4% (n = 5) had NGT. Approximately 1/3 of the male gender at Year 1 had progressed to DM (T2DM) [26.9% (n = 7)]. Further analysis of this data demonstrated that 60% of females had IFG versus 40% of males, 42.9% of females had IFG+IGT versus 57.1% of males, 40% of females had IGT versus 60% of males and 28.6% females had NGT versus 57.8% males. Interestingly, 100% males were found to have progressed to having DM (T2DM) at Year 1 (Figure 65: MIX Year 1 OGTT Outcome: Gender**

); no females had.



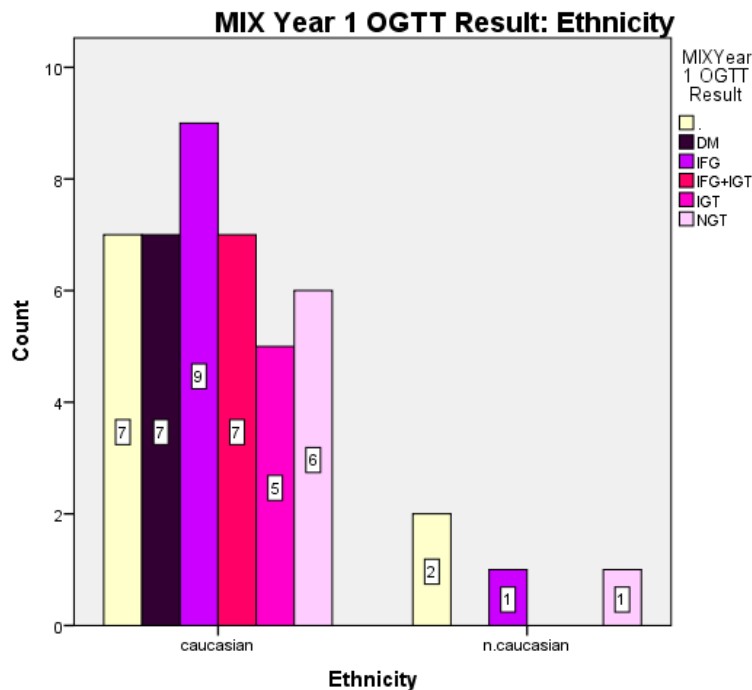


**Figure 65: MIX Year 1 OGTT Outcome: Gender**

-The OGTT outcome at Year 1 (n = 36), for female gender, taking into account those who did not return (cream bar), 31.6% (n = 6) had IFG, 15.8% (n = 3) had IFG+IGT, 10.5% (n = 2) had IGT, 0% had DM (T2DM) and 10.5% (n = 2) had NGT (normal glucose tolerance). If OGTT outcome at Year 1 was looked at for male gender, taking into account those who did not return (cream bar), 15.4% (n = 4) of the male gender had IFG, 15.4% (n = 4) had IFG+IGT, 11.5% (n = 3) had IGT and 4.4% (n = 5) had NGT.

**If one looked at OGTT outcome at Year 1 for ethnicity, out of the (n = 41) Caucasians subjects at baseline, 17.1% (n = 7) did not return or refused repeat OGTT at Year 1; 22% (n = 9) had IFG, 17.1% (n = 7) had IFG + IGT, 12.2 % (n = 5) had IGT, 17.1% (n = 7) had DM (T2DM) and 14.6% (n = 6) had NGT. With regard to (n = 4) Non Caucasian subjects at baseline, 50% (n = 2) of them did not return for repeat OGTT at Year 1; 25% (n = 1) had IFG, 0% had IFG+IGT, IGT and DM (T2DM) and 25% (n = 1) had NGT. Further analysis of this data demonstrated that 90% of Caucasians versus 10.0% of Non Caucasians had IFG, 100% of Caucasians versus 0% of Non Caucasians had IFG +IGT, IGT and DM (T2DM) respectively and 85.7% Caucasians versus 14.3% Non Caucasians had NGT. In this study, only Caucasians were seen to progress to DM (T2DM) at Year 1, but the numbers of non-Caucasians in this study group were very small, and this may be the reason for this (Figure 66: MIX Year 1 OGTT Outcome: Ethnicity**

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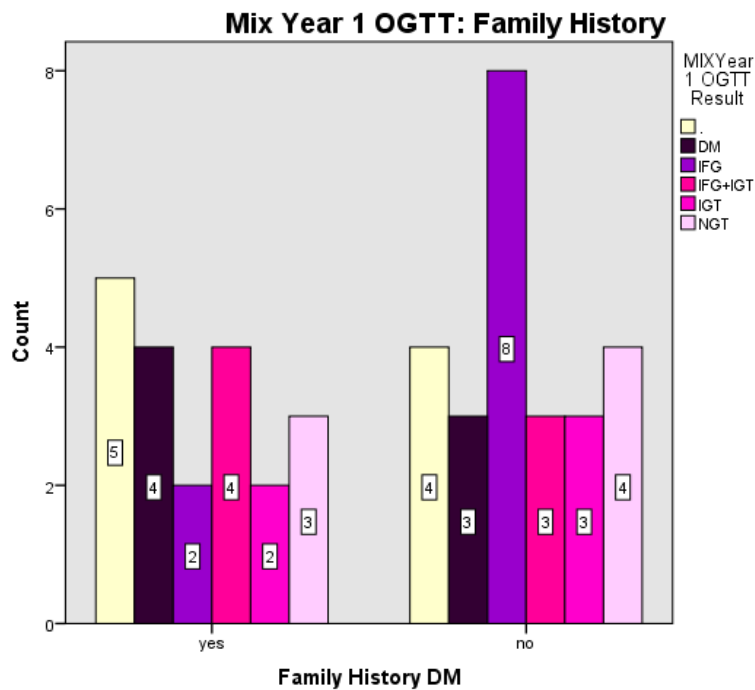


**Figure 66: MIX Year 1 OGTT Outcome: Ethnicity**

-The OGTT outcome at Year 1 for ethnicity, out of the (n = 41) Caucasians subjects at baseline, 17.1% (n = 7) did not return or refused repeat OGTT at Year 1 (cream bar); 22% (n = 9) had IFG, 17.1% (n = 7) had IFG + IGT, 12.2 % (n = 5) had IGT, 17.1% (n = 7) had DM (T2DM) and 14.6% (n = 6) had NGT. With regard to (n = 4) Non Caucasian subjects at baseline, 50% (n = 2) of them did not return for repeat OGTT at Year 1 (cream bar); 25% (n = 1) had IFG, 0% had IFG+IGT, IGT and DM (T2DM) and 25% (n = 1) had NGT.

**If one looked at OGTT outcome for subjects at Year 1 with regard to FHx of DM, (n = 9) subjects did not return or refused OGTT repeat at Year 1: (n = 5) of these had a FHx of DM and (n = 4) of these did not. Of the subjects with a FHx of DM and did not return for testing at Year 1, 10% (n = 2) had IFG, 20% (n = 4) had IFG+IGT, 10% (n = 2) had IGT, 20% (n = 4) had DM (T2DM) and 15% (n = 3) had NGT. Of those subjects who did not have a FHx of DM and did not return for testing at Year 1, 32% (n = 8) had IFG, 12% (n = 3) had IFG+IGT, 12% (n = 3) had IGT, 12% (n = 3) had DM (T2DM) and 16% (n = 4) had NGT. Further analysis of this data demonstrated that of those subjects that had IFG, 80% had no FHx of DM versus 20% that did. Of those subjects that had IFG+IGT, 42.9% did not have a FHx of DM while 57.1% did. Of those subjects that had IGT, 60% did not have a FHx of DM while 40% of them did. Of those subjects with DM (T2DM), 57.1% DM (T2DM), 57.1% did have a FHx of DM and 42.9% did not (Figure 67: MIX OGTT Year 1 Outcome: FHx**

). Of those subjects that had NGT, 42.9% did have FHx of DM and 57.1% did not. In this study, subjects found to be diabetic (T2DM) at Year 1, seemed to have a marginally increased presence of a FHx of DM.



**Figure 67: MIX OGTT Year 1 Outcome: FHx**

-The OGTT outcome for subjects at Year 1 with regard to FHx of DM, (n = 9) subjects did not return or refused OGTT repeat at Year 1 (cream bar): (n = 5) of these had a FHx of DM and (n = 4) of these did not. Of the subjects with a FHx of DM and did not return for testing at Year 1, 10% (n = 2) had IFG, 20% (n = 4) had IFG+IGT, 10% (n = 2) had IGT, 20% (n = 4) had DM (T2DM) and 15% (n = 3) had NGT. Of those subjects who did not have a FHx of DM and did not return for testing at Year 1, 32% (n = 8) had IFG, 12% (n = 3) had IFG+IGT, 12% (n = 3) had IGT, 12% (n = 3) had DM (T2DM) and 16% (n = 4) had NGT.

**If one looked at OGTT outcome for subjects at Year 1 with regard to BMI, (n = 9) subjects did not return or refused OGTT repeat at Year 1. If one looked at OGTT outcome for subjects at Year 1 (n = 37) with regard to BMI, of those subjects that had a normal BMI (n= 3), 33.3% (n = 1) had IFG, 33.3% (n = 1) had IGT and 33.3% (n = 1) had DM (T2DM). Of those subjects that were overweight (n = 8), 12.5% (n = 1) had IFG, 25% (n = 2) had IFG+IGT and 12.5% (n = 1) had IGT, 12.5% (n = 1) had DM (T2DM) and 37.5% (n = 3) had NGT. Of those subjects who were classed as obese (n = 26), 30.8% (n = 8) had IFG, 19.2% (n = 5) had IFG+IGT, 11.5% (n = 3) had IGT, 19.2% (n = 5) had DM (T2DM) and 15.4% (n = 4) had NGT; (n = 1) subject refused to be weighed at Year 1, but was obese at baseline. Interestingly, the IFG group demonstrated most obese subjects at Year 1. Further analysis of subjects at Year 1 demonstrated that of those with IFG, 10% had a normal BMI, 10% were overweight and 80% were classed as obese. Of those subjects with IFG+IGT, 0% had a normal BMI, 28.6% were overweight and 71.4% were classed as obese. Of those subjects with IGT, 20% had a normal BMI, 20% were overweight and 60% were classed as obese. Of those subjects with DM (T2DM) 14.3% had normal BMI, 14.3% were overweight and 71.4% were classed as obese. Of those subjects with NGT, 0% had a normal BMI, 42.9% were overweight and 57.1% were classed as obese (Figure 68: MIX OGTT Year 1 Outcome: BMI**

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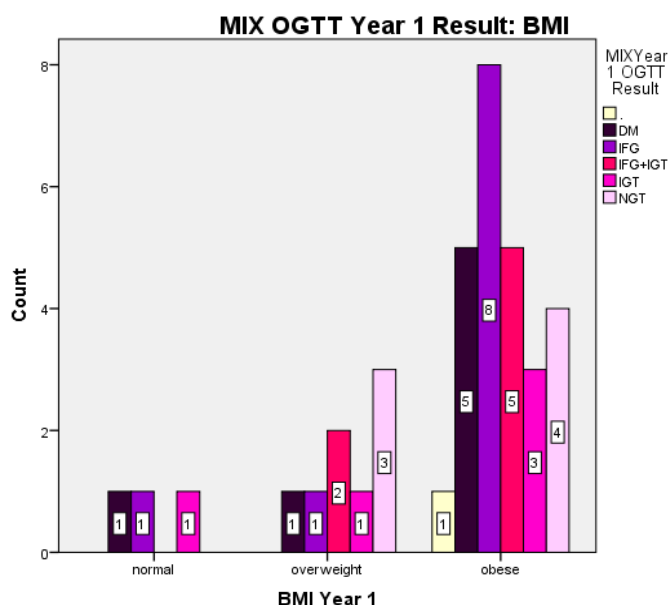


Figure 68: MIX OGTT Year 1 Outcome: BMI

-The OGTT outcome for subjects at Year 1 with regard to BMI, (n = 9) subjects did not return or refused OGTT repeat at Year 1. If one looked at OGTT outcome for subjects at Year 1 (n = 37) with regard to BMI, of those subjects that had a normal BMI (n= 3), 33.3% (n = 1) had IFG, 33.3% (n = 1) had IGT and 33.3% (n = 1) had DM (T2DM). Of those subjects that were overweight (n = 8), 12.5% (n = 1) had IFG, 25% (n = 2) had IFG+IGT and 12.5% (n = 1) had IGT, 12.5% (n = 1) had DM (T2DM) and 37.5% (n = 3) had NGT. Of those subjects who were classed as obese (n = 26), 30.8% (n = 8) had IFG, 19.2% (n = 5) had IFG+IGT, 11.5% (n = 3) had IGT, 19.2% (n = 5) had DM (T2DM) and 15.4% (n = 4) had NGT.

If one looked at OGTT outcome for subjects at Year 1 with regard to smoking status overall, (n = 9) subjects did not return or refused OGTT repeat at Year 1. If one looked at OGTT outcome for subjects at Year 1 (n = 36) with regard to smoking status, of those subjects that smoked and taking into account those subjects who smoked and did not return at Year 1, 16.7% (n = 1) had IFG, 16.7% (n = 1) had IFG+IGT, 0% had IGT, 16.7% (n = 1) had DM (T2DM) and 16.7% (n = 1) had NGT. Of those subjects that did not smoke and taking into account those subjects who did not smoke and did not return at Year 1, 29% (n = 9) at Year 1, 29% (n = 9) had IFG, 12.9% (n = 4) had IFG+IGT, 16.1% (n = 5) had IGT, 19.4% (n = 6) had DM (T2DM) and 12.9% (n = 4) had NGT. Of those subjects that were ex-smokers 0% had IFG, IGT and DM (T2DM) respectively, 25% (n = 2) had IFG+IGT and 25% (n = 2) had NGT. Further analysis of subjects at Year 1 demonstrated that of those subjects with IFG, 90% were non smokers and 10% were smokers; there were no ex-smokers; there were no ex-smokers. Of those subjects with IFG+IGT, 57.1% were non smokers, 14.3% were smokers, 14.3% were smokers and 28.6% were ex-smokers. Of those subjects with IGT, 100% were non smokers. What was encouraging here, was that of those subjects with DM (T2DM) 87.5% DM (T2DM) 87.5% were non smokers and .of those subjects with NGT, 57.1% were non smokers, 14.3% were non smokers, 14.3% were smokers and 28.6% were ex-smokers (Figure 69: MIX OGTT Year 1 Outcome: Smoking Status

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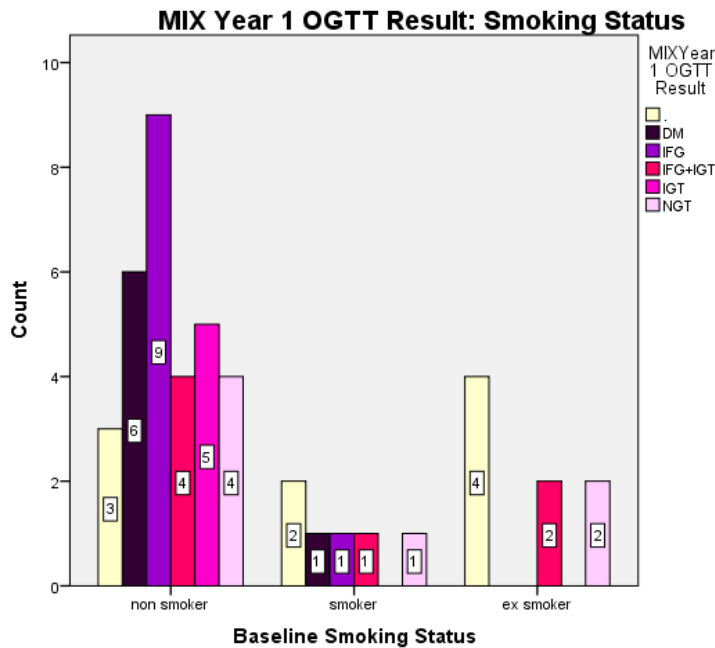
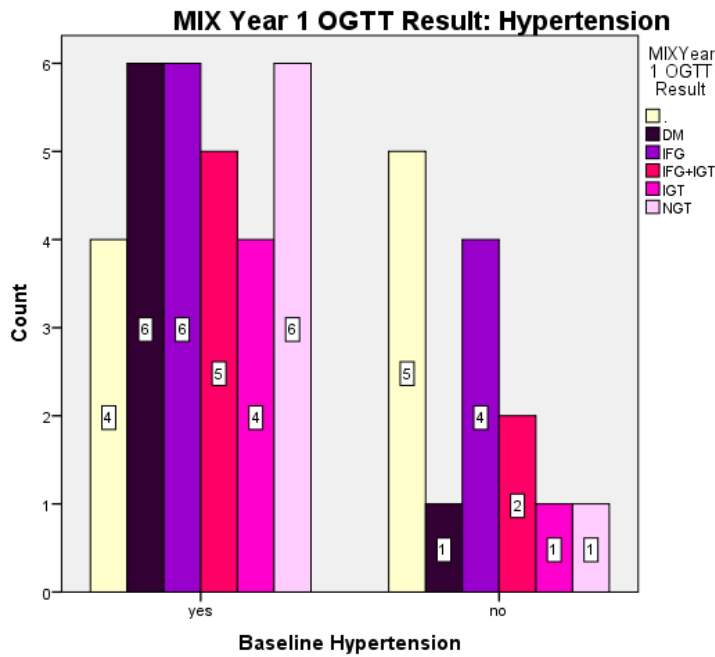


Figure 69: MIX OGTT Year 1 Outcome: Smoking Status

-The OGTT outcome for subjects at Year 1 with regard to smoking status, (n = 9) subjects did not return or refused OGTT repeat at Year 1 (cream bar). Of those subjects that smoked and taking into account those subjects who smoked and did not return at Year 1, 16.7% (n = 1) had IFG, 16.7% (n = 1) had IFG+IGT, 0% had IGT, 16.7% (n = 1) had DM (T2DM) and 16.7% (n = 1) had NGT. Of those subjects that did not smoke and taking into account those subjects who did not smoke and did not return at Year 1, 29% (n = 9) had IFG, 12.9% (n = 4) had IFG+IGT, 16.1% (n = 5) had IGT, 19.4% (n = 6) had DM (T2DM) and 12.9% (n = 4) had NGT. Of those subjects that were ex-smokers 0% had IFG, IGT and DM (T2DM) respectively, 25% (n = 2) had IFG+IGT and 25% (n = 2) had NGT.

**If one looked at OGTT outcome for subjects at Year 1 with regard to hypertension, (n = 9) subjects did not return or refused repeat OGTT at Year 1. If one looked at OGTT outcome for subjects at Year 1 with a history of hypertension and taking into account the non returners at Year 1 with a history of hypertension at baseline, 19.4% (n = 6) had IFG, 16.1% (n = 5) had IFG+IGT, 12.9% (n = 4) had IGT, and 19.4% (n = 6) had both DM (T2DM) and NGT respectively. Of those subjects who had hypertension at baseline, (n = 4) did not return at Year 1. Of those subjects who did not have hypertension at Year 1 and taking into account the non returners who did not have hypertension at baseline, 28.6% (n = 4) had IFG, 14.3% (n = 2) had IFG+IGT, 7.1% (n = 1) had IGT, DM (T2DM) and NGT, respectively. Further analysis of subjects at Year 1 demonstrated that of those subjects with IFG, 60% had hypertension and 40% did not. Of those subjects that had IFG+IGT, 71.4% did have hypertension and 28.6% did not. Of those subjects that had IGT, 80% had hypertension compared to 20% who did not. Interestingly, 85.7% of the subjects at Year 1 found to have progressed to DM (T2DM) had hypertension, while in those subjects who had reverted to NGT 14.3% of them had hypertension (Figure 70: MIX OGTT Year 1 Outcome: Hypertension**

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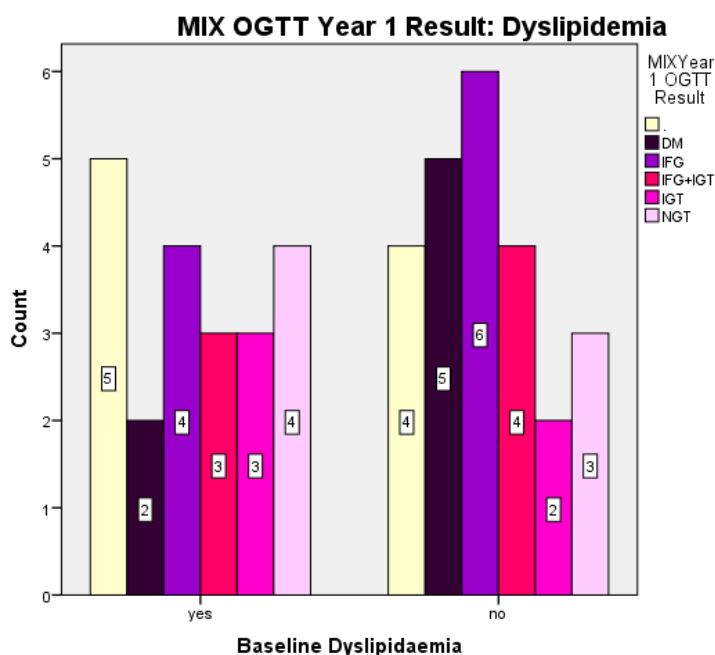


**Figure 70: MIX OGTT Year 1 Outcome: Hypertension**

-The OGTT outcome for subjects at Year 1 with regard to hypertension, (n = 9) subjects did not return or refused repeat OGTT at Year 1 (cream bar). If one looked at OGTT outcome for subjects at Year 1 with a history of hypertension and taking into account the non returners at Year 1 with a history of hypertension at baseline, 19.4% (n = 6) had IFG, 16.1% (n = 5) had IFG+IGT, 12.9% (n = 4) had IGT, and 19.4% (n = 6) had both DM (T2DM) and NGT respectively. Of those subjects who did not have hypertension at Year 1 and taking into account the non returners who did not have hypertension at baseline, 28.6% (n = 4) had IFG, 14.3% (n = 2) had IFG+IGT, 7.1% (n = 1) had IGT, DM (T2DM) and NGT, respectively.

**If one looked at OGTT outcome for subjects at Year 1 with regard to lipid status, (n = 9) subjects did not return or refused repeat OGTT at Year 1. If one looked at OGTT outcome for subjects at Year 1 with regard to lipid status, of those subjects that had dyslipidemia, 19% (n = 4) had IFG, 14.3% (n = 3) had both IFG+IGT and IGT, respectively; 9.5% (n = 2) had DM (T2DM) and 19% (n = 4) had NGT. Of those subjects that did not return for Year 1 assessment, (n = 5) had dyslipidemia and (n = 4) did not; these values were taken into account in the calculations. Of those subjects that did not have dyslipidemia, 25% (n = 6) had IFG, 16.7% (n = 4) had IFG+IGT, 8.3% (n = 2) had IGT, 20.8% (n = 5) had DM (T2DM) and 12.5% (n = 3) had NGT. Further analysis of subjects demonstrated that of those subjects with IFG, 40% had dyslipidemia and 60% did not. Of those subjects that had IFG+IGT, 42.9% had dyslipidemia and 57.1% did not. Of those 57.1% did not. Of those subjects that had IGT, 60% had dyslipidemia and 40% did not. In the group of subjects that had progressed to DM (T2DM), a third of them had dyslipidemia. Interestingly, of those subjects that had NGT, 57.1% had dyslipidemia and 42.9% did not and 42.9% did not (Figure 71: MIX OGTT Year 1 Outcome: Dyslipidemia**

).



**Figure 71: MIX OGTT Year 1 Outcome: Dyslipidemia**

-The OGTT outcome for subjects at Year 1 with regard to lipid status, (n = 9) subjects did not return or refused repeat OGTT (cream bar). If one looked at OGTT outcome for subjects at Year 1 with regard to lipid status, of those subjects that had dyslipidemia, 19% (n = 4) had IFG, 14.3% (n = 3) had both IFG+IGT and IGT, respectively; 9.5% (n = 2) had DM (T2DM) and 19% (n = 4) had NGT. Of those subjects that did not return for Year 1 assessment, (n = 5) had dyslipidemia and (n = 4) did not; these values were taken into account in the calculations. Of those subjects that did not have dyslipidemia, 25% (n = 6) had IFG, 16.7% (n = 4) had IFG+IGT, 8.3% (n = 2) had IGT, 20.8% (n = 5) had DM (T2DM) and 12.5% (n = 3) had NGT.

### 5.1.9 Blood Assay Analysis: Glucose Biomarkers

As already discussed, an attempt was made to identify any biomarkers that predict progression from abnormal glucose tolerance to T2DM. Therefore, for each study subject that attended Year 1 follow up, biomarkers of beta cell function C-peptide and Insulin were assayed in the fasting state, as described by Varvel *et al* (2014) (40) (Table 32); GAD Antibody was also tested at this time. This aspect of the study ideally would have been conducted at baseline, however, the original ethical approval didn't include this and so in order to look at these parameters, further ethical approval was sought via an amendment, which allowed this to occur at Year 1.

**Table 32: Analysis: GAD Ab, Insulin and C- Peptide**

		GAD Ab (U/ml)	Insulin (pmol/L)	C-peptide (pmol/ml)
N		36	36	36
	Non returners	9	9	9

Mean	0.96	118.22	0.58
Std. Deviation	0.52	57.92	0.27
Range	2.10	193.80	1.17
Minimum	0.30	33.80	0.05
Maximum	2.40	227.60	1.22

At Year 1, the mean level of GAD Ab in the (n = 36) fasted subjects was 0.96 u/ml. The maximum level was 2.40 U/ml and the minimum level was 0.30 U/ml. This gave a range of 2.10 U/ml and a Std Dev of 0.52. Given all of the subjects had GAD Ab levels < 5 U/ml (69), none of the subjects demonstrated serological marker positivity for LADA or T1DM (Table 32).

At Year 1, the mean level of Insulin in the (n = 36) fasted subjects was 118.22 pmol/L. The maximum level was 227.6 pmol/L and the minimum level was 33.8 pmol/L, which gave a range of 193.8 pmol/L and a Std Dev of 57.92 (Table 32). As biochemically, the mean level of Insulin in the subjects tested at Year 1 was above 100 pmol/L (the reference range of fasting plasma for this test was 6 - 100 pmol/L) (72), it's possible that this reflected a concurrent increase in insulin production and blood glucose levels in the subjects tested and thus could be used as a marker to assist diagnosing early T2DM.

At Year 1, the mean level of fasting C-peptide in the (n = 36) subjects was 0.58 pmol/ml. The maximum level was 1.22 pmol/ml and the minimum level was 0.05 pmol/ml. This gave a range of 1.17 pmol/ml and a Std Dev of 0.27 pmol/ml (Table 32). In theory, a high fasting blood sugar with a high C-peptide value should point to T2DM primarily caused by insulin resistance. That is because the high C-peptide value would suggest a lot of insulin was being produced but insulin resistance was keeping it from lowering blood sugar. The fact that the maximum fasting C-peptide observed in the Year 1 subjects was above the reference range, could suggest the presence of insulin resistance (71).

## 5.2 Analysing Progression Data

### 5.2.1 Analysing Data

Since a number of the most common statistical tests rely on the normality of a sample or population, it is often useful to test whether the underlying distribution is normal, or at least symmetric. In order to proceed with a statistical analysis of the results in this study, the distribution of data was looked at. In this study, there was a screening



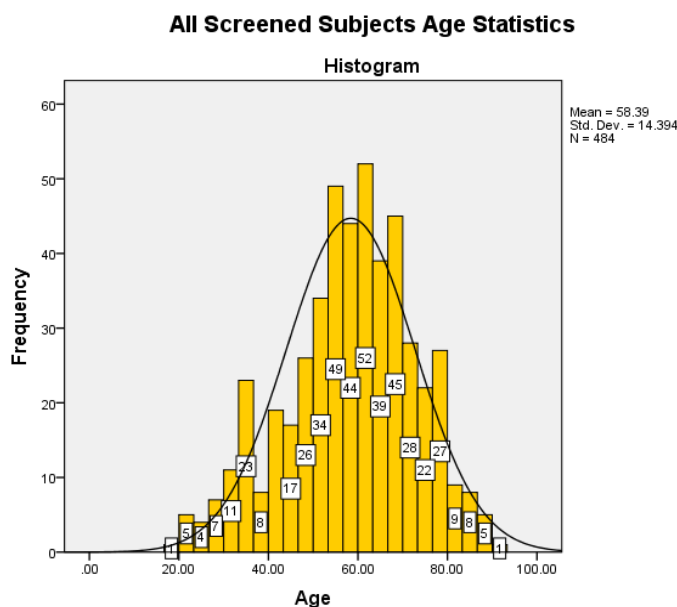
population and the study sample, which came from the screening population. Technically, one assumed that the distribution of the study sample mean was normal. In order to check this assumption, the population data and the sample data was looked at in more detail. In SPSS, the general trend of the data distribution was visually inspected for normality; the frequency distribution (histogram) and the P-P plot (probability-probability plot) were also used for checking this, as described by Ghasemi *et al* (2010) (77).

Ideally, data should be distributed symmetrically around a centre point i.e. be normally distributed. In SPSS the P-P plot plotted the cumulative probability of a variable against the cumulative probability of a particular distribution (e.g. normal distribution). After data were ranked and sorted, the corresponding z-score was calculated for each rank (this is the expected value that the score should have in a normal distribution). The scores were then themselves converted to z-scores and the actual z-scores were plotted against the expected z-scores, as described by Ghasemi *et al* (2010) (77).

For the screening population, the data appeared to be distributed symmetrically, around the centre of all the centre of all scores in a bell shape and therefore graphically, the screening population appeared to be normally distributed for age (age being the parameter uniform to the screening to the screening population) (Figure 72: The Normality of Age in the Screening Population

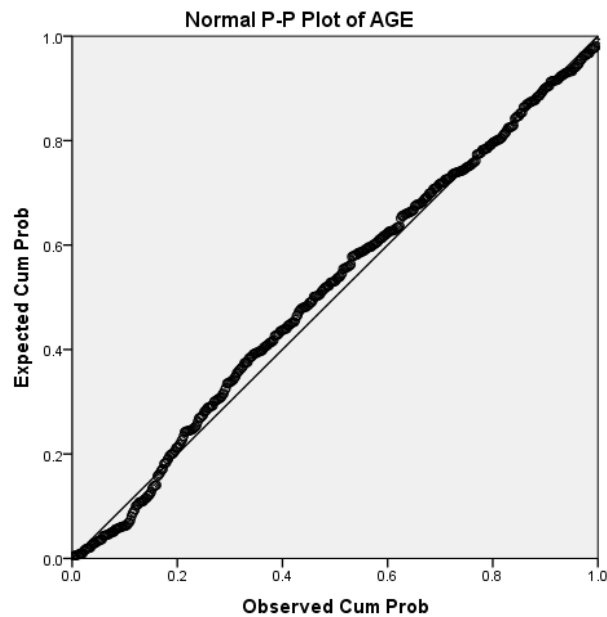
). With regard to the P-P plot for age in the screening population, as the result demonstrated a straight diagonal line, it was proposed that this data was normally distributed (Figure 73: P-P Plot of Normality in the Screening Population by Age

).



**Figure 72: The Normality of Age in the Screening Population**

-In the screening population (n = 484), the mean age was 58.38 years (X axis) and the range was 18.49 – 90.47 years. Visually, the screened data set followed a bell shaped distribution i.e. was normally distributed.



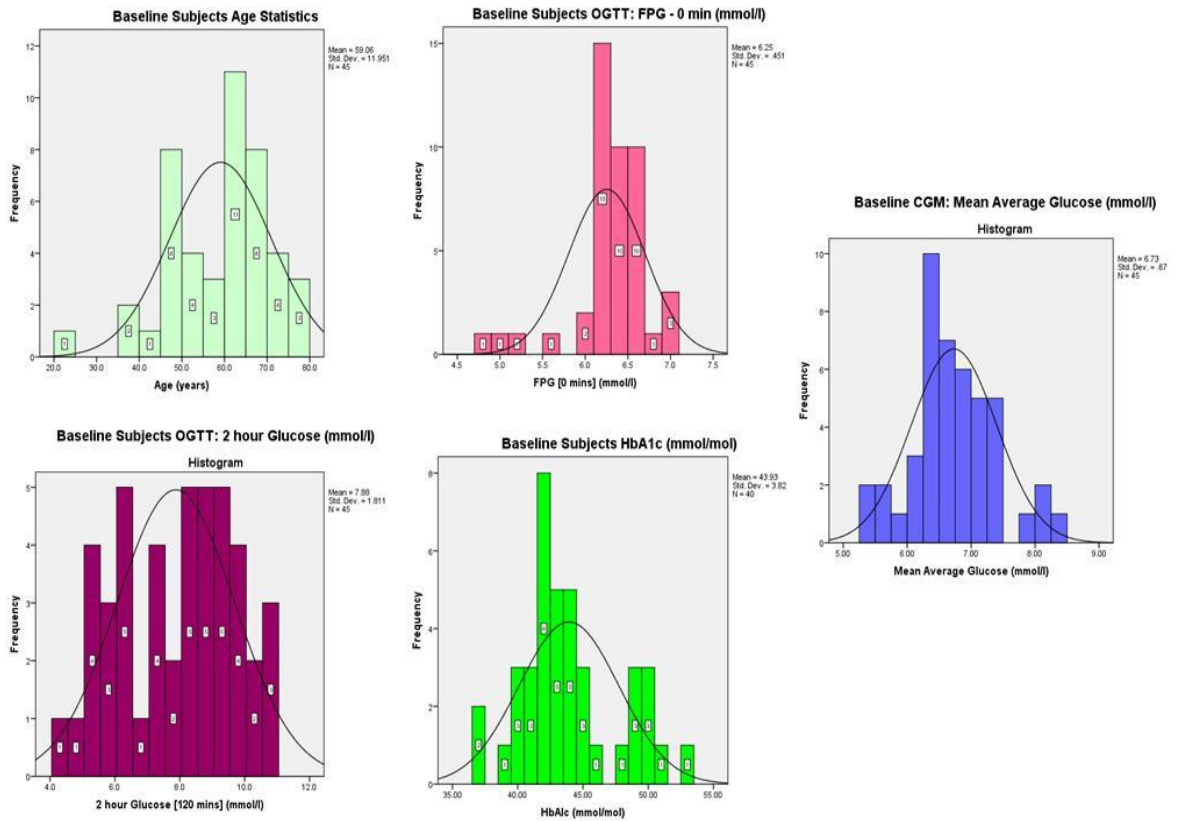
**Figure 73: P-P Plot of Normality in the Screening Population by Age**

-In the screening population (n = 484) the mean was 58.38 years and the range was 18.49 – 90.47 years. Visually, the screened data set appeared to follow a fairly straight line and therefore, it was proposed that this data was normally distributed; (\*Cum Prob = Cumulative Probability).

**For the sample population, a number of parameters were looked at to assess for normality visually: baseline age (years), baseline OGTT 0 mins (mmol/l), baseline OGTT 120 mins (mmol/l), baseline HbA1c (mmol/l), mean baseline Av Gl (mmol/l). For each of these parameters, the data appeared to be distributed fairly symmetrically, around the centre of all scores in a bell shape and therefore graphically, the sample population appeared to be normally distributed for these parameters, as can be seen below (**

**Figure 74: Normality in the Sample Population**

).



**Figure 74: Normality in the Sample Population**

-Visually, the sample data set follow a bell shaped distribution i.e. was normally distributed for each of the five parameters investigated i.e. **Age (years)**, **OGTT: FPG [0 mins] (mmol/l)**, **OGTT 2 hour Glucose [120 mins] (mmol/l)**, **HbA1c (mmol/mol)** and **Mean Average Glucose (mmol/l)**.

P-P plots were then constructed for the above parameters from the sample population. As can be seen from (Figure 75), (Figure 76), (Figure 77), (Figure 78) and (Figure 79) the result demonstrated fairly straight diagonal line, it was therefore proposed that this data was normally distributed.

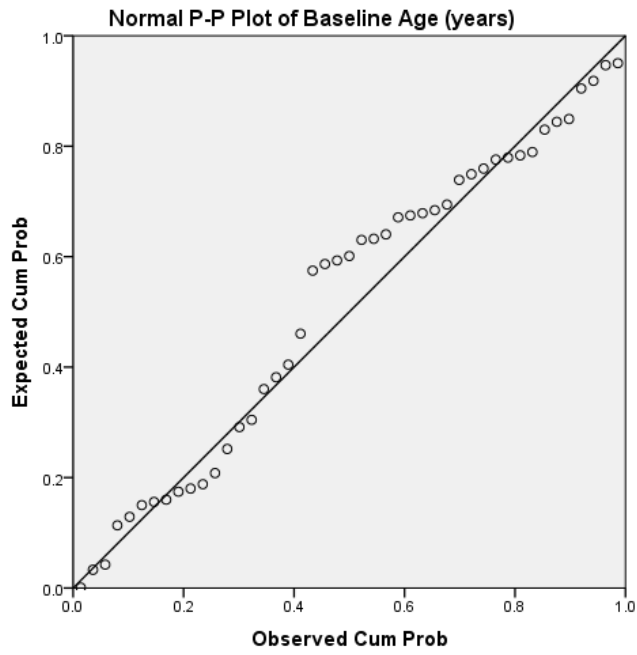


Figure 75: P-P Plots in the Sample Population at Baseline: Age

**Error! Reference source not found.** (\*Cum Prob = Cumulative Probability).

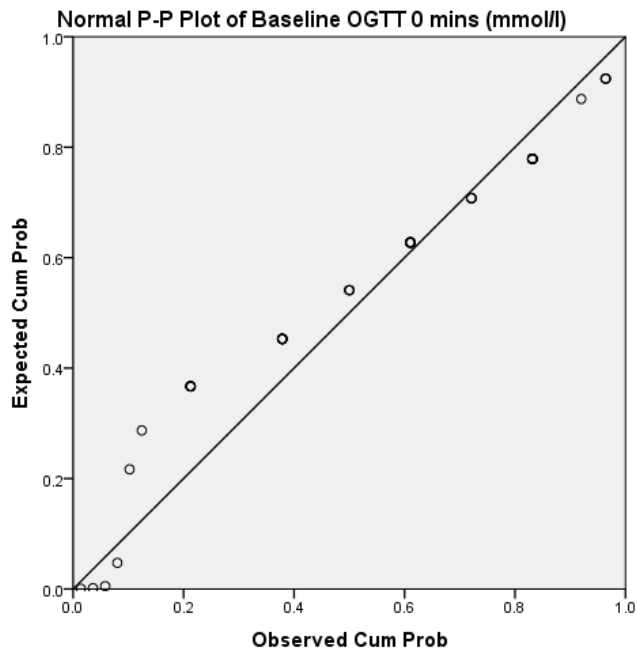


Figure 76: P-P Plots in the Sample Population at Baseline: FPG

**Error! Reference source not found.** (\*Cum Prob = Cumulative Probability).

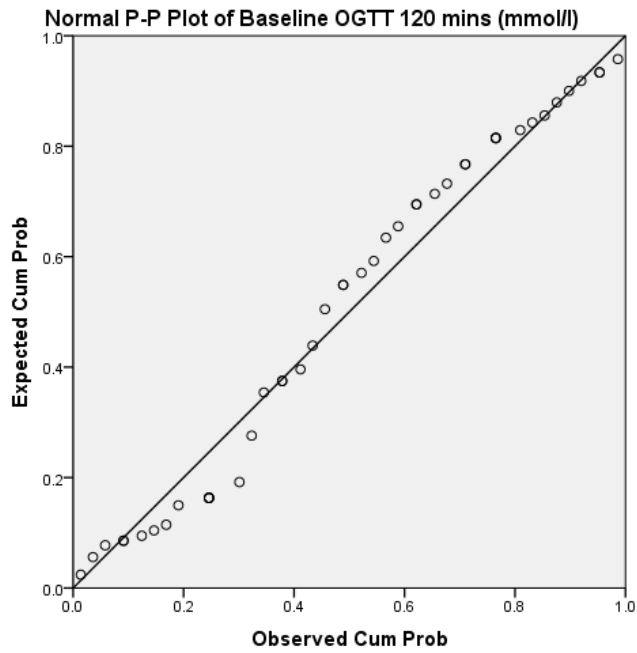


Figure 77: P-P Plots in the Sample Population at Baseline: 2 hr OGTT

-Error! Reference source not found. (\*Cum Prob = Cumulative Probability).

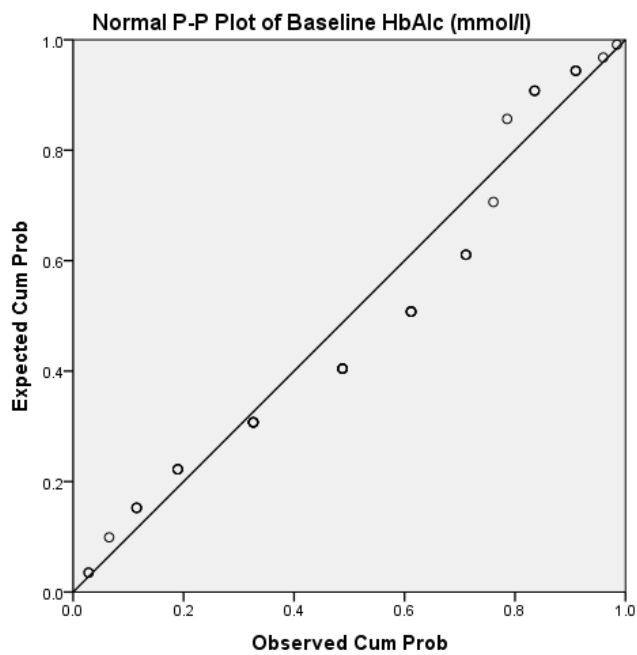
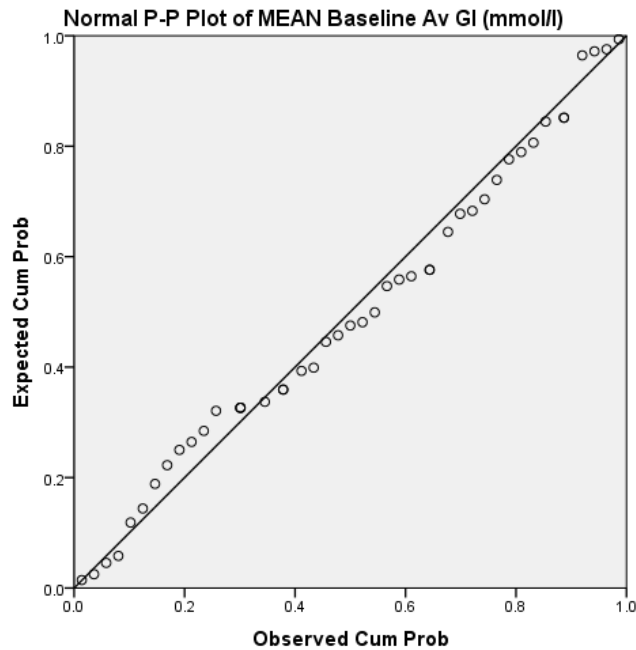


Figure 78: P-P Plots in the Sample Population at Baseline: HbA1c

-Error! Reference source not found. (\*Cum Prob = Cumulative Probability).



**Figure 79: P-P Plots in the Sample Population at Baseline: Mean Average Glucose**

-Visually, the data set appeared to follow a fairly straight line and therefore, it was proposed that this data was normally distributed for mean baseline average glucose (\*Cum Prob = Cumulative Probability).

### 5.2.2 Comparing Means – Paired Sample *t*-Test (Dependent *t*-Test)

Assuming the data were normally distributed, as the same subjects were tested at different time intervals (baseline, Year 1 and Year 3), the paired sample *t*-test (or dependent *t*-test) was used to compare differences in two means of the set parameters, as the study progressed in time. The means of the following 17 pairs of samples, at specific time intervals (stated below) were tested.

A table of summary statistics for the two experimental conditions was demonstrated for each pair tested [pair 1 - 17]. For each condition, the mean, the number of participants (N), the Std Dev of the sample and the standard error (i.e. the standard deviation divided by the square root of the sample size) was seen (Table 33).

- Pair 1 Baseline weight (Kg) with Year 1 weight (Kg)
- Pair 2 Baseline weight (Kg) with Year 3 weight (Kg)
- Pair 3 Year 1 weight (Kg) with Year 3 weight (Kg)
- Pair 4 Baseline BMI (Kg/m<sup>2</sup>) with Year 1 BMI (Kg/m<sup>2</sup>)
- Pair 5 Baseline BMI (Kg/m<sup>2</sup>) with Year 3 BMI (Kg/m<sup>2</sup>)
- Pair 6 Year 1 BMI (Kg/m<sup>2</sup>) with Year 3 BMI (Kg/m<sup>2</sup>)
- Pair 7 Baseline OGTT 0 mins (mmol/l) with Year 1 OGTT 0 mins (mmol/l)
- Pair 8 Baseline OGTT 0 mins (mmol/l) with Year 3 FPG 0 mins (mmol/l)
- Pair 9 Year 1 OGTT 0 mins (mmol/l) with Year 3 FPG 0 mins (mmol/l)
- Pair 10 Baseline OGTT 2 hours (mmol/l) with Year 1 OGTT 2 hours (mmol/l)
- Pair 11 Baseline HbA1c (mmol/mol) with Year 1 HbA1c (mmol/mol)
- Pair 12 Baseline HbA1c (mmol/mol) with Year 3 HbA1c (mmol/mol)
- Pair 13 Year 1 HbA1c (mmol/mol) with Year 3 HbA1c (mmol/mol)
- Pair 14 Mean Baseline Average Glucose (mmol/l) with Mean Year 1 Average Glucose (mmol/l)
- Pair 15 Mean Baseline Average Glucose Excursion (mmol/l) from Average with Mean Year 1 Average Glucose Excursion from Average (mmol/l)
- Pair 16 Mean Baseline Average above Normal Glucose (mmol/l) with Mean Year 1 Average above Normal Glucose (mmol/l)
- Pair 17 Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l) with Mean Year 1 Average Glucose Excursion from Normal Glucose (mmol/l)

**Table 33: Paired t-Tests: Paired Sample Statistics**

VARIABLES		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline Weight	96.85	37	20.66	3.39
	Year 1 Weight	97.37	37	20.06	3.29
Pair 2	Baseline Weight	94.47	38	21.30	3.45
	Year 3 Weight	94.61	38	22.26	3.61
Pair 3	Year 1 Weight	97.05	34	20.66	3.54
	Year 3 Weight	96.59	34	22.20	3.80
Pair 4	Baseline BMI	33.25	37	6.53	1.07
	Year 1 BMI	33.48	37	6.49	1.06
Pair 5	Baseline BMI	32.53	38	6.69	1.08
	Year 3 BMI	32.54	38	6.93	1.12
Pair 6	Year 1 BMI	33.37	34	6.77	1.16
	Year 3 BMI	33.13	34	7.03	1.20
Pair 7	Baseline OGTT 0 mins	6.27	36	.44	.073
	Year 1 OGTT 0 mins	6.24	36	.710	.11
Pair 8	Baseline OGTT 0 mins	6.25	37	.48	.079
	Year 3 FPG	6.96	37	1.83	.30
Pair 8	Year 1 OGTT 0 mins	6.23	32	.75	.13
	Year 3 FPG	6.94	32	1.91	.33
Pair 10	Baseline OGTT 120 mins	7.74	36	1.88	.31
	Year 1 OGTT 120mins	8.04	36	2.06	.34
Pair 11	Baseline HbA1c	44.03	33	4.01	.69
	Year 1 HbA1c	45.12	33	4.89	.85
Pair 12	Baseline HbA1c	44.00	34	3.89	.66
	Year 3 HbA1c	48.08	34	12.35	2.11
Pair 13	Year 1 HbA1c	45.41	34	4.82	.82
	Year 3 HbA1c	47.94	34	12.27	2.10
Pair 14	MEAN BL Av GI	6.72	37	.68	.11
	MEAN Y1 AvGI	6.89	37	1.04	.17
Pair 15	MEAN BL AvGI exc from Av	1.01	37	.36	.06
	MEAN Y1 AvGI exc from Av	1.15	37	.58	.09
Pair 16	MEAN BL Av above norm GI	2.22	37	.68	.11
	MEAN Y1 Av above norm GI	2.39	37	1.04	.17
Pair 17	MEAN BL Av GI exc from norm GI	2.48	37	.66	.11
	MEAN Y1 AvGI exc from norm GI	2.70	37	1.10	.18

A summary of paired samples tested was also demonstrated. The Null Hypothesis in this instance was that there was no significant difference (i.e. no variation) between specified variables in each pair. For example, regarding PAIR 1- weight, the Null



Hypothesis would be that there was no significant difference in the mean weight observed at baseline compared to the mean weight observed at Year 1.

For each pair, the mean differences between the scores were demonstrated, together with the Std Dev of the differences between the means and also the standard error (Std Err) of the differences between scores within each pair. The test statistic  $t$  was then obtained for each pair by dividing the mean of differences by the Std Err of differences. The size of the  $t$  was then compared against known values based on degrees of freedom (i.e. sample size -1). SPSS 20, used the degrees of freedom to calculate the exact probability that a value of  $t$  was big as the one obtained could occur if the Null Hypothesis were true (i.e. no difference between the means tested). In this instance, the two-tailed probability was demonstrated (the probability when no prediction was made about the direction of differences). In this test, at 95% Confidence Interval (CI), a  $p < 0.05$  was classed as statistically meaningful and significant and the Null Hypothesis was rejected at this point.

Table 34: T Tests: Paired Differences

VARIABLES		Paired Differences					T	Df	Sig. (2-tailed)
		Mean	Std. Dev	Std. Err Mean	95% CI of the Difference Lower Upper				
Pair 1	Baseline Weight - Year 1 Weight	-.52	5.59	.91	-2.38	1.34	-.56	36	.57
Pair 2	Baseline Weight - Year 3 Weight	-.13	5.56	.90	-1.96	1.69	-.14	37	.88
Pair 3	Year 1 Weight - Year 3 Weight	.45	6.78	1.16	-1.91	2.82	.39	33	.69
Pair 4	Baseline BMI - Year 1 BMI	-.22	1.95	.32	-.88	.421	-.71	36	.48
Pair 5	Baseline BMI - Year 3 BMI	-.01	2.02	.32	-.67	.65	-.03	37	.97
Pair 6	Year 1 BMI - Year 3 BMI	.23	2.43	.41	-.61	1.08	.57	33	.57
Pair 7	Baseline OGTT 0 mins - Year 1 OGTT 0 mins	.036	.61	.10	-.17	.24	.35	35	.72
Pair 8	Baseline OGTT 0 mins - Year 3 FPG	-.71	1.77	.29	-1.30	-.12	-2.44	36	.02
Pair 9	Year 1 OGTT 0 mins - Year 3 FPG	-.71	1.74	.30	-1.34	-.08	-2.32	31	.02
Pair 10	Baseline OGTT 120 mins - Year 1 OGTT 120mins	-.29	2.02	.33	-.98	.38	-.88	35	.38
Pair 11	Baseline HbA1c - Year 1 HbA1c	-1.09	5.03	.87	-2.87	.69	-1.24	32	.22
Pair 12	Baseline HbA1c - Year 3 HbA1c	-4.08	11.82	2.02	-8.21	.03	-2.01	33	.05
Pair 13	Year 1 HbA1c - Year 3 HbA1c	-2.52	11.82	2.02	-6.65	1.59	-1.24	33	.22
Pair 14	MEAN Baseline Av GI - MEAN Year1 AvGI	-.16	.835	.13	-.44	.11	-1.21	36	.23
Pair 15	MEAN Baseline AvGI excursion from Av - MEAN Year1 AvGI excursion from Av	-.14	.62	.10	-.35	.068	-1.36	36	.18
Pair 16	MEAN Baseline Av above norm GI - MEAN Year1 Av above norm GI	-.16	.83	.13	-.44	.11	-1.21	36	.23
Pair 17	MEAN Baseline Av GI excursion from norm GI - MEAN Year1 AvGI excursion from norm GI	-.21	.90	.14	-.51	.08	-1.43	36	.15

Results were as follows:

**PAIR 1:** (M 96.85, SE 3.397)  $t(36) = -0.56$ ,  $p > 0.05$  (0.574). There is a 57.3% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 2:** (M 94.47, SE 3.456)  $t(37) = -0.149$ ,  $p > 0.05$  (0.883). There is an 88.3% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 3:** (M 97.05, SE 3.544)  $t(33) = 0.393$ ,  $p > 0.05$  (0.697). There is a 69.7% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 4:** (M 33.25, SE 1.074)  $t(36) = -0.714$ ,  $p > 0.05$  (0.480). There is a 48% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 5:** (M 32.53, SE 1.086)  $t(37) = -0.037$ ,  $p > 0.05$  (0.971). There is a 97.1% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 6:** (M 33.37, SE 1.160)  $t(33) = 0.570$ ,  $p > 0.05$  (0.573). There is a 57.3% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted. Therefore, for weight and BMI, there was no significant difference between the means of these parameters at baseline, Year 1 or Year 3, as the study progressed with time to its outcome (PAIRS 1-6).

**PAIR 7:** (M 6.27, SE 0.073)  $t(35) = 0.350$ ,  $p > 0.05$  (0.728). There is a 72.8% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 8:** (M 6.25, SE 0.790)  $t(36) = -2.44$ ,  $p < 0.05$  (0.020). There is a 2% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was rejected, as there was a significant difference between the FPG at baseline and Year 3, with a 95% CI of -1.3 to -1.2. This indicated the boundaries within which the true mean difference lay and the fact that this interval did not contain a zero meant the true value of the mean difference was unlikely to be zero.

**PAIR 9:** (M 6.23, SE 0.132)  $t(31) = -2.32$ ,  $p < 0.05$  (0.027). There is a 2.7% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was rejected, as there was a significant difference between the FPG at Year 1 (OGTT) and Year 3, with a 95% CI of -1.34 to -0.86. This indicated the boundaries within which the true mean difference lay and the fact that this interval did not contain a zero meant the true value of the mean difference was unlikely to be zero. This demonstrated that a significant

difference was seen for between the means of FPG at baseline and Year 3 and also at Year 1 and Year 3, as the subjects progressed through the study with time. The difference between the mean FPG at baseline and Year 1 was not significant (PAIRS 7-9).

**PAIR 10:** (M 7.74, SE 0.313)  $t(35) = -0.881$ ,  $p > 0.05$  (0.385). There is a 38.5% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted. This demonstrated at baseline and Year 1, there was no significant difference between the means at 2hr OGTT as the study progressed with time (PAIR 10).

**PAIR 11:** (M 44.03, SE 0.698)  $t(32) = -1.24$ ,  $p > 0.05$  (0.223). There is a 22.3% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 12:** (M 44.00, SE 0.667)  $t(33) = -2.016$ ,  $p > 0.05$  (0.052). There is a 5.2% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted. This pairing, between the HbA1c at baseline and Year 3 was almost significant.

**PAIR 13:** (M 45.41, SE 0.827)  $t(33) = -1.24$ ,  $p > 0.05$  (0.221). There is a 22.1% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted. This demonstrated that the mean of the HbA1c at baseline and Year 3 approached the significance level to be different, but the HbA1c means at baseline and Year 1 and again at Year 1 and Year 3 were not significantly different, as the subjects progressed through the study with time (PAIRS 11-13).

**PAIR 14:** (M 6.72, SE 0.112)  $t(36) = -1.21$ ,  $p > 0.05$  (0.231). There is a 23.1% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 15:** (M 1.01, SE 0.060)  $t(36) = -1.36$ ,  $p > 0.05$  (0.180). There is an 18% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 16:** (M 2.22, SE 0.112)  $t(36) = -1.21$ ,  $p > 0.05$  (0.231). There is a 23% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted.

**PAIR 17:** (M 2.48, SE 0.110)  $t(36) = -1.43$ ,  $p > 0.05$  (0.159). There is a 15.9% chance that this could happen if the Null Hypothesis was true. The Null Hypothesis was accepted. This demonstrated that the 4 CGM parameters used in this study to look at progression, when looked at individually, their means were not significantly different at baseline to the means for each at Year 1 (PAIRS 14-17).

### 5.3 Summary: Chapter 5

In summary, at Year 1, all subjects from the baseline study were invited back for re-analysis. A number of parameters were analysed at Year 1, including age, study follow up interval, BMI, OGTT 0 hrs (FPG), OGTT 2 hours, HbA1c, CGM Glucose Excursion Parameters and CGM Profiles. At Year 3, variables which were analysed included age and follow up interval, BMI, OGTT 0 hrs (FPG) and HbA1c. The aim of this was to identify any parameter that demonstrated trends associated with progression to DM (T2DM). With regard to the study subjects, the majority of them were in the older adulthood or of adult retirement age. In general, the majority of subjects in this study were either overweight or obese and interestingly all of the subjects that were obese at baseline were still obese at Year 3. With regard to attendance rates, 82.2% of baseline subjects re-attended at Year 1 and 84.4% re-attended at Year 3, a response rate of over 80%. All of the subjects had GAD Ab levels < 5 U/ml (69) demonstrating that none of the subjects demonstrated serological marker positivity for LADA or T1DM.

As the study progressed with time, it was demonstrated that the mean subject FPG, the mean 2 Hour Glucose (mmol/l) and the mean subject HbA1c (n = 37) all increased in a stepwise fashion. With regard to CGM parameters: CGM Mean Average Glucose, CGM Mean Average Glucose Excursion from the Average Glucose (mmol/l), CGM Mean Average Glucose above Normal Glucose (mmol/l) and CGM Mean Average Glucose Excursion from Normal Glucose (mmol/l), again all increased in a stepwise fashion as the study progressed with time. With regard to the visually inspected CGM (SAP) Profiles, 17.8% of them were observed to demonstrate least variability, 35.6% were observed to demonstrate medium variability and 28.9% were observed to demonstrate most variability. These results closely mirrored the independent observer assessment of variability. At Year 1, each of the four CGM parameters, were looked at with regard to their relationship with the CGM profile groups. As the degree of variability of the visually inspected subject CGM profiles (SAP) increased from least variability to most variability, the mean of each of the four CGM parameters was observed to increase also (Figure 63: Summary: Year 1 Subjects CGM Parameters v CGM Profiles (SAP) - Mean Plots

a-d). The mean plots demonstrated a fairly positive linear correlation for each of these graphically, indicating a likely relationship between these two variables.

One assumed that the distribution of the study sample mean was normal. In order to check this assumption, the population data and the sample data was looked at in more detail. For the screening population, the data appeared to be distributed symmetrically, around the centre of all scores in a bell shape and therefore graphically, the screening population appeared to be normally distributed for age (age being the parameter uniform to the screening population) (Figure 72: The Normality of Age in the Screening Population

). With regard to the P-P plot for age in the screening population, as the result demonstrated a straight diagonal line, it was proposed that this data was normally distributed (Figure 73: P-P Plot of Normality in the Screening Population by Age

). Assuming the data were normally distributed, as the same subjects were tested at different time intervals (baseline, Year 1 and Year 3), the paired sample *t*-test (or dependent *t*-test) was used to compare differences in two means of the set parameters. This included demographic data, biochemical tests and CGM parameters, as the study progressed with time. The Null Hypothesis in this instance was that there was no significant difference (i.e. no variation) between specified variables in each pair with time. The Null Hypothesis was accepted when at the 95% confidence interval ( $p > 0.05$ ). However, when  $p < 0.05$ , this was classed as statistically meaningful and significant and the Null Hypothesis was rejected (Table 33) (Table 34).

**Pair 1** Baseline weight (Kg) with Year 1 weight (Kg) = The Null Hypothesis was accepted.

**Pair 2** Baseline weight (Kg) with Year 3 weight (Kg) = The Null Hypothesis was accepted.

**Pair 3** Year 1 weight (Kg) with Year 3 weight (Kg) = The Null Hypothesis was accepted.

**Pair 4** Baseline BMI ( $\text{Kg}/\text{m}^2$ ) with Year 1 BMI ( $\text{Kg}/\text{m}^2$ ) = The Null Hypothesis was accepted.

**Pair 5** Baseline BMI ( $\text{Kg}/\text{m}^2$ ) with Year 3 BMI ( $\text{Kg}/\text{m}^2$ ) = The Null Hypothesis was accepted.

**Pair 6** Year 1 BMI ( $\text{Kg}/\text{m}^2$ ) with Year 3 BMI ( $\text{Kg}/\text{m}^2$ ) = The Null Hypothesis was accepted.

**Pair 7** Baseline OGTT 0 mins (mmol/l) with Year 1 OGTT 0 mins (mmol/l) = The Null Hypothesis was accepted.

**Pair 8** Baseline OGTT 0 mins (mmol/l) with Year 3 FPG 0 mins (mmol/l) = The Null Hypothesis was rejected, as there was a significant difference between the FPG at baseline and Year 3.

**Pair 9** Year 1 OGTT 0 mins (mmol/l) with Year 3 FPG 0 mins (mmol/l) = The Null Hypothesis was rejected, as there was a significant difference between the FPG at Year 1 (OGTT) and Year 3.

**Pair 10** Baseline OGTT 2 hours (mmol/l) with Year 1 OGTT 2 hours (mmol/l) = The Null Hypothesis was accepted.

**Pair 11** Baseline HbA1c (mmol/mol) with Year 1 HbA1c (mmol/mol) = The Null Hypothesis was accepted.

**Pair 12** Baseline HbA1c (mmol/mol) with Year 3 HbA1c (mmol/mol) = The Null Hypothesis was accepted. However, this pairing, between the HbA1c at baseline and Year 3 was almost significant.

**Pair 13** Year 1 HbA1c (mmol/mol) with Year 3 HbA1c (mmol/mol) = The Null Hypothesis was accepted.

**Pair 14** Mean Baseline Average Glucose (mmol/l) with Mean Year 1 Average Glucose (mmol/l) = The Null Hypothesis was accepted.

**Pair 15** Mean Baseline Average Glucose Excursion (mmol/l) from Average with Mean Year 1 Average Glucose Excursion from Average (mmol/l) = The Null Hypothesis was accepted.

**Pair 16** Mean Baseline Average above Normal Glucose (mmol/l) with Mean Year 1 Average above Normal Glucose (mmol/l) = The Null Hypothesis was accepted.

**Pair 17** Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l) with Mean Year 1 Average Glucose Excursion from Normal Glucose (mmol/l) = The Null Hypothesis was accepted.

Data was analysed and for each pair one can see that in the majority of cases (for example for weight and BMI), the Null Hypothesis was accepted i.e. there was no significant difference between each of the set variables with time; time being baseline to Year 1, baseline to Year 3 and Year 1 to Year 3. However, a significant difference was seen between the means of FPG at baseline and Year 3 and also at Year 1 and Year 3, as the subjects progressed through the study with time; however the difference between the mean FPG at baseline and Year 1 was not significant. The pairing, between the HbA1c at baseline and Year 3 was almost significant and this demonstrated that the mean of the HbA1c at baseline and Year 3 approached the significance level to be different, but the

HbA1c means at baseline and Year 1 and again at Year 1 and Year 3 were not significantly different, as the subjects progressed through the study with time. With regard to the 4 CGM parameters used in this study to look at progression, when looked at individually, their means were not significantly different at baseline to the means for each at Year 1.



## 6.0 Results: Study Outcome

### 6.1 Analysing Outcome Data

A number of pre-selected parameters were analysed at baseline, Year 1 and Year 3 with regard to looking at progression towards an outcome of diabetes (T2DM) or non diabetes. These included a mix of glycaemic status indicators: standard biochemical indicators (FPG, HbA1c and OGTT 2 Hour), observed graphical indicators (CGM Profiles) and CGM parameter (CGM Sensor Data) indicators. Baseline data included information regarding subject age, ethnicity gender, FHx of DM, smoking status, hypertension status and dyslipidemia status. At Year 1, an outcome of DM (T2DM) or non diabetes was established for all study subjects following testing with OGTT. In addition to this, a number of parameters were analysed and compared to corresponding baseline data. These included FPG, OGTT 2 hours, HbA1c, plus the four CGM parameters (Mean Baseline Average Glucose; Mean Baseline Average Glucose Excursion from the Average, Mean Baseline Average above Normal and Mean Baseline Average Glucose Excursion from the Normal Glucose). At Year 3, an outcome of DM (T2DM) or non diabetes was established for all study subjects following FPG testing. In addition to this, the following parameters were analysed and compared to corresponding baseline data and Year 1 data, in order to look at progression or change over time.

A breakdown of the outcome at Year 1 and Year 3 can be seen in (Table 35) and Table 36) respectively. At year 1, 17.8% (n = 8) subjects had progressed to DM (T2DM). At Year 3, (n = 21) 46.6% of subjects had progressed to DM (T2DM). This would be in keeping with current literature with regard to the rate of progression.

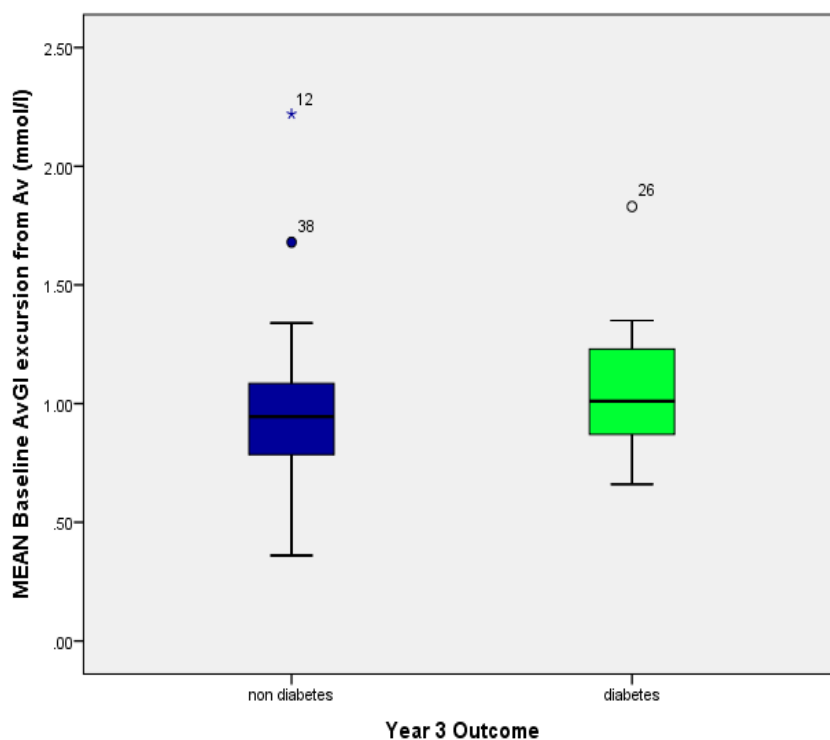
Table 35: Outcome: Year 1

		Frequency	Percent
Valid	non diabetes	29	64.4
	Diabetes	8	17.8
	Total	37	82.2
Missing	System	8	17.8
Total		45	100.0

**Table 36: Outcome: Year 3**

		Frequency	Percent
Valid	non diabetes	16	35.6
	Diabetes	21	46.7
	Total	37	82.2
Missing	System	8	17.8
Total		45	100.0

At the start of this study, 4 CGM Parameters were constructed to look at whether they, within the CGMS framework, were able to predict progression to DM (T2DM). One of the most promising parameters to look at this specifically during this study was seen to be ‘Mean Baseline AvGI Excursion from Av’. However, when one looked at this particular, parameter - ‘Mean Baseline AvGI excursion from Av’ with regard to Year 3 outcome of diabetes (T2DM), the median values were almost the same for the subjects with diabetes as those without. The resulting inter-quartile ranges and lowest and highest values of data were for each outcome was almost identical also (Figure 80).



**Figure 80: Year 3 Outcome v Mean Baseline AvGI excursion from Av**

-MEAN Baseline AvGI excursion from Av was looked at with regard to Year 3 Outcome, i.e. non diabetes or diabetes using a box-plot, in an attempt to graphically display the distribution of data through quartiles for each possible outcome. Each box was made up of a lower 25<sup>th</sup> percentile, a thick central tendency line (the median) and the upper 75<sup>th</sup> percentile line. The lines which extended vertically from the boxes indicated variability outside the lower and upper quartiles i.e. they represented the lowest and highest value of data, respectively. Outliers (which were values greater than 1.5 inter-quartile ranges away from the 25<sup>th</sup> or the 75<sup>th</sup> percentile), were demonstrated as individual points (circles = mild outlier, star = extreme outlier).

## 6.2. Independent *t* Test

Assuming the data were normally distributed, the Independent Sample *t*-Test, or *t* –test for short, was used to compare the means of two independent groups in order to determine whether there was a statistically significant difference between them. The two outcome groups were non diabetes and diabetes. The outcome of both groups at Year 1 and Year 3 was investigated with regard to a number of baseline variables:

- Baseline Age (years)
- Baseline OGTT 0 mins (mmol/l)
- Baseline OGTT 2 hours (mmol/l)
- Baseline HbA1c (mmol/mol)
- Mean Baseline Average Glucose (mmol/l)
- Mean Baseline Average Glucose Excursion (mmol/l) from Average
- Mean Baseline Average above Normal Glucose (mmol/l)
- Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l)
- Baseline BMI (Kg/m<sup>2</sup>)
- At Year 3, the outcome of both groups was investigated with regard to Year 1 Insulin and Year 1 C-peptide.

A table of summary statistics for the two experimental conditions was demonstrated for each variable tested. For each condition, the mean, the number of participants (N), the Std Dev of the sample and the standard error (i.e. the standard deviation divided by the square root of the sample size) was seen. For example, one can see that non diabetes had 29 participants and diabetes had 8 participants at Year 1 Outcome for all groups except HbA1c, who had 25 and 8 respectively. With regard to age, the mean age of those who were DM (T2DM) at Year 1 was 63.17 years compared to the non diabetics at Year 1, with a mean age of 59.7 years. On inspection of this data further, at Year 1, the mean of all variables (except HbA1c) was higher in the diabetic compared to the non diabetic group (Table 37).

A summary of the independent samples tested was also demonstrated. The Null Hypothesis in this instance was that there was no significant difference (i.e. no variation) with regard to the specified variable in each group, with regard to outcome. In parametric testing, one assumes that the variances in experimental groups are roughly equal. Levene's test was used to see whether variances were different in different

groups and this test tested the hypothesis that the variances in the two groups were equal. Where Levene's test was  $p > 0.05$ , then equal variances were assumed and the Null Hypothesis was accepted, assuming the variances were roughly equal and equal variances was assumed (EVA). This was the case for all variables except Baseline OGTT 120 mins and HbA1c, respectively. With regard to these two variables, the test statistics in the row labelled Equal Variances Not Assumed (EVNA) were used (Table 38).

**Table 37: Independent *t*-test: Sample Statistics: Year 1 Outcome**

Variable	Year 1 Outcome	N	Mean	Std. Deviation	Std. Error Mean
Baseline Age (years)	non diabetes	29	59.70	12.00	2.22
	Diabetes	8	63.17	9.08	3.21
Baseline OGTT 0 mins (mmol/l)	non diabetes	29	6.26	.48	.090
	Diabetes	8	6.36	.17	.062
Baseline OGTT 120 mins (mmol/l)	non diabetes	29	7.46	1.88	.35
	Diabetes	8	8.81	1.37	.48
Baseline HbA1c (mmol/l)	non diabetes	25	44.48	4.41	.88
	Diabetes	8	42.62	1.92	.67
MEAN Baseline Av GI (mmol/l)	non diabetes	29	6.65	.67	.12
	Diabetes	8	6.99	.68	.24
MEAN Baseline AvGI excursion from Av (mmol/l)	non diabetes	29	.95	.28	.05
	Diabetes	8	1.22	.54	.19
MEAN Baseline Av above norm GI (mmol/l)	non diabetes	29	2.15	.67	.12
	Diabetes	8	2.49	.68	.24
MEAN Baseline Av GI excursion from norm GI (mmol/l)	non diabetes	29	2.38	.65	.12
	Diabetes	8	2.85	.63	.22
Baseline BMI (kg/m <sup>2</sup> )	non diabetes	29	33.18	6.35	1.18
	Diabetes	8	33.50	7.59	2.68

Table 38: Independent t test: Equality of Means: Year 1 Outcome

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Dif	Std. Error Dif	95% Confidence Interval of the Difference	
									Lower	Upper
Baseline Age (years)	*EVA	.48	.49	-.75	35	.45	-3.46	4.58	-12.77	5.83
	**EVNA			-.88	14.52	.38	-3.46	3.90	-11.82	4.88
Baseline OGTT 0 mins (mmol/l)	EVA	1.85	.18	-.54	35	.58	-.09	.17	-.45	.26
	EVNA			-.88	32.00	.38	-.09	.11	-.32	.12
Baseline OGTT 120 mins (mmol/l)	EVA	4.86	.03	-1.88	35	.06	-1.34	.71	-2.80	.10
	EVNA			-2.25	15.18	.03	-1.34	.59	-2.61	-.07
Baseline HbA1c (mmol/l)	EVA	5.09	.03	1.14	31	.26	1.85	1.62	-1.45	5.16
	EVNA			1.66	27.62	.10	1.85	1.11	-.42	4.13
MEAN Baseline Av Gl (mmol/l)	EVA	.04	.84	-1.27	35	.21	-.34	.26	-.89	.20
	EVNA			-1.26	11.03	.23	-.34	.27	-.94	.25
MEAN Baseline AvGl excursion from Av (mmol/l)	EVA	3.97	.05	-1.96	35	.05	-.27	.14	-.564	.00
	EVNA			-1.39	8.13	.19	-.27	.19	-.73	.17
MEAN Baseline Av above norm Gl (mmol/l)	EVA	.04	.84	-1.27	35	.21	-.34	.26	-.89	.20
	EVNA			-1.26	11.03	.23	-.34	.27	-.94	.25
MEAN Baseline Av Gl excursion from norm Gl (mmol/l)	EVA	.00	.92	-1.82	35	.07	-.47	.25	-.99	.05
	EVNA			-1.85	11.48	.08	-.47	.25	-1.02	.084
Baseline BMI (kg/m <sup>2</sup> )	EVA	.20	.65	-.12	35	.90	-.31	2.64	-5.68	5.05
	EVNA			-.10	9.87	.91	-.31	2.93	-6.86	6.23

\*EVA = Expected Variances Assumed; \*\*EVNA = Expected Variances Not Assumed

The test statistic  $t$  was then obtained for each pair by dividing the mean of differences by the Std Err of differences. The size of the  $t$  was then compared against known values based on degrees of freedom (i.e. sample size -1). SPSS 20 used the degrees of freedom to calculate the exact probability that a value of  $t$  was as big as the one obtained could occur if the Null Hypothesis were true (i.e. no difference between the means tested). In this instance, the two-tailed probability was demonstrated, which was the probability when no prediction was made about the direction of differences. In this test, at 95% CI, a  $p < 0.05$  was classed as statistically meaningful and significant and the Null Hypothesis was rejected. In this study, the effect size was also demonstrated, which gave an objective measure of the importance of an effect and the strength of the relationship between variables. Pearson's correlation coefficient  $r$ , is a measure of effect size and it is constrained to lie between 0 (no effect) and 1 (a perfect effect). Cohen's (1992) (56) reference regarding effect size was used in this study i.e.  $r = .10$  (small effect);  $r = .30$  (medium effect) and  $r = 0.50$  (large effect). Results were as follows for Year 1 outcome (Table 37, Table 38):

- **Baseline Age (years):** DM (M 63.17, SE 3.21) were older compared to non diabetics (M 59.70, SE 2.22) at Year 1, but this difference was not significant  $t(35) = -0.75$ ,  $p > 0.05$  (0.45). The Null Hypothesis was accepted, as there was no significant difference between the groups in age at Year 1. In this instance the effect was small ( $r = 0.12$ ).
- **Baseline OGTT 0 mins (mmol/l):** DM (M 6.36, SE 0.62) had higher OGTT 0 mins compared to non diabetics (M 6.26, SE 0.90) at Year 1, but this was not significant  $t(35) = -0.54$ ,  $p > 0.05$  (0.58) and the size effect was small ( $r = 0.09$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for OGTT 0 mins.
- **Baseline OGTT 2 hours (mmol/l):** DM (M 8.81, SE 0.48) had higher OGTT 120 mins compared to non diabetics (M 7.46, SE 0.88) at Year 1, which **was significant**  $t(15.18) = -2.25$ ,  $p < 0.05$  (0.039). The Null Hypothesis was rejected, as there was a significant difference between the groups at Year 1 for OGTT 2 hours. The effect size was also **large** in this instance ( $r = 0.50$ ).
- **Baseline HbA1c (mmol/mol):** Non diabetics (M 44.4, SE 0.88) had higher HbA1c compared to diabetics (M 42.62, SE 0.67) at Year 1, but this was not significant  $t(27.62) = 1.66$ ,  $p > 0.05$  (0.10). The Null Hypothesis was accepted, as

there was no significant difference between the groups at Year 1 for HbA1c. The effect size was of medium in this instance ( $r = 0.30$ ).

- **Mean Baseline Average Glucose (mmol/l):** DM (M 6.99, SE 0.24) had higher Mean Baseline Average Glucose compared to non diabetics (M 6.65, SE 0.12) at Year 1, but this was not significant  $t(35) = - 1.274$ ,  $p >0.05$  (0.21). The effect size approached a medium effect ( $r = 0.20$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose (mmol/l).
- **Mean Baseline Average Glucose Excursion (mmol/l) from Average:** DM (M 1.22, SE 0.19) had higher Mean Baseline Average Glucose Excursion (mmol/l) from Average compared to non diabetics (M 0.95, SE 0.05) at Year 1. This result **was just about significant**, as  $t(35) = - 1.96$ ,  $p >0.05$  (0.05) and the effect size was seen to be medium in size ( $r = 0.31$ ). The Null Hypothesis was rejected, as there was a significant difference between the groups at Year 1 for Mean Baseline Average Glucose Excursion (mmol/l) from Average.
- **Mean Baseline Average above Normal Glucose (mmol/l):** DM (M 2.49, SE 0.24) had higher Mean Baseline Average Glucose above Normal Glucose compared to non diabetics (M 2.15, SE 0.12 ) at Year 1, but this was not significant  $t(35) = - 1.27$ ,  $p >0.05$  (0.21). The effect size approached a medium effect ( $r = 0.20$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose above Normal Glucose (mmol/l).
- **Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l):** DM (M 2.85, SE 0.22) had higher Mean Baseline Average Glucose Excursion from Normal Glucose compared to non diabetics (M 2.38, SE 0.12) at Year 1, which was demonstrated to be **approaching significance**. However, although it was not significant  $t(35) = - 1.82$ ,  $p >0.05$  (0.77) the effect size was medium ( $r = 0.29$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose above Normal Glucose (mmol/l).
- **Baseline BMI (Kg/m<sup>2</sup>):** DM (M 33.50, SE 2.68) had higher BMI compared to non diabetics (M33.18, SE 1.18 ) at Year 1, but this was not significant  $t(35) = - 0.12$ ,  $p >0.05$  (0.905) and the effect size was small ( $r = 0.02$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for BMI.

Results were as follows for Year 3 Outcome (Table 39, Table 40):

- **Baseline Age (years):** Non Diabetics (M 59.59, SE 2.21) were older compared to DM (T2DM) (M 58.44, SE 2.92) at Year 1, but this difference was not significant  $t(35) = 0.235$ ,  $p > 0.05$  (0.76) and the effect size was small ( $r = 0.04$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups in age at Year 1.
- **Baseline OGTT 0 mins (mmol/l):** DM (T2DM) (M 6.34, SE 0.08) had higher OGTT 0 mins compared to non diabetics (M 6.13, SE 0.14) at Year 1, but this was not significant  $t(35) = -1.33$ ,  $p > 0.05$  (0.19). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for OGTT 0 mins; the effect size approached a medium result here ( $r = 0.21$ ).
- **Baseline OGTT 2 hours (mmol/l):** DM (T2DM) (M 8.09, SE 0.37) had higher OGTT 120 mins compared to non diabetics (M 7.58, SE 0.45) at Year 1, which not significant  $t(35) = -0.87$ ,  $p > 0.05$  (0.39) and the effect size was small ( $r = 0.14$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for OGTT 2 hours.
- **Baseline HbA1c (mmol/mol):** DM (T2DM) (M 44.78, SE 0.92) had higher HbA1c compared to non diabetics (M 43.42.62, SE 0.87) at Year 1, but this was not significant  $t(32) = -1.03$ ,  $p > 0.05$  (0.31). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for HbA1c; the effect size approached a medium effect here ( $r = 0.17$ ).
- **Mean Baseline Average Glucose (mmol/l):** DM (T2DM) (M 6.88, SE 0.13) had higher Mean Baseline Average Glucose compared to non diabetics (M 6.65, SE 0.20) at Year 1, but this was not significant  $t(35) = -1.02$ ,  $p > 0.05$  (0.31). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose (mmol/l); the effect size approached a medium effect here ( $r = 0.16$ ).
- **Mean Baseline Average Glucose Excursion (mmol/l) from Average:** DM (T2DM) (M 1.05, SE 0.08) had higher Mean Baseline Average Glucose Excursion (mmol/l) from Average compared to non diabetics (M 0.96, SE 0.07) at Year 1, but this was not significant  $t(35) = -0.75$ ,  $p > 0.05$  (0.45) and the effect size was small ( $r = 0.12$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose Excursion (mmol/l) from Average.



- **Mean Baseline Average above Normal Glucose (mmol/l):** DM (T2DM) (M 2.38, SE 0.13) had higher Mean Baseline Average Glucose above Normal Glucose compared to non diabetics (M 2.14, SE 0.20 ) at Year 1, but this was not significant  $t(35) = - 1.02$ ,  $p >0.05$  (0.31). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose above Normal Glucose (mmol/l); the effect size approached a medium effect here ( $r = 0.16$ ).
- **Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l):** DM (T2DM) (M 2.65, SE 0.12) had higher Mean Baseline Average Glucose Excursion from Normal Glucose compared to non diabetics (M 2.39, SE 0.19) at Year 1, but this was not significant  $t(35) = - 1.13$ ,  $p >0.05$  (0.26) and so the Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for Mean Baseline Average Glucose above Normal Glucose (mmol/l); the effect size approached a medium effect here ( $r = 0.18$ ).
- **Baseline BMI (Kg/m<sup>2</sup>):** Non diabetics (M 32.77, SE 1.61) had higher BMI compared to DM (T2DM) (M 32.46, SE 1.56) at Year 1, but this was not significant  $t(35) = - 0.13$ ,  $p >0.05$  (0.896), with a small effect size ( $r = 0.02$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for BMI.
- **Year 1 Insulin (pmol/L):** Non diabetics (M 125.34, SE 16.62) had higher venous Insulin levels in peripheral blood compared to DM (T2DM) (M 112.37, SE 13.60) at Year 1, but this was not significant  $t(30) = 0.60$ ,  $p >0.05$  (0.54), with a small effect size ( $r = 0.11$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for venous Insulin (pmol/L).
- **Year 1 C-peptide (pmol/ml):** Non diabetics (M 0.630, SE 0.072) had higher venous C-peptide levels in peripheral blood compared to DM (T2DM) (M 0.55, SE 0.06) at Year 1, but this was not significant  $t(30) = 0.78$ ,  $p >0.05$  (0.43), with a small effect size ( $r = 0.14$ ). The Null Hypothesis was accepted, as there was no significant difference between the groups at Year 1 for venous C-peptide (pmol/ml).

**Table 39: Independent t-tests: Sample Statistics: Year 3 Outcome**

Variable	Year 3 Outcome	N	Mean	Std. Deviation	Std. Error Mean
Baseline Age (years)	non diabetes	16	59.59	8.87	2.21
	Diabetes	21	58.44	13.42	2.92
Baseline OGTT 0 mins (mmol/l)	non diabetes	16	6.13	.57	.14
	Diabetes	21	6.34	.37	.08
Baseline OGTT 120 mins (mmol/l)	non diabetes	16	7.58	1.83	.45
	Diabetes	21	8.09	1.70	.37
Baseline HbA1c (mmol/l)	non diabetes	14	43.42	3.27	.87
	Diabetes	19	44.78	4.04	.92
MEAN Baseline Av GI (mmol/l)	non diabetes	16	6.64	.81	.20
	Diabetes	21	6.88	.59	.13
MEAN Baseline AvGI excursion from Av (mmol/l)	non diabetes	16	.96	.30	.07
	Diabetes	21	1.05	.39	.08
MEAN Baseline Av above norm GI (mmol/l)	non diabetes	16	2.14	.81	.20
	Diabetes	21	2.38	.59	.13
MEAN Baseline Av GI excursion from norm GI (mmol/l)	non diabetes	16	2.39	.78	.19
	Diabetes	21	2.65	.59	.12
Baseline BMI (kg/m <sup>2</sup> )	non diabetes	16	32.77	6.46	1.61
	Diabetes	21	32.46	7.16	1.56
Year 1 Insulin (pmol/L)	non diabetes	15	125.34	64.37	16.62
	Diabetes	17	112.37	56.08	13.60
Year 1 C-peptide (pmol/ml)	non diabetes	15	.63	.27	.072
	Diabetes	17	.55	.28	.069

At Year 3, none of the variables tested demonstrated significance difference in means, when the outcome of the diabetes and non diabetes groups was investigated, respectively. This differed to what was seen at Year 1, where a significant difference in means between the two groups was seen for Baseline OGTT 2 hours (mmol/l) and Mean Baseline Average Glucose Excursion (mmol/l) from Average. In addition, at Year 1, Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l) also approached significance.

**Table 40: Independent t-test: Equality of Means: Year 3 Outcome**

			Levene's Test for Equality of Variances		t-test for Equality of Means						
			F	Sig.	T	Df	Sig. (2-tailed)	Mean Dif	Std Err Dif	95% Confidence Interval of the Difference	
										Lower	Upper
Baseline Age (years)	*EVA	1.46	.23	.29	35	.76	1.14	3.88	-6.73	9.02	
	**EVNA			.31	34.42	.75	1.14	3.67	-6.31	8.61	
Baseline OGTT 0 mins (mmol/l)	EVA	1.67	.20	-1.33	35	.19	-.21	.15	-.53	.11	
	EVNA			-1.26	24.42	.22	-.21	.16	-.55	.13	
Baseline OGTT 120 mins (mmol/l)	EVA	.68	.41	-.87	35	.39	-.50	.58	-1.69	.67	
	EVNA			-.86	31.07	.39	-.50	.59	-1.71	.69	
Baseline HbA1c (mmol/l)	EVA	1.77	.19	-1.03	31	.31	-1.36	1.31	-4.05	1.32	
	EVNA			-1.06	30.67	.29	-1.36	1.27	-3.96	1.24	
MEAN Baseline Av Gl (mmol/l)	EVA	1.10	.30	-1.02	35	.31	-.23	.23	-.70	.23	
	EVNA			-.98	26.49	.33	-.23	.24	-.73	.25	
MEAN Baseline AvGl excursion from Av (mmol/l)	EVA	.36	.55	-.75	35	.45	-.08	.11	-.32	.15	
	EVNA			-.77	34.95	.44	-.08	.11	-.32	.14	
MEAN Baseline Av above norm Gl (mmol/l)	EVA	1.10	.30	-1.02	35	.31	-.23	.23	-.70	.23	
	EVNA			-.98	26.49	.33	-.23	.24	-.73	.25	
MEAN Baseline Av Gl excursion from norm Gl (mmol/l)	EVA	1.11	.29	-1.13	35	.26	-.25	.22	-.71	.20	
	EVNA			-1.09	26.95	.28	-.25	.23	-.74	.22	
Baseline BMI (kg/m <sup>2</sup> )	EVA	.34	.56	.13	35	.89	.30	2.28	-4.32	4.93	
	EVNA			.13	33.93	.89	.30	2.24	-4.26	4.87	
Year 1 Insulin (pmol/L)	EVA	.78	.38	.60	30	.54	12.97	21.28	-30.50	56.44	
	EVNA			.60	28.03	.55	12.97	21.47	-31.02	56.96	
Year 1 C-peptide (pmol/ml)	EVA	.01	.90	.78	30	.43	.078	.10	-.12	.28	
	EVNA			.78	29.65	.43	.078	.10	-.12	.28	

\*EVA = Expected Variances Assumed; \*\*EVNA = Expected Variances Not Assumed

### **6.3 Chi Square Test**

In this study, a number of categorical variables were assessed at baseline, to see if they had any bearing on the outcome of DM (T2DM) at Year 1 or Year 3. Pearson's-chi square test is used to compare the frequency observed in certain categories to the frequencies one may expect to get in those categories by chance. In this study, the Pearson's chi-square statistic tested whether the two variables were independent when tested. If the significance was small enough ( $p < 0.05$ ), then the Null Hypothesis was rejected and confidence was gained in the hypothesis that the variables in some way were related. In order to use to use the chi-square test the expected frequency in each cell should be greater than 5, otherwise there may be a loss of statistical power (and may fail to detect a genuine effect); i.e. the sample size may be too small and the sampling distribution of the test statistic may be too deviated from a chi-square distribution to be of any use. In these circumstances, when the sample sizes were deemed small, for an accurate exact probability of the chi sq statistic being accurate, Fishers exact test was used.

#### **Outcome: Year 1: Gender**

Chi sq (1) = 2.786,  $p < 0.05$ ; p value 0.102 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, gender does not significantly affect risk of DM (T2DM) at Year 1. The odds ratio demonstrated that for females, at Year 1 their odds of being non diabetic were 5.7 times higher than if they had been a male; (likelihood ratio 3.162) (Figure 81).

#### **Outcome: Year 3 Gender**

Chi sq (1) = 2.733,  $p < 0.05$ ; p value 0.098 (Pearson's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline gender does not significantly affect risk of DM (T2DM) at Year 3 even though the trend suggests that it would be so for males; (likelihood ratio = 2.73) (Figure 82).

#### **Outcome: Year 1 Ethnicity**

Chi sq (1) = 0.583,  $p < 0.05$ ; p value 0.610 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, ethnicity did not significantly affect risk of DM (T2DM) at Year 1. It was difficult to make any conclusions about the odds ratio, given the few numbers of non Caucasians (Figure 83).

### **Outcome: Year 3 Ethnicity**

Chi sq (1) = 0.083,  $p < 0.05$ ; p value 0.587 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, ethnicity did not significantly affect risk of DM (T2DM) at Year 3 (Figure 84).

### **Outcome: Year 1 Smoking Status**

Chi sq (2) = 0.035,  $p < 0.05$ ; p value 0.982 (Pearson's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, smoking status did not significantly affect risk of DM (T2DM) at Year 1; (likelihood ratio = 0.035) (Figure 85).

### **Outcome: Year 3 Smoking Status**

Chi sq (2) = 0.71,  $p < 0.05$ ; p value 0.701 (Pearson's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, smoking status does not significantly affect risk of DM (T2 DM) at Year 3; (likelihood ratio = 0.721) (Figure 86).

### **Outcome: Year 1 Hypertension**

Chi sq (1) = 0.775,  $p < 0.05$ ; p value 0.649 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline hypertension does not significantly affect risk of DM (T2DM) at Year 1. (likelihood ratio = 0.864) (Figure 87).

### **Outcome: Year 3 Hypertension**

Chi sq (1) = 1.64,  $p < 0.05$ ; p value 0.199 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline hypertension does not significantly affect risk of DM (T2DM) at Year 3; (likelihood ratio = 1.64) (Figure 88).

### **Outcome: Year 1 Dyslipidemia**

Chi sq (1) = 0.293,  $p < 0.05$ ; p value 0.701 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline dyslipidemia does not significantly affect risk of DM (T2DM) at Year 1. The odds ratio demonstrated that for subjects that had dyslipidemia, the odds of being non diabetic

were 1.5 times higher than if they didn't have dyslipidemia at Year 1 (likelihood ratio = 1.20) (Figure 89).

**Outcome: Year 3 Dyslipidemia**

Chi sq (1) = 1.205,  $p < 0.05$ ; p value 0.272 (Pearson's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline dyslipidemia does not significantly affect risk of DM (T2DM) at Year 3; (likelihood ratio = 1.20) (Figure 90).

**Outcome: Year 1 FHx of DM**

Chi sq (1) = 0.379,  $p < 0.05$ ; p value 0.690 (Fisher's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, baseline FHx of DM does not significantly affect risk of DM (T2DM) at Year 1. The odds ratio demonstrated that for subjects that had a FHx of DM at baseline, the odds of being non diabetic were 0.61 times higher than if they didn't have a FHx of DM (likelihood ratio = 0.374) (Figure 91).

**Outcome: Year 3 FHx of DM**

Chi sq (1) = 0.379,  $p < 0.05$ ; p value 0.538 (Pearsons's exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore, a baseline FHx of DM does not significantly affect risk of DM (T2 DM) at Year 3; (likelihood ratio 0.381) (Figure 92).

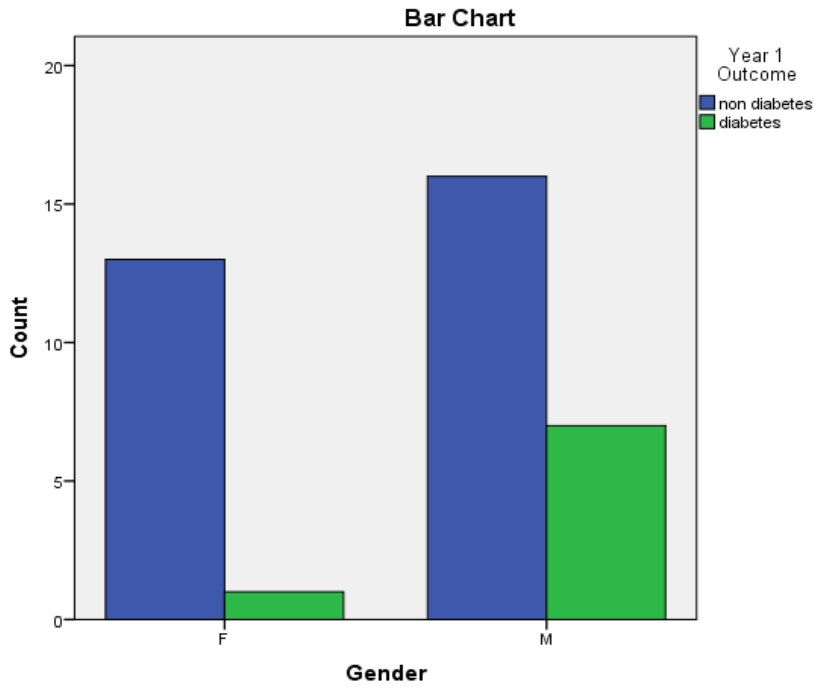


Figure 81: Chi-sq Year 1 Outcome: Gender

-In the bar chart above, baseline gender does not significantly affect risk of DM (T2DM) at Year 1 (F = female; M = male).

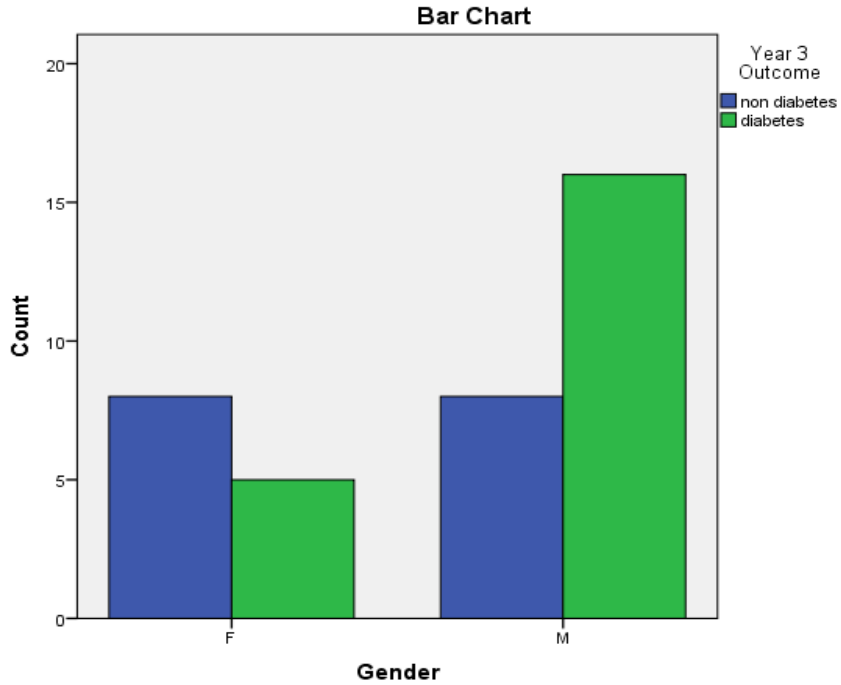


Figure 82: Chi-sq Year 3 Outcome: Gender

-In the bar chart above, baseline gender does not significantly affect risk of DM (T2DM) at Year 3 (F = female; M = male).

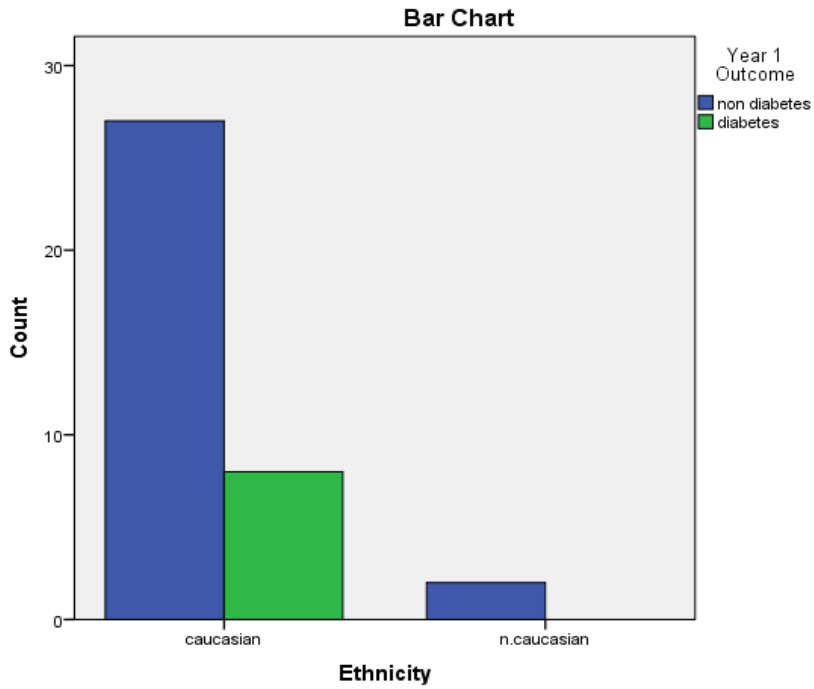


Figure 83: Chi-sq Year 1 Outcome: Ethnicity

-In the bar chart above, baseline ethnicity did not significantly affect risk of DM (T2DM) at Year 1; (n. caucasian = non caucasian).

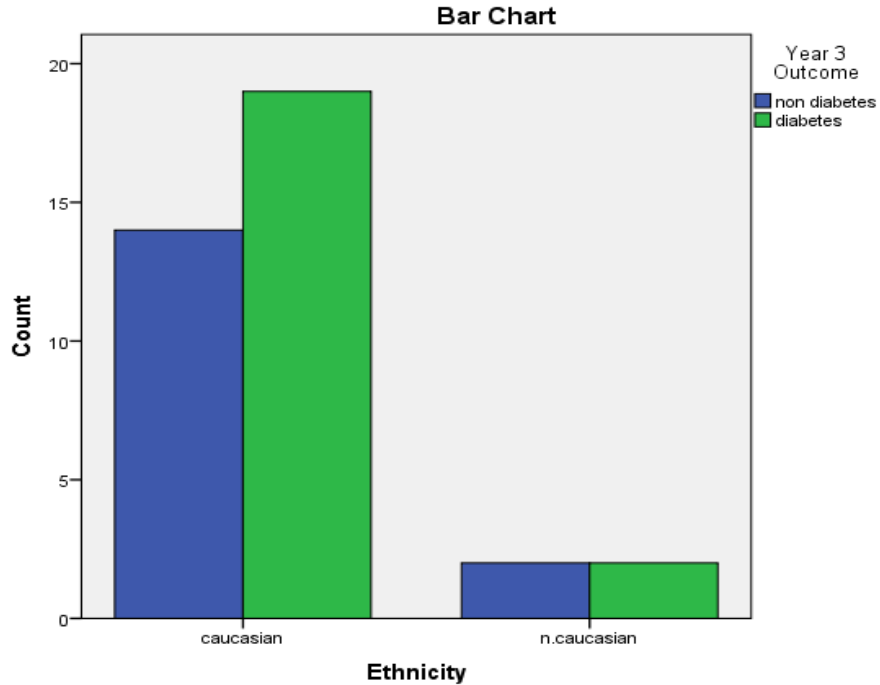


Figure 84: Chi-sq Year 3 Outcome: Ethnicity

In the bar chart above, baseline ethnicity did not significantly affect risk of DM (T2DM) at Year 3; (n. caucasian = non caucasian).



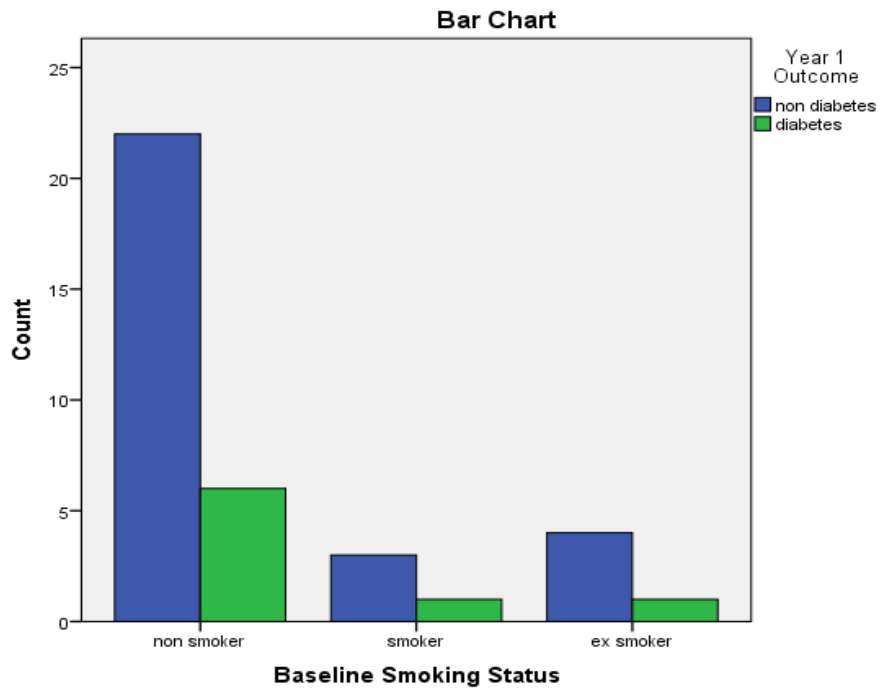


Figure 85: Chi-sq Year 1 Outcome: Smoking Status

-In the bar chart above, smoking status at baseline did not significantly affect risk of DM (T2DM) at Year 1.

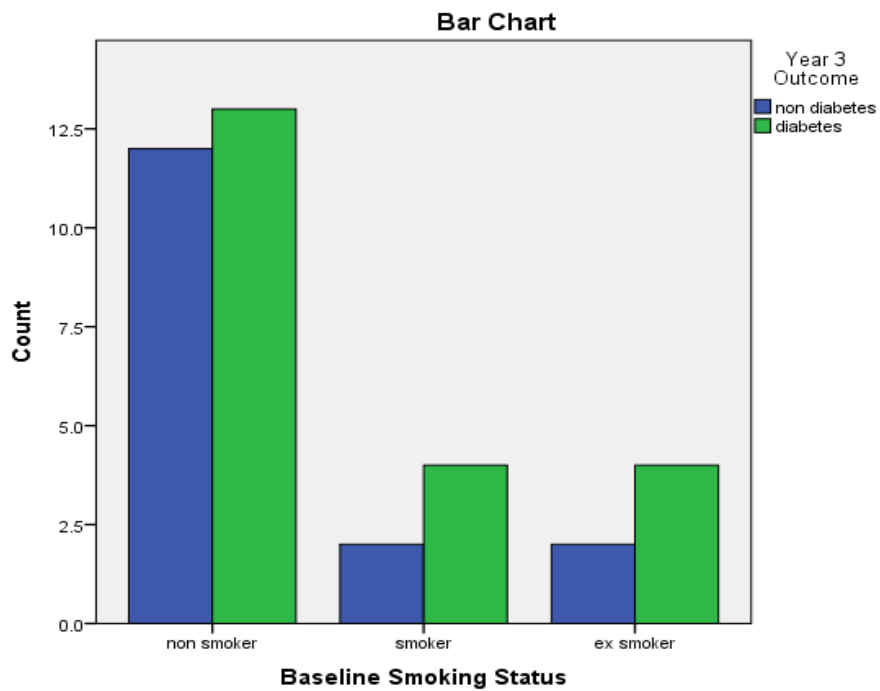


Figure 86: chi-sq Year 3 Outcome: Smoking Status

-In the bar chart above, smoking status at baseline did not significantly affect risk of DM (T2DM) at Year 3.

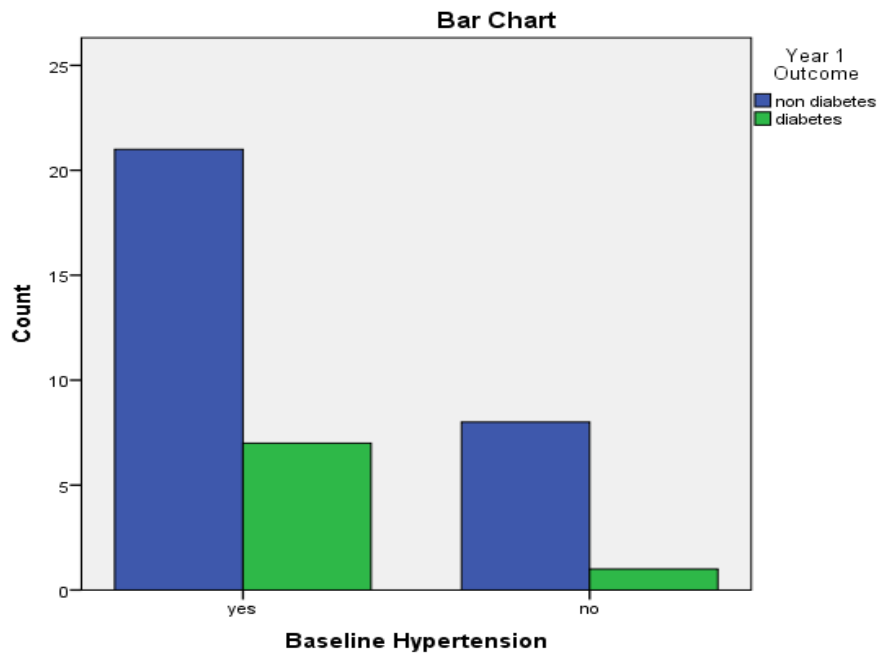


Figure 87: Chi-sq Year 1 Outcome: Hypertension

-In the bar chart above, the presence of hypertension at baseline does not significantly affect risk of DM (T2DM) at Year 1.

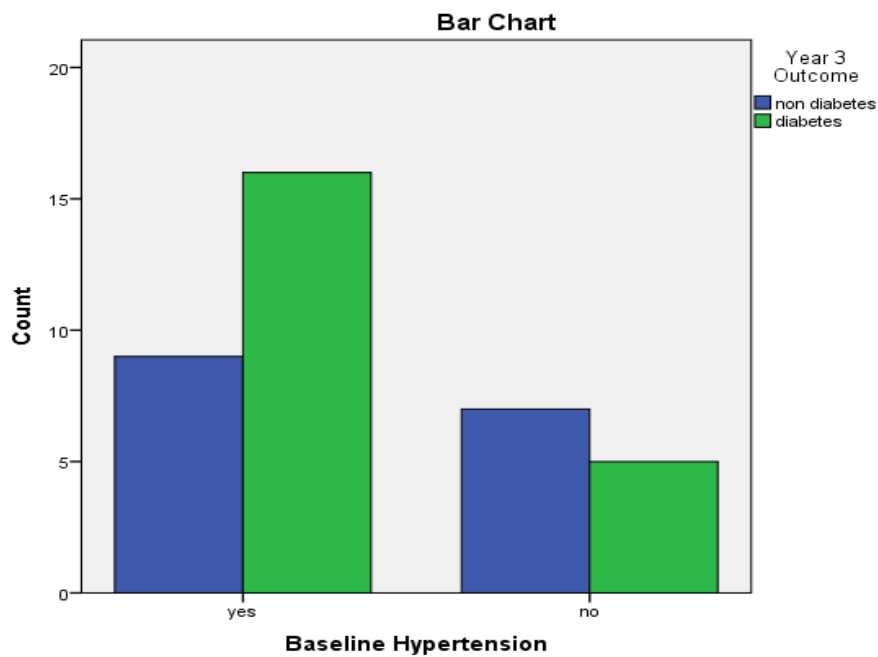


Figure 88: Chi-sq Year 3 Outcome: Hypertension

-In the bar chart above, the presence of hypertension at baseline does not significantly affect risk of DM (T2DM) at Year 3.

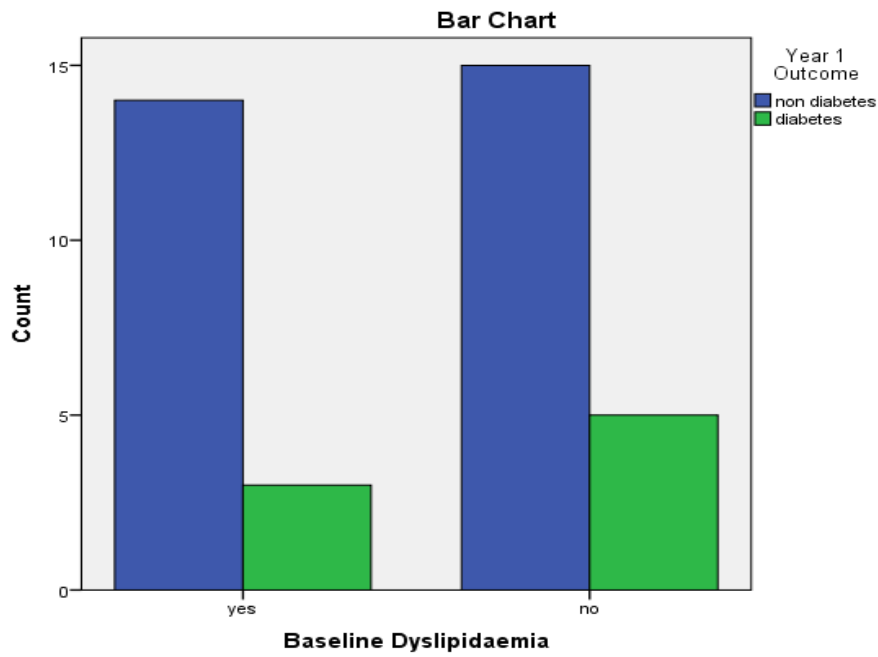


Figure 89: Chi-sq Year 1 Outcome: Dyslipidemia

-In the bar chart above, the presence of dyslipidemia at baseline does not significantly affect risk of DM (T2DM) at Year 1.

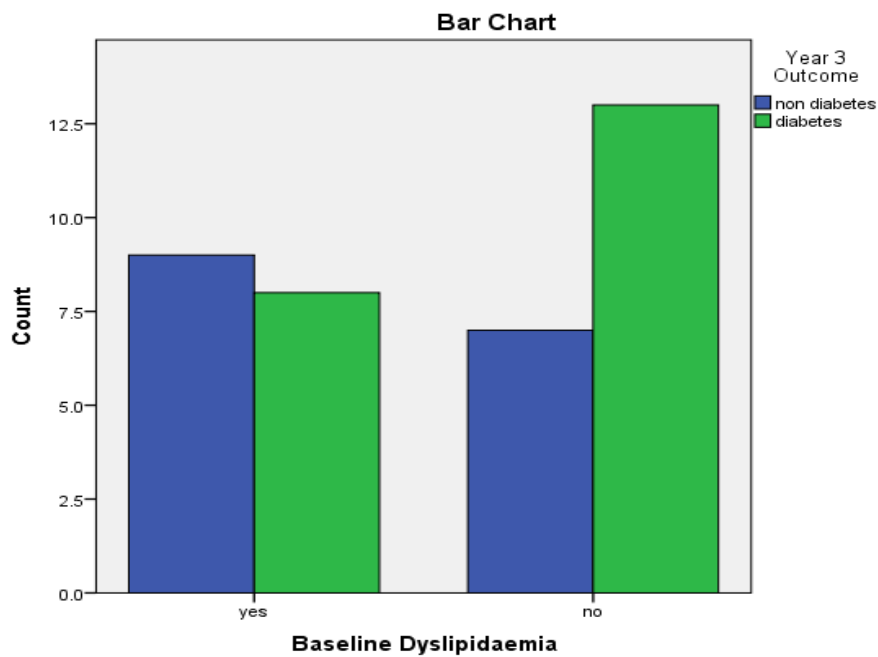


Figure 90: Chi-sq Year 3 Outcome: Dyslipidemia

-In the bar chart above, the presence of dyslipidemia at baseline does not significantly affect risk of DM (T2DM) at Year 3.

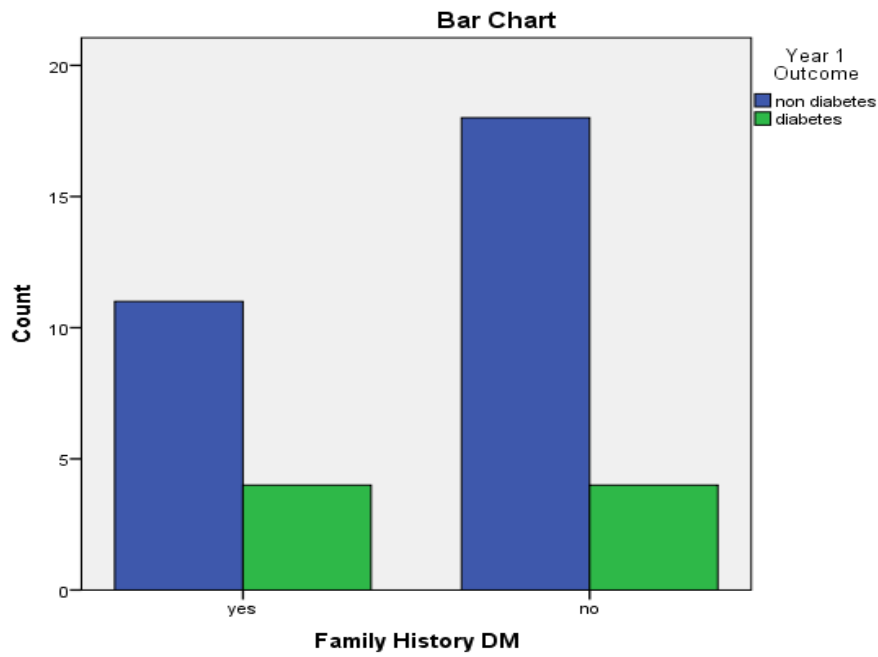


Figure 91: Chi-sq Year 1 Outcome: FHx DM

-In the bar chart above, a positive FHx of DM at baseline does not significantly affect risk of DM (T2DM) at Year 1.

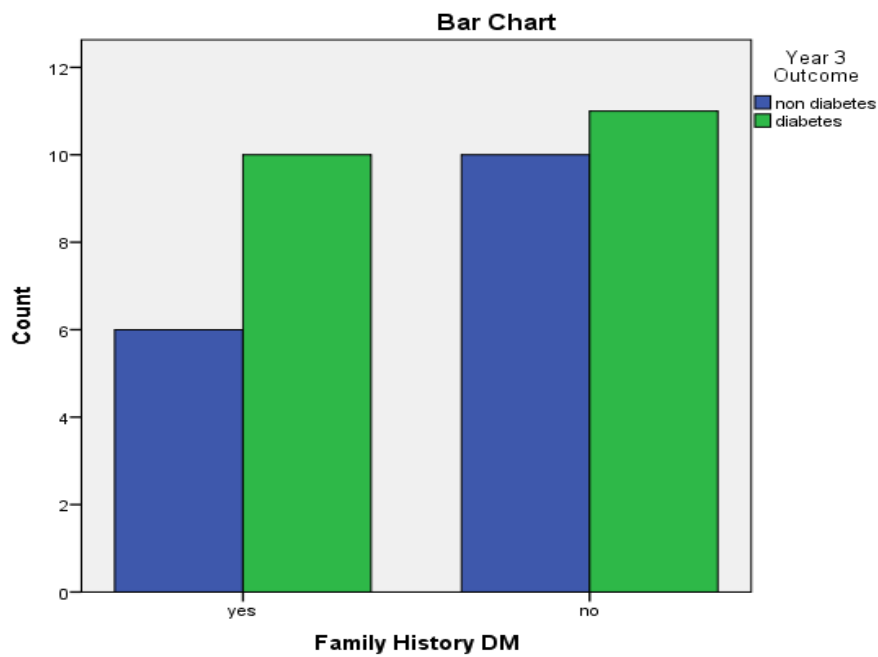


Figure 92: Chi-sq Year 3 Outcome: FHx DM

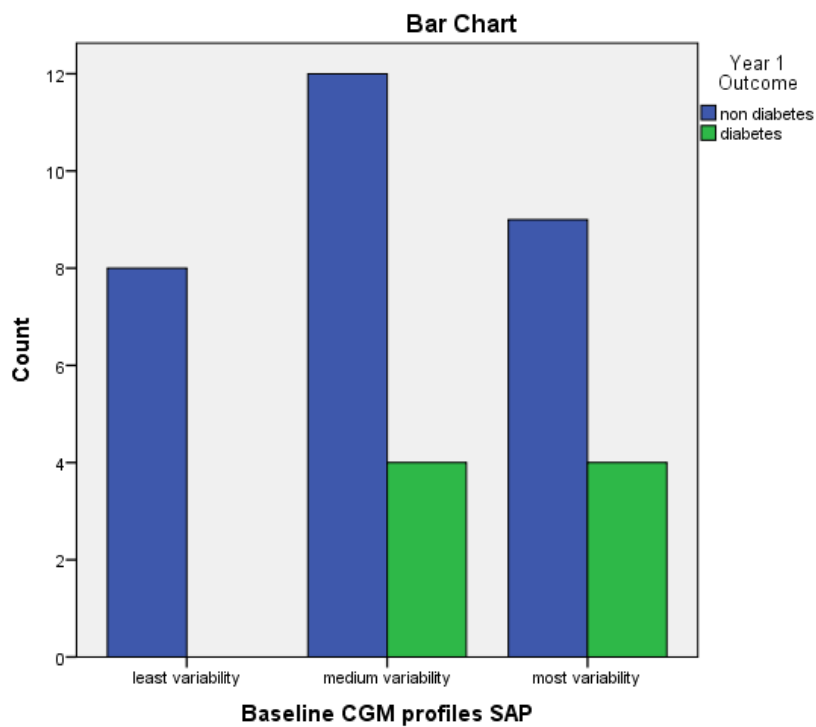
-In the bar chart above, a positive FHx of DM at baseline does not significantly affect risk of DM (T2DM) at Year 3.

Outcome: Baseline CGM Profiles (SAP)

Chi sq (2) = 2.957, p <0.05; p value 0.228 (Pearsons’s exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore baseline CGM Profiles (SAP) did not significantly affect risk of DM (T2DM) at Year 1; (likelihood ratio 4.59). Interestingly, at Year 1, there were no subjects with T2DM and least variability CGM profiles (Table 41, Figure 93).

**Table 41: Baseline CGM Profile SAP - Year 1 Outcome**

		Baseline CGM profiles SAP			Total
		least variability	medium variability	most variability	
Year 1 Outcome	non diabetes	8	12	9	29
	Diabetes	0	4	4	8
Total		8	16	13	37



**Figure 93: Baseline CGM Profiles (SAP): Year 1 Outcome**

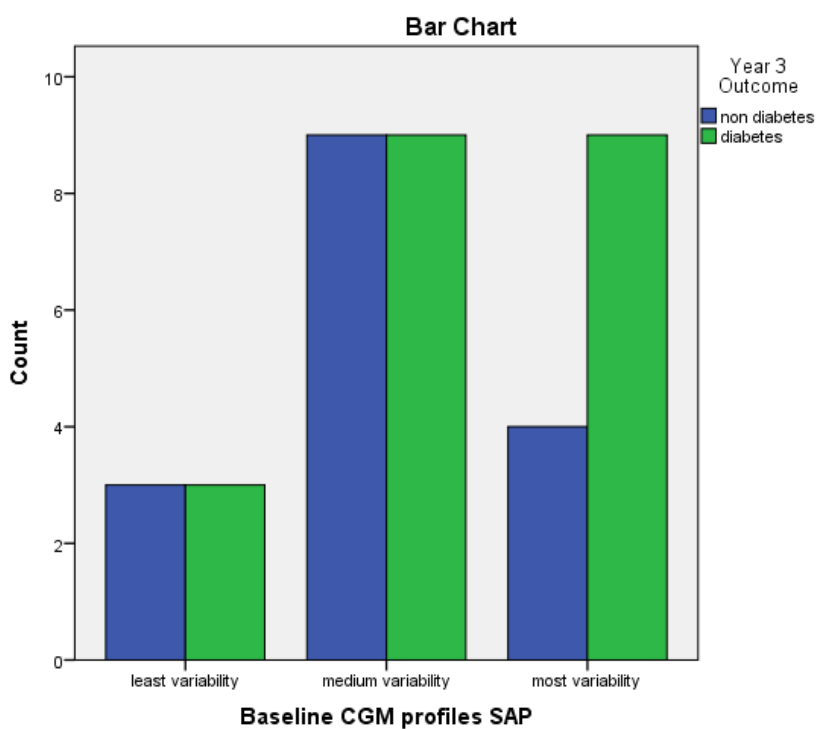
-From the bar chart above, baseline CGM Profiles (SAP) (least, medium and most variability) did not significantly affect risk of DM (T2DM) at Year 1.

Outcome: Baseline CGM Profiles (SAP)

Chi sq (2) = 1.271, p <0.05; p value 0.530 (Pearsons’s exact test). This was not significant, there was no association and the Null Hypothesis was accepted. Therefore baseline CGM Profiles (SAP) did not significantly affect risk of DM (T2DM) at Year 3; (likelihood ratio 1.29) (Table 42, Figure 94).

**Table 42: Baseline CGM Profiles SAP - Year 3 Outcome**

		Baseline CGM profiles SAP			Total
		least variability	medium variability	most variability	
Year 3 Outcome	non diabetes	3	14	7	24
	Diabetes	3	4	6	13
Total		6	18	13	37



**Figure 94: Baseline CGM Profiles (SAP): Year 3 Outcome**

-From the bar chart above, baseline CGM Profiles (SAP) (least, medium and most variability) did not significantly affect risk of DM (T2DM) at Year 3.

## 6.4 CGM Profiles

### 6.4.1 Significance of CGM Profiles by Inspection

The CGM profiles for each subject that were inspected by eye (SAP) (APPENDIX 2 – least variability, APPENDIX 3 - medium variability, APPENDIX 4 – most variability). The CGM profiles for each subject that were inspected by eye were investigated to see if there was a significant difference in them with regard to each of the four devised CGM parameters that were used to assess CGM subject data. The Null Hypothesis was that there was no significant difference in CGM profiles by eye per individual CGM parameter.

At baseline (n = 45), the mean can be seen to increase as the degree of variability by eye was thought to increase. This was the case for all four CGM parameters investigated (Table 43). At baseline, the Levene Statistic was > 0.05 for all CGM parameters (range of 1.2-2.9) and so the assumption of homogeneity of variance had not been violated and the variances were not significantly different (

Table 44). A one way analysis of variance (ANOVA) was conducted at baseline to evaluate the Null Hypothesis that there was no significant difference in the CGM profiles, inspected by eye (least, medium and most variability) based on the four CGM parameters respectively. A cut off point of 0.05 was used as a criterion for used as a criterion for statistical significance. The observed significance value was less than 0.05 (p<0.05) with than 0.05 (p<0.05) with regard to variability (least, medium and most) for all 4 CGM parameters, respectively parameters, respectively (

Table 45). Therefore, there was a significant difference between the variability of the eyeballed CGM profiles for each of the CGM parameters studied at baseline; the Null Hypothesis was thus rejected.

At Year 1 (n = 37) and the mean can be seen to increase as the degree of variability by eye was thought to increase. This was the case for all four CGM parameters investigated (Table 46). At Year 1, as at baseline, the Levene Statistic was > 0.05 for all CGM parameters (range of 3.2-5.7) and so the assumption of homogeneity of variance had not been violated and the variances were not significantly different (Table 47). A one way ANOVA was conducted at Year 1 to evaluate the Null Hypothesis that there was no significant difference in the CGM profiles inspected by eye (least, medium and most variability) based on the four CGM parameters respectively. A cut off point of 0.05 was used as a criterion for statistical significance. The observed significance value is less than 0.05 (p < 0.05) with regard to variability (least, medium and most) for all 4 CGM parameters, respectively (Table 48). Therefore, there was a significant difference and evidence to reject the Null Hypothesis at this level of significance. There is a significant

difference for each of the four CGM parameters, respectively per observed CGM profile inspected by eye at Year 1.

**Table 43: Baseline CGM Parameters v Degree of CGM Profile Variability by Eye**

CGM PARAMETERS DEGREE OF VARIABILITY BY EYE	v	N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
						MEAN Baseline Av GI (mmol/l)	Least		
	Medium	22	6.69	.50	.10	6.47	6.92	5.42	7.43
	Most	14	7.16	.69	.18	6.76	7.56	6.14	8.40
	Total	45	<b>6.73</b>	<b>.66</b>	.09	6.53	6.93	5.27	8.40
MEAN Baseline AvGI excursion from Av (mmol/l)	Least	9	.64	.18	.06	.50	.78	.36	.90
	Medium	22	.92	.15	.03	.85	.99	.54	1.19
	Most	14	1.34	.34	.09	1.14	1.54	.98	2.22
	Total	45	<b>.99</b>	<b>.34</b>	.05	.89	1.10	.36	2.22
MEAN Baseline Av above norm GI (mmol/l)	Least	9	1.63	.53	.17	1.22	2.04	.77	2.31
	Medium	22	2.19	.50	.10	1.97	2.42	.92	2.93
	Most	14	2.66	.69	.18	2.26	3.06	1.64	3.90
	Total	45	<b>2.23</b>	<b>.66</b>	.09	2.03	2.43	.77	3.90
MEAN Baseline Av GI excursion from norm GI (mmol/l)	Least	9	1.80	.45	.15	1.45	2.15	1.03	2.44
	Medium	22	2.41	.44	.09	2.21	2.60	1.32	3.10
	Most	14	3.03	.60	.16	2.68	3.38	2.30	4.05
	Total	45	<b>2.48</b>	<b>.65</b>	.09	2.28	2.68	1.03	4.05

**Table 44: Baseline Test of Homogeneity of Variances**

CGM PARAMETERS	Levene Statistic	df1	df2	Sig.
MEAN Baseline Av GI (mmol/l)	<b>1.27</b>	2	42	.29
MEAN Baseline AvGI excursion from Av (mmol/l)	<b>2.99</b>	2	42	<b>.06</b>
MEAN Baseline Av above norm GI (mmol/l)	<b>1.27</b>	2	42	.29
MEAN Baseline Av GI excursion from norm GI (mmol/l)	<b>1.54</b>	2	42	.22

**Table 45: Baseline ANOVA of CGM Profile Variability by Eye and CGM Parameters**



CGM PARAMETERS		Sum of Squares	Df	Mean Square	F	Sig
MEAN Baseline Av GI (mmol/l)	Between Grps	5.84	2	2.92	8.85	<b>.00</b>
	Within Grps	13.87	42	.33		
	Total	19.72	44			
MEAN Baseline AvGI excursion from Av (mmol/l)	Between Grps	2.89	2	1.45	25.88	<b>.00</b>
	Within Grps	2.35	42	.05		
	Total	5.25	44			
MEAN Baseline Av above norm GI (mmol/l)	Between Grps	5.84	2	2.92	8.85	<b>.00</b>
	Within Grps	13.87	42	.33		
	Total	19.72	44			
MEAN Baseline Av GI excursion from norm GI (mmol/l)	Between Grps	8.53	2	4.26	17.17	<b>.00</b>
	Within Grps	10.43	42	.24		
	Total	18.97	44			

Table 46: Year 1 CGM Parameters v Degree of CGM Profile Variability by Eye

CGM PARAMETERS V DEGREES OF VARIABILITY BY EYE		N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
MEAN Year1 AvGI (mmol/l)	Least	8	5.98	.66	.23	5.42	6.53	5.03	6.82
	Medium	16	6.64	.42	.10	6.42	6.87	6.17	7.66
	Most	13	7.75	1.15	.32	7.05	8.45	6.07	10.47
	Total	37	<b>6.89</b>	<b>1.04</b>	.17	6.54	7.24	5.03	10.47
MEAN Year1 AvGI excursion from Av (mmol/l)	Least	8	.64	.20	.072	.47	.81	.41	1.00
	Medium	16	.97	.22	.056	.85	1.09	.72	1.47
	Most	13	1.68	.64	.17	1.29	2.07	1.03	3.41
	Total	37	<b>1.15</b>	<b>.58</b>	.09	.95	1.34	.41	3.41
MEAN Year1 Av above norm GI (mmol/l)	Least	8	1.48	.66	.23	.92	2.03	.53	2.32
	Medium	16	2.14	.42	.10	1.92	2.37	1.67	3.16
	Most	13	3.25	1.15	.32	2.55	3.95	1.57	5.97
	Total	37	<b>2.39</b>	<b>1.04</b>	.17	2.04	2.74	.53	5.97
MEAN Year1 AvGI excursion from norm GI (mmol/l)	Least	8	1.67	.54	.19	1.22	2.12	1.03	2.44
	Medium	16	2.38	.35	.08	2.19	2.57	1.82	3.25
	Most	13	3.72	1.16	.32	3.02	4.43	2.33	6.19
	Total	37	<b>2.70</b>	<b>1.10</b>	.18	2.33	3.07	1.03	6.19

Table 47: Year 1 Test of Homogeneity of Variances

VARIABLES	Levene Statistic	df1	df2	Sig.
MEAN Year1 AvGI (mmol/l)	3.27	2	34	.050
MEAN Year1 AvGI excursion from Av (mmol/l)	3.94	2	34	.029
MEAN Year1 Av above norm GI (mmol/l)	3.27	2	34	.050
MEAN Year1 AvGI excursion from norm GI (mmol/l)	5.76	2	34	.007

**Table 48: Year 1 ANOVA of CGM Profile Variability by Eye and CGM**

CGM PARAMETERS		Sum of Squares	Df	Mean Square	F	Sig.
MEAN Year1 AvGI (mmol/l)	Between Groups	17.36	2	8.68	13.48	<b>.00</b>
	Within Groups	21.89	34	.64		
	Total	39.25	36			
MEAN Year1 AvGI excursion from Av (mmol/l)	Between Groups	6.24	2	3.12	17.71	<b>.00</b>
	Within Groups	5.98	34	.17		
	Total	12.22	36			
MEAN Year1 Av above norm GI (mmol/l)	Between Groups	17.36	2	8.68	13.48	<b>.00</b>
	Within Groups	21.89	34	.64		
	Total	39.25	36			
MEAN Year1 AvGI excursion from norm GI (mmol/l)	Between Groups	23.71	2	11.85	19.84	<b>.00</b>
	Within Groups	20.31	34	.59		
	Total	44.02	36			

#### **6.4.2 CGM Profiles & Outcome: Year 1 and Year 3**

At Year 1, subjects were classed as DM (T2DM) according to their OGTT result (FPG  $\geq 7.0$  mmol/l) and at Year 3, a FPG  $\geq 7.0$  mmol/l. With regard to the subjects who became DM (T2DM) at Year 1 (n = 8) (Table 49), 100% of those that underwent repeat testing with OGTT at Year 1, had FPG  $\geq 6.1$  mmol/l and 62.5% of them had a Year 3 FPG of  $\geq 7.0$  mmol/l. With regard to the corresponding CGM profiles (SAP) that were inspected by eye, 87.5% of the subjects that were classed as DM (T2DM) at Year 1 on OGTT results had CGM profiles (at baseline or Year 1) that were thought to be at least

of medium variability (apart from subject IPRO-03, who refused a repeat OGTT). At Year 1, for those (n = 8) subjects found to be DM (T2DM), 50% of them (n = 4) had been prescribed medication by their General Practitioner (GP); the other 50% (n = 4) had received diet and lifestyle advice. At Year 3, all subjects who had been commenced on medication at Year 1 by their GP had a FPG of  $\leq 7$  mmol/l; these subjects were scored as having DM (T2DM) at Year 3. With regard to the five subjects (n = 5) that received lifestyle advice from the GP at Year 1, less than 50% had improvement in their FPG at Year 3 on testing. The GP was contacted immediately by telephone, regarding subject IPRO-22, who had a FPG at Year 3 of 22.0 mmol/l. Interestingly, two subjects IPRO-13 and IPRO-19 did not attend for Year 1 repeat OGTT testing, but did attend at Year 3 and were both found to both have FPG  $\geq 7.0$  mmol/l i.e. DM (T2DM). IPRO-06 was DM at Year 1 and had a most variable CGM profile, which remained most variable at Year 3 (Figure 95).

With regard to the subjects who became NGT at Year 1 (n = 7) all of them had FPG  $\leq 6.1$  mmol/l ([Error! Reference source not found.](#)). With regard to the corresponding CGM profiles (SAP) that were inspected by eye, the majority (57.14%) (n = 4) of subjects profiles became less variable with time; 14.28% (n=1) of the CGM profiles (SAP) stayed the same and 28.57% (n = 2) of the CGM profiles (CGM) increased in variability with time) (Figure 96). Interestingly, at Year 3, two subjects went on to become DM (T2DM) i.e. FPG  $\geq 7.0$  mmol/l (IPRO-34 and IPRO-36) and both of their corresponding CGM profiles were observed by eye to have increased in variability also none of these subjects had been prescribed medication by their GP.

With regard to the subjects who were found to be IFG at Year 1 (n = 10), as expected all of them had FPG  $\geq 6.1$  mmol/l (Table 51). With regard to the corresponding CGM profiles (SAP) that were inspected by eye, the majority 40% (n = 4) of subjects profiles became increasingly variable with time; 30% (n=3) of the CGM profiles (SAP) stayed the same and 30% (n = 3) of the CGM profiles (CGM) decreased in variability with time. Interestingly, three subjects had been prescribed medication or given lifestyle advice by their GP, all of which had between medium to most variability on their corresponding CGM profiles (SAP), the two subjects (IPRO-35 and IPRO-38) with the most variability on CGM profiles (SAP) at Year 3 went on to have FPG at this point  $\geq 7.0$  mmol/l (Figure 97).

**With regard to the subjects who were found to be IFG+IGT at Year 1 (n = 7), all of them had FPG  $\geq$  6.1 mmol/l (**

Table 52). With regard to the corresponding CGM profiles (SAP) that were inspected by eye, the majority 57.1% (n = 4) of subjects profiles became increasingly variable with time; 28.5% (n=2) of the CGM profiles (SAP) stayed the same and 14.2% (n = 1) of the CGM profiles (CGM) decreased in variability with time. At Year 3, three subjects (IPRO-07, IPRO-11 and IPRO-21) had FPG  $\geq$ 7 mmol/l and all of them had received lifestyle advice from the GP. Subject IPRO-18 had been placed on medication by the GP based on his baseline abnormal OGTT; interestingly, the corresponding CGM profile (SAP) appeared to increase in variability (Figure 98).

**With regard to the subjects who became IGT at Year 1 (n = 5) (**

Table 53), all of them had FPG  $\leq$  6.1 mmol/l at Year 1. With regard to the corresponding CGM profiles (SAP) that were inspected by eye, the majority (60%) (n = 3) of subjects profiles became less variable with time; 40% (n=2) of the CGM profiles (SAP) stayed the same and none of the CGM profiles (CGM) increased in variability with time. None of these subjects had been prescribed medication by their GP. Based on FPG at Year 3, only one subject (IPRO-02) had a FPG  $\geq$ 7.0 mmol/l (Figure 99).

**Table 49: DM (T2DM) at Year 1 with regard to CGM Profiles**

Subject	Y1 OGTT Result (FPG) (mmol/l)	CGM Profile Baseline	CGM Profile Year 1	GP Advice	Year 3 FPG (mmol/l)
*IPRO-03	/	Least	Most	Medication	6.10
<b>IPRO-06</b>	7.2	Most	Most	Medication	7.0
*IPRO-13	/	/	/	/	7.7
IPRO-14	6.1	Most	Most	Lifestyle	6.5
*IPRO-19	/	/	/	/	9.3
IPRO-22	7.0	Most	Medium	Lifestyle	22.0
IPRO-24	7.0	Most	Medium	Medication	6.5
IPRO-26	7.2	Medium	Medium	Lifestyle	6.9
IPRO-28	7.7	Most	Most	Lifestyle	8.0
IPRO-41	7.3	Medium	Medium	Lifestyle	6.6

\*Subject IPRO-03 refused repeat OGTT but agreed to CGM. Subjects IPRO-13 and IPRO-19 did not attend for Year 1 repeat OGTT testing, but attended for Year 3 FPG check.

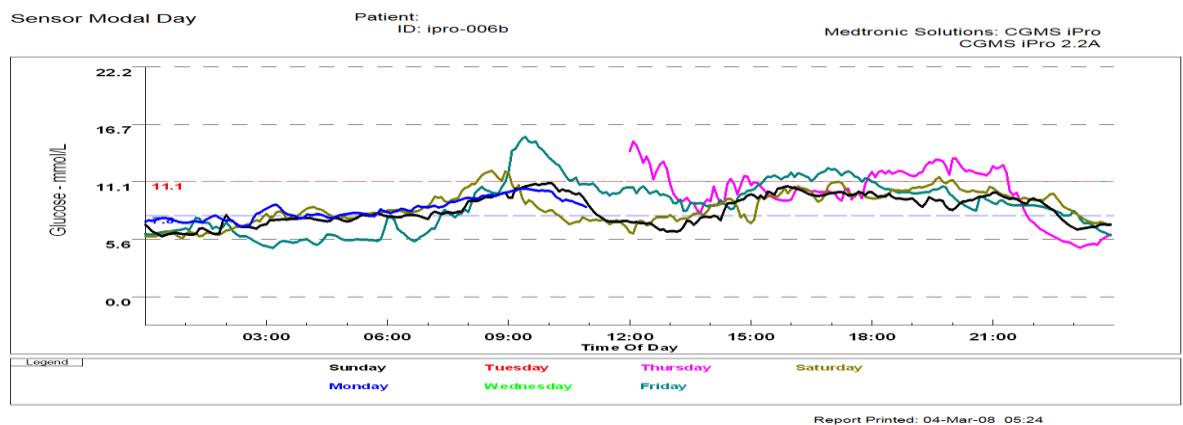
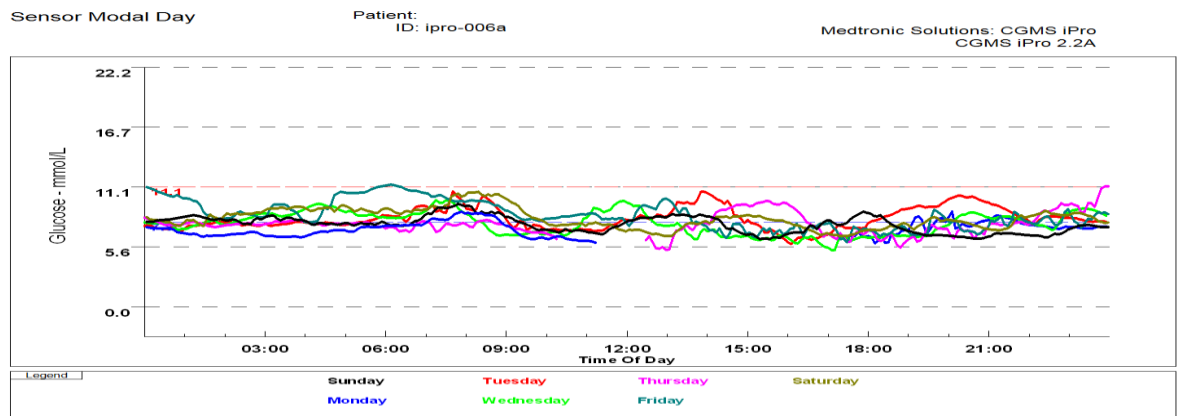


Figure 95: DM: Sensor Modal Day Subject: IPRO-06a and IPRO-06b

-\*IPRO-06a: DM at Year 1: most variability demonstrated on CGMS at baseline and Year 1, as can be observed from the subject profiles above.

Table 50: NGT at Year 1 with regard to CGM Profile

Subject	Y1 OGTT Result (FPG) (mmol/l)	CGM Profile Baseline	CGM Profile Year 1	GP Advice	Year 3 FPG (mmol/l)
<b>I PRO-23</b>	4.2	Medium	Least	/	4.7
I PRO-30	5.3	Medium	Least	/	6.3
I PRO-34	5.7	Least	Medium	/	7.6
I PRO-36	5.7	Medium	Most	/	8.0
I PRO-37	4.6	Medium	Medium	/	5.2
I PRO-39	5.9	Most	Medium	/	6.8
I PRO-43	5.8	Medium	Least	/	6.2

\*Y1 = year

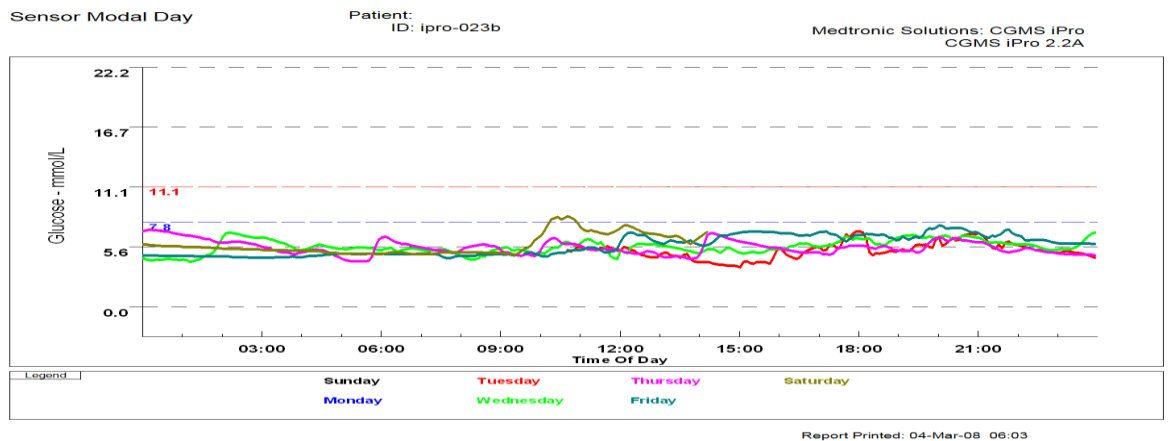
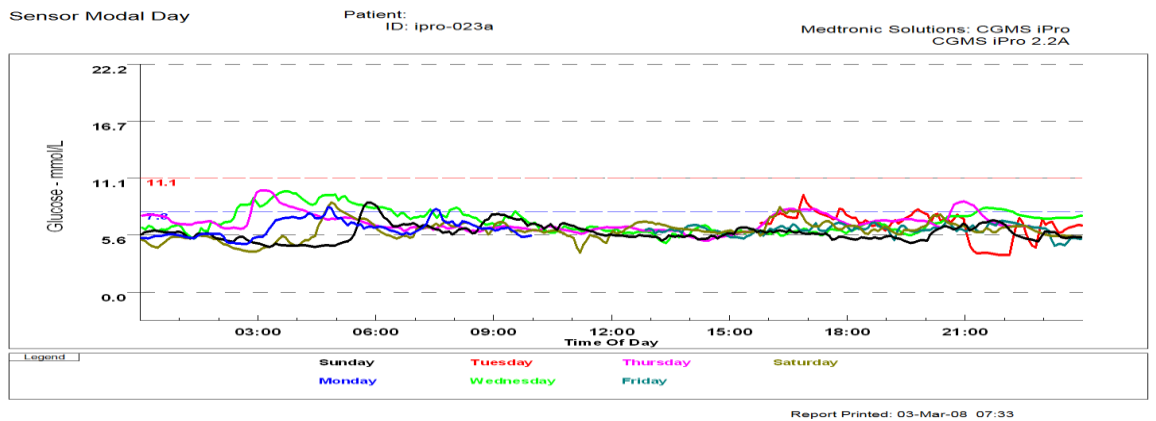


Figure 96: NGT Sensor Modal Day Subject: I PRO-23a and I PRO-23b

-Medium variability at baseline on CGM Profiles by eye, least variability at Year 1, as can be observed from the subject profiles above.

Table 51: IFG at Year 1 with regard to CGM Profiles

Subject	Y1 OGTT Result (FPG) (mmol/l)	CGM Profile Baseline	CGM Profile Year 1	GP Advice	Year 3 FPG (mmol/l)
IPro-16	6.2	Medium	Least	/	6.5
IPro-17	6.3	Medium	Least	/	6.6
IPro-20	6.2	Least	Medium	/	/
IPro-31	6.2	Most	Most	Medication	6.6
<b>IPro-35</b>	6.3	Medium	Most	Medication	7.2
IPro-38	6.8	Medium	Most	Lifestyle	7.5
IPro-42	6.3	Most	Medium	/	/
IPro-44	6.4	Medium	Medium	/	6.5
IPro-45	6.5	Medium	Most	/	6.3
IPro-46	6.6	Medium	Medium	/	/

\*Y1 = Year 1

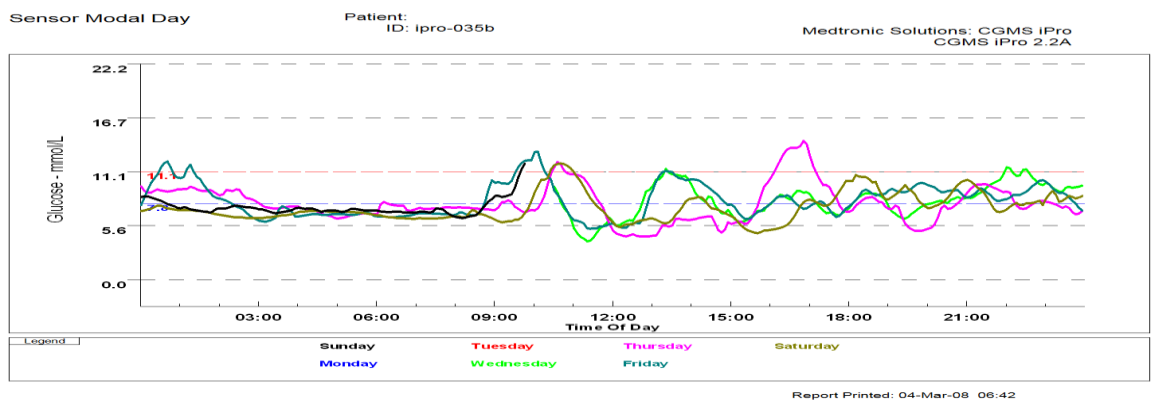
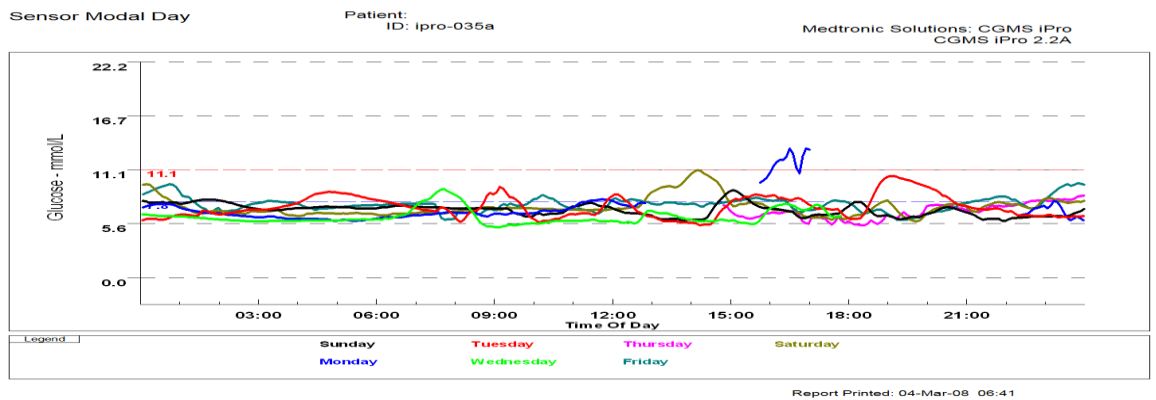


Figure 97: IFG Sensor Modal Day Subject IPro-35a and IPro-35b

--Medium variability at baseline on CGM Profiles by eye, most variability at Year 1, as can be observed from the subject profiles above.

Table 52: IFG+IGT at Year 1 with regard to CGMS Profiles

Subject	Y1 OGTT Result (FPG) (mmol/l)	CGM Profile Baseline	CGM Profile Year 1	GP Advice	Year 3 FPG (mmol/l)
IPro-05	6.8	Least	Medium	/	6.6
IPro-07	6.3	Least	Least	Lifestyle	8.3
IPro-09	6.2	Least/Medium	Medium	/	/
<b>IPro-11</b>	6.3	Least	Most	Lifestyle	7.6
IPro-18	6.3	Medium	Most	Medication	6.0
IPro-21	6.4	Most	Medium	Lifestyle	7.2
IPro-46	6.9	Medium	Medium	/	6.4

\*Y1 =Year 1

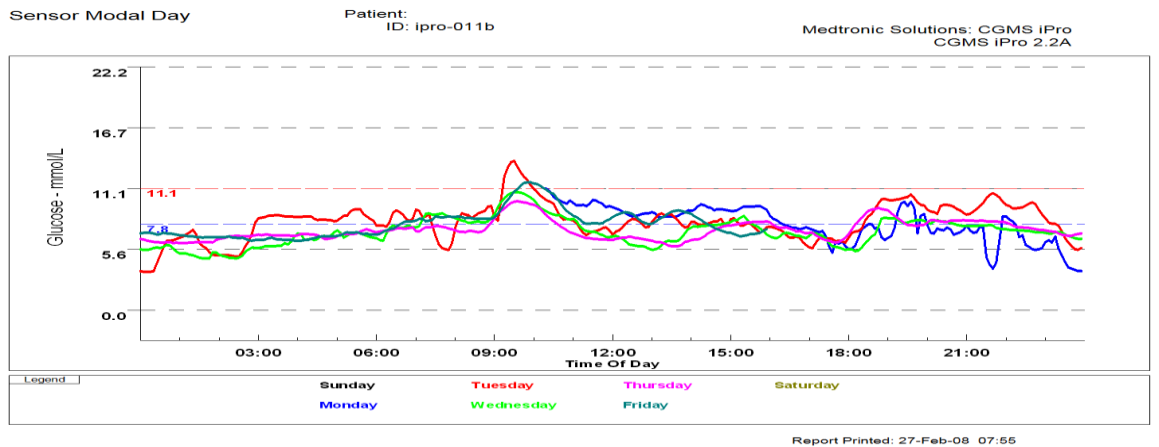
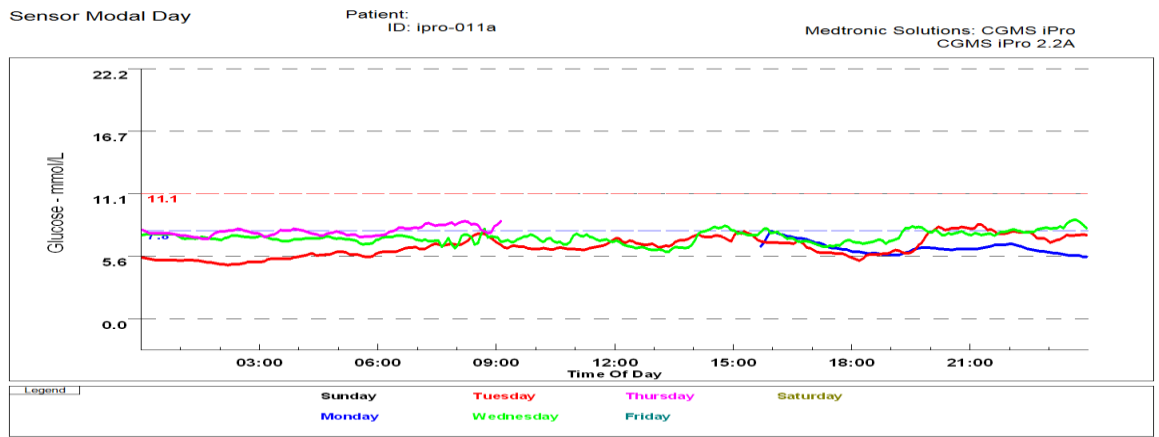


Figure 98: IFG+IGT Sensor Modal Day Subject: IPro-11a and IPro-11b

-Least variability at baseline on CGM Profiles by eye, most variability at Year 1, as can be observed from the subject profiles above.



Table 53: IGT at Year 1 with regard to CGM Profiles

Subject	Y1 OGTT Result (FPG) (mmol/l)	CGM Profile Baseline	CGM Profile Year 1	GP Advice	Year 3 FPG (mmol/l)
IPRO-01	5.9	Most	Most	/	6.3
I <b>PRO-02</b>	6.0	Most	Medium	/	7.4
IPRO-10	5.9	Most	Least	/	5.4
IPRO-29	5.2	Least	Least	/	4.6
IPRO-40	6.0	Most	Medium	/	6.7

\*Y1 = Year 1

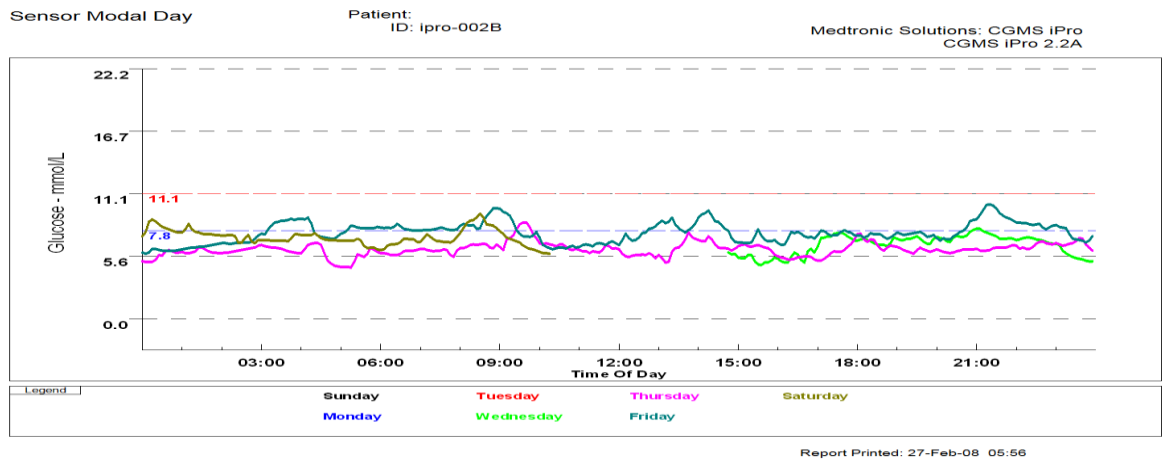
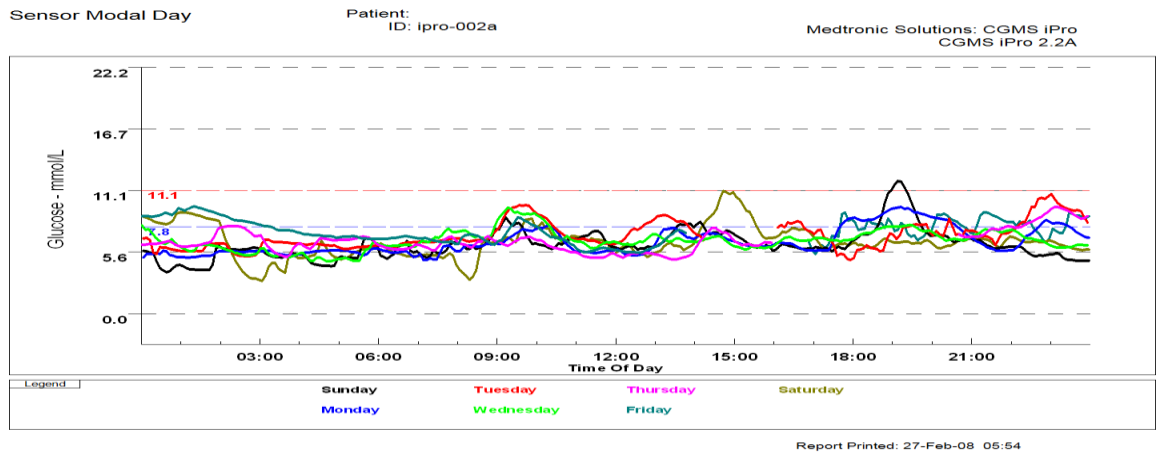


Figure 99: IGT Sensor Modal Day Subject IPRO-02a and IPRO-02b

-Most variability at baseline on CGM Profiles by eye, medium variability at Year 1, as can be observed from the subject profiles above.

## 6.5 Regression Analysis

Regression analysis is a statistical process used to estimate the relationships among variables. The focus is on the relationship between the dependent variable (outcome) and one or more independent variables or predictors. It is used to understand which among the independent variables are related to the dependent variables. In this study, the outcome (dependent variable) was Year 3 FPG.

Baseline Age, baseline BMI, baseline Gender, baseline FPG, baseline 2hr glucose, baseline HbA1c, baseline CGM for 4 parameters respectively, GAD Ab, C-peptide and insulin were all tested with regard to Year 3 Outcome, using a binary logistical statistical programme, to see if any of them affected the outcome or influenced each other. The Null Hypothesis being that there was no association with any of the measurement variables discussed above, at the  $p < 0.05$  level and the outcome (DM i.e. FPG Year 3) (Table 54).

In logistic regression, the Wald statistic (which has a Chi-square distribution), was used to assess the contribution of predictors. The Wald statistic was the value of the regression coefficient divided by its associated standard error; the odds ratio [Exp(B)] was an indicator of the change in odds resulting from a unit change in the predictor (78). In this study, none of the parameters tested reached significance (at the  $p < 0.05$  level), with regard to outcome affect and the Null Hypothesis was accepted.

$$R = (1) 2.69 \quad p > 0.05 (0.1)$$

## **6.6 Summary: Chapter 6**

A number of pre-selected parameters were analysed at baseline, Year 1 and Year 3 with regard to looking at progression towards an outcome of diabetes (T2DM) or non diabetes. At Year 1, an outcome of DM (T2DM) or non diabetes was established for all study subjects following testing with OGTT; at Year 3, an outcome of DM (T2DM) or non diabetes was established for all study subjects following FPG testing. The parameters were analysed in order to look at progression or change over time. At baseline and Year 1, parameters analysed included FPG, OGTT 2 hours, HbA1c, plus the four CGM parameters (Mean Baseline Average Glucose; Mean Baseline Average Glucose Excursion from the Average, Mean Baseline Average above Normal and Mean Baseline Average Glucose Excursion from the Normal Glucose); at Year 3, FPG and

HbA1c were analysed. At Year 1, 17.8% of subjects had progressed to DM (T2DM) (Table 35) and at Year 3, 46.6% of subjects had progressed to DM (T2DM) Table 36).

Assuming the data to be normally distributed, the Independent Sample *t*-Test was used to compare the means of two independent groups in order to determine whether there was a statistically significant difference between them. The two outcome groups were non diabetes and diabetes. The outcome of both groups at Year 1 and Year 3 was investigated with regard to a number of baseline variables:

- Baseline Age (years)
- Baseline OGTT 0 mins (mmol/l)
- Baseline OGTT 2 hours (mmol/l)
- Baseline HbA1c (mmol/mol)
- Mean Baseline Average Glucose (mmol/l)
- Mean Baseline Average Glucose Excursion (mmol/l) from Average
- Mean Baseline Average above Normal Glucose (mmol/l)
- Mean Baseline Average Glucose Excursion from Normal Glucose (mmol/l)
- Baseline BMI (Kg/m<sup>2</sup>)

The outcome of both groups at Year 3 was investigated with regard to:

- Year 1 Insulin and Year 1 C-peptide.

The Null Hypothesis in this instance was that there was no significant difference (i.e. no variation) with regard to the specified variable in each group, with regard to outcome. Where Levene's test was  $p > 0.05$ , the Null Hypothesis was accepted. With regard to Year 1 outcome, a 95% CI, a  $p < 0.05$  was classed as statistically meaningful and significant and the Null Hypothesis was rejected. With regard to Baseline OGTT 120 mins and Mean Baseline Average Glucose Excursion (mmol/l) from Average, the results were significant and the Null Hypothesis was rejected. There was a significant difference between the groups at Year 1 for Baseline OGTT 120 mins and Mean Baseline Average Glucose Excursion (mmol/l) from Average (Table 37, Table 38). With regard to Year 3 outcome, none of the variables tested demonstrated significance difference in means, when the outcome of the diabetes and non diabetes groups was investigated, respectively (Table 39, Table 40). Of the 4 CGM Parameters constructed to look at whether they were able to predict progression to DM (T2DM), one of the

most promising parameters was seen to be ‘Mean Baseline AvGl Excursion from Av’. However, when one looked at this particular parameter - ‘Mean Baseline AvGl excursion from Av’ with regard to Year 3 outcome of diabetes (T2DM), the median values were almost the same for the subjects with diabetes as those without, which was disappointing. The resulting inter-quartile ranges and lowest and highest values of data were for each outcome was almost identical also (Figure 80).

In this study, a number of categorical variables were also assessed at baseline, to see if they had any bearing on the outcome of DM (T2DM) at Year 1 or Year 3. Pearson’s-chi square test was used to compare the frequency observed in certain categories to the frequencies one may expect to get in those categories by chance. Ethnicity, smoking status, hypertension, dyslipidemia and FHx DM did not significantly affect risk of DM (T2DM) at Year 1 or Year 3 and the Null Hypothesis was accepted in these cases. Baseline CGM Profiles (SAP) were also studied and did not significantly affect risk of DM (T2DM) at Year 1 and Year 3.

The CGM profiles for each subject that were inspected by eye (SAP) were investigated to see if there was a significant difference in them with regard to each of the four devised CGM parameters that were used to assess CGM subject data. ANOVA was conducted at baseline and Year 1 to evaluate the Null Hypothesis, in that there was no significant difference in the CGM profiles inspected by eye (least, medium and most variability) based on the four CGM parameters respectively. The observed significance value was less than 0.05 ( $p < 0.05$ ) with regard to variability (least, medium and most) for all 4 CGM parameters, respectively (

Table 44) (

Table 45) (Table 47) (Table 48). Therefore, there was a significant difference between the variability of the visually inspected CGM profiles for each of the CGM parameters studied at baseline and at Year 1; the Null Hypothesis was thus rejected.

Finally, regression analysis was used to understand which among the independent variables were related to the dependent variables. In this study, the dependent variable was Year 3 FPG and the independent variables were baseline Age, BMI, Gender, FPG, 2hr glucose, HbA1c, CGM for 4 respective parameters, GAD Ab, C-peptide and insulin. The independent variables were all tested with regard to Year 3 Outcome, using a

binary logistical regression analysis to see if any of them affected the outcome or influenced each other. The Null Hypothesis being that there was no association with any of the measurement variables discussed above at the  $p < 0.05$  level and the outcome (DM i.e. FPG Year 3) (Table 54). In this study, none of the parameters tested reached significance (at the  $p < 0.05$  level), with regard to outcome affect and the Null Hypothesis was accepted.

Table 54: Regression Analysis - Binary Logistic

		Score	Df	Sig.	
Step 0	Variables	FHxDM(1)	.012	1	.913
		Gender(1)	3.155	1	.076
		Ethnicity(1)	.229	1	.632
		BaselinesmokingStatus(1)	.002	1	.965
		BaselineHypertension(1)	.440	1	.507
		BaselineDyslipidaemia(1)	1.357	1	.244
		BaselineAgeyears	.045	1	.831
		BaselineWeightkg	.153	1	.696
		BaselineBMIkgm2	.188	1	.665
		BaselineOGTT0minsmmol	1.118	1	.290
		BaselineOGTT120minsmmol	.939	1	.332
		MIXBaselineOGTTresult	2.174	2	.337
		MIXBaselineOGTTresult(1)	.018	1	.893
		MIXBaselineOGTTresult(2)	.944	1	.331
		BaselineHbA1cmmol	.906	1	.341
		BaselineCGMprofilesSAP	1.402	2	.496
		BaselineCGMprofilesSAP(1)	.081	1	.775
		BaselineCGMprofilesSAP(2)	1.357	1	.244
		Year1GADuml	.004	1	.948
		Year1InsulinpmolL	.702	1	.402
		Year1Cpeptidpmolml	.396	1	.529
		MEANBaselineAvGImmolL	3.837	1	.050
		MEANBaselineAvGlexcursionfromAvmmolL	.161	1	.688
		MEANBaselineAvabovenormGImmolL	3.837	1	.050
		MEANBaselineAvGlexcursionfromnormGImmolL	3.235	1	.072
		baseline_BMI_categories	.307	2	.858
		baseline_BMI_categories(1)	.229	1	.632
		baseline_BMI_categories(2)	.136	1	.713
		BaseAgeGrp	.672	2	.715
		BaseAgeGrp(1)	.545	1	.460
		BaseAgeGrp(2)	.028	1	.868

a. Residual Chi-Squares are not computed because of redundancies.

		B	S.E.	Wald	Df	Sig.	Exp(B)
Step 0	Constant	-.642	.391	2.699	1	.100	.526

## 7.0 Discussion

During the recruitment period between October 2009 and June 2011, 486 subjects were screened for this study. All subjects were invited to participate in the study if they met the study inclusion criteria and were found to have impaired glucose tolerance on testing. Out of the 486 subjects screened 47 subjects were recruited to study (cohort group), almost meeting our study objective of  $n = 50$ . Of these 47 subjects, all have completed baseline (A)  $t = 0$  investigations; two subjects requested to leave the study following completion of baseline  $t = 0$  investigations. One year later, all 45 subjects of the cohort group were invited to return for  $t = 12$  month follow up investigations. Of the 45 subjects contacted, 37 subjects responded and have completed 12 month follow up (B) investigations. The 8 outstanding subjects were withdrawn from the study at this point either at their request (due to personal time commitments) or being untraceable via all forms of communication. Of note, a 10-fold difference was observed in the subjects attending for OGTT to those who actually participated in the study. This data may be useful when planning future studies with regard to consideration of target recruitment numbers. At Year 3, similar numbers returned for assessment, limiting the 'drop out' rate.

There is no screening programme in Wales for T2DM. Patients referred in for OGTT testing in this study form a group of individuals deemed to be 'intermediate risk', this being based on the fact that GP and secondary care physicians selected them from the population as a whole and referred them in. The risk of picking up positive OGTT initially from a general screen of the population as a whole would be lower. As previously discussed, IFG and IGT classify a pre-diabetic state and define individuals who may develop progressive deterioration in glucose homeostasis, leading to frank T2DM, as described by Gabor *et al* (2000) (4). Many people with IFG or IGT will not progress to T2DM but appear to spontaneously revert to normal, as described by Lim *et al* (2000) (5). Others may progress only very slowly, whereas in some cases the progression may be rapid, as described by Alberti *et al* (1996) (6). At Year 1, 37 subjects ( $n = 37$ ) completed 12 month follow up and ( $n = 8$ ) subjects (22%) were shown to have progressed to diabetes on repeat OGTT testing, at Year 3, approximately half of the study group had become diabetic (T2DM). This result would be in excess of the projected numbers thought to progress in this population from initial calculations at 12 months. Of the remaining 29 subjects, one would expect half of them to revert to normal, as described by Barr *et al* (2007) (20) and the other half to stay as IFG/IGT. In

this instance, 7 subjects (n =7) (19%) returned to normal glycaemia, which is less than anticipated at 12 months. This may be due to the clinical advice and information received by the patient from their referring clinician at baseline OGTT testing and also if any advice given was carried out. However, it would be consistent with the projected estimated target number at 2 years.

As already discussed, therapeutic interventions have been shown to reduce the risk, or at least the pace of deterioration from IGT and IFG to T2DM (Riccardi *et al* 1985) (7) (Ferrannini *et al* 2004) (8) and (Unger and Orci 2010) (9). To target interventions specifically to prevent progression in those at greatest risk, further information as to which individuals are most likely to progress is needed. A marker of deteriorating carbohydrate homeostasis would be increased fluctuations in blood glucose levels. The use of continuous glucose sensing to quantify the fluctuations was proposed in this study to assess whether CGMS could help identify people with abnormal glucose tolerance who progress to T2DM. Unfortunately, the analysis here demonstrated here, as seen in Results Chapters 4, 5 and 6 that CGMS were unable to predict progression to diabetes in this study population. In this study, none of the analysis conducted reached statistical significance ( $p < 0.05$ ). It may be that in a larger data set, CGM may have greater predictive power to predict progression to diabetes from a pre diabetic state. CGM, did however, identify a group, IFG subjects, who between baseline and Year 1 appeared to demonstrate an increased risk of progression towards diabetes, which was also reflected in the subjects CGM profiles level of variability ie medium variability. This may be the group on which to focus, with regard to targeted intervention. The apparent baseline high risk group identified from this study was IFG. The CGM parameter mean average glucose excursion above the average seemed at times to be approaching significance in the various statistical tests, unfortunately, it was not a significant predictor of progression.

This study had a number of strengths and weaknesses

### **Strengths**

- The study was ethically approved.
- The study subjects were reflective of a normal population.
- The cohort of individuals with a positive OGTT, that gave consent to be included in the study were a precious resource.



- Demographic data was obtained on all subjects for past medical history (hypertension, dyslipidemia, cardiovascular disease, pancreatic disease and thyroid disease), social history (smoking history), family history of diabetes, medications and ethnicity.
- The study data was analysed at baseline, Year 1 and Year 3 respectively, according to outcome. In order to enable a Year 3 analysis and obtain increased data regarding disease progression, an ethical amendment was successfully obtained.
- At Year 1, 37 subjects (n = 37) out of the original 45 baseline subjects (n = 45) returned (82.2%), with similar numbers at Year 3 (84.4%); this demonstrated minimal ‘drop out’ rate.
- As part of the study, subjects were identified that had progressed in all the possible combination of ways: i.e. progressed from IFG and IGT to T2DM; progressed from IFG and IGT to NGT, progressed from IFG to IGT or remained static.
- The outcome of the 12 months repeat OGTT, which ever direction it progressed appeared to be reflected in the HbA1c value.
- The CGM sensor was able to read 288 readings of interstitial glucose in any 24 hour period.
- There was a 10-fold difference observed in subject number attending for OGTT to those who actually participated in this study. This data may be useful in planning future studies, as it may have bearing when attempting to recruit a set target number.
- In the future, the ‘raw data set’, could be used for further studies. For example, re-analysis at different time points, which may yield beneficial information:-
  - (1) Complete 24 hour data sets (midnight to midnight)
  - (2) Complete day time data (12 hour data) (9am-9pm)
  - (3) Complete night time data (12 hour data) (9pm-9am)
  - (4) Meal time data. This can be split into pre-prandial (fasting) and 2 hour postprandial for breakfast, lunch and supper time.

### **Weaknesses**

- The patients referred in for OGTT testing in this study form a group of individuals deemed to be ‘intermediate risk’, as GPs have already ‘selected’

them from the population as a whole and referred them. The risk of picking up positive OGTT initially from a general screen of the population as a whole would be lower.

- A larger cohort of subjects (i.e.  $n > 45$ ) at the start would have added power to the study.
- A robust plan to analyse the subjects who withdrew from the study at each stage, would have provided added information to this study.
- The age range of the subject cohort was approximately 45-70 years, which appeared to be biased towards the older age group. However, this may be a reflection of real life with regard to T2DM.
- Initially, the study was to look at progression of disease from baseline to Year 1 and Year 3. However, in reality some subjects returned up to 6 months after their exact date for re analysis. Ideally, exact date re-analysis would have been preferred.
- A complete data set is optimal. In this study, the data set was incomplete in places; for example, (n = 1) subject out of (n = 37) subjects at Year 1 refused the OGTT but agreed to CGM. At Year 3, OGTT was not conducted due to patient choice and CGM was not conducted due to patient choice and PI/funding availability. Any incomplete data was accounted for during the statistical analysis.
- All subjects should have had GAD Ab test at baseline rather than at Year 1. All subjects should also have had insulin and C-peptide testing at baseline and at Year 3. This was due to availability and limitation in study funding.
- There is only a single day of complete 24 hour CGM sensor data in some study subjects; ideally, there should be 4-5 days of complete data to get the most accurate results. One could have increased the chances of achieving complete CGM sensor data if the subjects had agreed to wear the sensor for longer. However, this would have meant the subjects returning multiple times for a fresh sensor (which was not favoured by the subjects). If this was stipulated, then it may have resulted in an increased 'drop out' rate.
- The CGM system requires calibration using a blood glucose value obtained from a home glucose meter. Therefore, any inaccuracies of the value obtained from the reference meter would also affect the accuracy of the value calculated by the CGM system. Since the sensitivity of the sensor changes also, any failure to

calibrate the sensor by the study subjects (at least four times daily as instructed) would result in inaccurate glucose readings.

With the study aim in mind, a set of specific objectives were set out that were measurable, potentially achievable, realistic and timely. With regard to them, ethical approval was successfully obtained from the relevant bodies in order to conduct the study. Statistical advice was obtained from Professor Robert Newcombe, Cardiff University School of Medicine and a proposed sample size of ( $n = 50$ ) with confirmed IFG/IGT on OGTT was considered justifiable. Demographic data, a thorough medical history, medication history, family history and social history was obtained at baseline and on all study subjects that returned for repeat testing at Year 1 and Year 3. Instead of ideally, obtaining GAD antibody status, C-peptide and Insulin levels on all study subjects at baseline, this was obtained at Year 1. This was due to the fact that the original ethical approval did not include GAD Ab, C-peptide or Insulin testing. An amended ethical approval was successful in order to achieve this. At baseline and Year 1, OGTT, check HbA1c and CGM was performed on all study subjects. At Year 3, FPG was performed and used as outcome indicator, instead of OGTT. Although ethical approval was obtained for an OGTT and CGM at Year 3, when the study subjects contacted and invited back, they were not keen to have OGTT or CGM; however, they were happy to attend for a FPG. With the assistance of Professor Richard Ollerton, Professor of Statistics (Sydney Australia), glycaemic excursion variables to reflect glucose fluctuations observed in CGM were constructed. These were successfully applied in Excel 2007 to the raw CGM sensor data. Baseline, Year 1 and Year 3 data were successfully analysed, with regard to the study parameters and the CGM profiles for each study subject were separated by eye into 3 groups based on variability (least variability, medium variability and most variability). The paired Sample  $t$ -Test was then successfully used to compare differences in two means of the set parameters as the study progressed with time and the independent sample  $t$  Test was successfully used to compare the means of two independent groups (diabetes and non diabetes) at Year 1 and Year 3, in order to determine any significant difference between them, when tested against a number of baseline parameters. ANOVA was used at baseline to evaluate any significant difference between the CGM profiles by inspection and the statistically constructed CGM parameters and baseline parameters were tested successfully for Year 1 and Year 3 outcome, using binary logistic regression analysis, to see if any of them affected the outcome or influenced each other.

In general, the research design worked well and was guided by the objectives in a stepwise fashion. This contributed to achieving the overall aim of the study, which was to assess whether CGMS can help identify people with abnormal glucose tolerance that progress to T2DM. In addition to this, this study enabled the PI to have an opportunity to educate and improve the awareness in study subjects, with regard to T2DM risk factor modification and prevention. In addition, this study enabled the PI to gain experience and knowledge in the use of new technologies, currently at the forefront of diabetes clinical care and research. In this study, the data was not significant and unfortunately CGM was not able to be used to predict progression from abnormal glucose tolerance (pre-diabetes) to T2DM; the Null Hypothesis was rejected.

To date, there are a number of high risk patients in which the use of CGM has demonstrated beneficial information, to diagnose early glycaemic abnormalities, as described by Soliman *et al* (2014) (79):

- **Morbid obesity:** Studies have demonstrated that in obese children and adolescents, CGM is superior to OGTT and HbA1C in detecting early glycaemic abnormalities El Awwa *et al* (2012).
- **Polycystic ovary syndrome (PCOS):** Tao *et al* (2009) investigated 20 PCOS women with NGT and 20 age-matched healthy women with normal menstruation using OGTT and CGM. Results demonstrated CGM diagnosed an abnormal mode of daily glucose change in the PCOS group, characterized by a delayed peak of post-breakfast plasma glucose level.
- **Cystic fibrosis (CF):** A long pre-diabetic phase of abnormal glucose tolerance is described in subjects with CF since childhood. Under certain circumstances, OGTT screening, used to diagnose CF-related diabetes (CFRD), fails to reveal early glucose tolerance abnormalities. In this situation, CGM could be a useful tool for evaluating early abnormalities of glucose tolerance in CF patients as described by Schiaffini *et al* (2010).
- **Thalassemia major (TM):** Both insulin deficiency and resistance are reported in patients with  $\beta$ -thalassemia major. Studies have demonstrated that CGM is a useful method to detect the variability of glucose fluctuations and offers the

opportunity for better assessment of glucose homeostasis in TM patients, as described by Soliman *et al* (2013).

- **Gestational diabetes (GDM):** A study by Bühling *et al* (2004), demonstrated that CGM detected more frequent and longer durations of hyperglycemia in GDM compared to non diabetic women than the SMBG and women with an IGT exhibited higher glucose levels than patients with gestational diabetes.
- **Acute coronary syndrome (ACS):** Intensive monitoring for hyperglycemia is essential during care for ACS. Radermecker *et al* (2009) demonstrated that CGM disclosed early and frequent hyperglycaemia in non-diabetic patients with ACS compared to SMBG.
- **After renal transplantation:** New onset of diabetes after transplantation (NODAT) and IGT are well-known complications of immunosuppressive therapy after transplantation, being a risk factor for cardiovascular disease affecting patient and graft survival. Therefore, early identification and treatment are of high importance. A study by Pasti *et al* (2013) (80) demonstrated that CGM analysis showed that IGT patients had higher “lowest glucose” level and the incidence of hypoglycemic episodes was significantly lower compared with patients with normal OGTT result. In IGT patients, glucose variability tended to be lower.
- **In critically ill and in perioperative, intraoperative and postoperative periods:** Given the demonstrated benefit of euglycemia in critically ill patients as well as the risk for hypoglycemia during their management, Piper *et al* (2006) (81) used CGM in this instance and reported effective glucose monitoring by CGM; the sensor performance was also not affected by body temperature, ionotrope dose, or body-wall oedema.

In summary, the use of CGM in the diagnosis of early dysglycemia (pre-diabetes) in high risk patients, described above, appears to be promising and in many occasions superior to other known diagnostic modalities namely OGTT and measurement of HbA1C. Its use in combination with intermittent glucose monitoring adds to its accuracy and reliability.

With regard to using CGM as an early detection test for the masses at high risk of T2DM, the main disadvantages and limiting factors here would be patient factors, practical issues, technical issues and cost. CGM systems are an emerging technology that allows frequent glucose measurements to monitor glucose trends in real time. Their use as a diagnostic tool is still developing, but the real benefit of CGM currently lies in the field of clinical diabetes management. CGM is especially beneficial for individuals who (i) want to reduce their HbA1c target without increasing hypoglycemia risk; (ii) aid identification of hypo- unawareness; (iii) in pregnancy and (iv) in children and adolescents at or above their target HbA1c (82). When this study began in 2009, there was a paucity of research studies that had used CGM to look at predication of progression from pre-diabetes to T2DM. However, over the years the use of CGM has become more commonplace and is now used widely in diabetes research and clinical management, as described by Field (2013) (79).

In the future, there are many exciting technologies that are being developed in the field of diabetes; for example, the Smart digital contact lens® that aims to measure blood glucose levels from tears (83), the Apple Inc Smart Watch® that encompasses a CGM Application (App) (84) and the IBG Star® Diabetes Management App (85), but to name a few. There are now glucose monitors that can be worn for longer time periods, improved sensors (Enlite) (86) and inserters and advancing pump technology, such as the Minimed connect (87). Abbot Freestyle Libre CGM (88) has also arrived and with the aid of sensor augmented pump therapy the loop towards an artificial pancreas is closing slowly. However, despite the benefits and uses of CGM in a clinical setting, this study was unable to demonstrate that CGM could predict progression from pre diabetes to diabetes, which is what our aim was.

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## **APPENDIX**

APPENDIX 1: STUDY Documents

APPENDIX 2: CGM Profiles – Least Variability

APPENDIX 3: CGM Profiles – Medium Variability

APPENDIX 4: CGM Profiles- Most Variability

## **APPENDIX 1**

## STUDY PROTOCOL

### **Continuous subcutaneous glucose monitoring to predict progression from abnormal glucose toleration to Type 2 diabetes mellitus**

**PROTOCOL: V3, 7<sup>th</sup> September, 2011**

**REC reference number: 09/WSE03/31**

#### **Aim:**

To assess whether continuous glucose monitoring (CGMS) can help identify people with abnormal glucose tolerance who progress to Type 2 diabetes mellitus (T2DM) within 24 months.

#### **Relevance to diabetes and Background information for the project:**

Type 2 diabetes mellitus (T2DM) is diagnosed when a person is found to have a fasting plasma glucose  $\geq 7.0$  mmol/l or a random glucose  $\geq 11.1$  mmol/l (Alberti KG *et al*, 1998). These consensus threshold values are based on evidence which suggests the risk of specific complications increases significantly amongst people with plasma glucose levels consistently above these levels (Gabir MM *et al*, 2000). However, both fasting and post-prandial (or random) plasma glucose levels broadly follow a normal distribution in the general population and it is also known that increased morbidity (especially from macrovascular disease) is associated with more modest levels of glycaemia (Lim SC *et al*, 2000). The categories of impaired fasting glycaemia (IFG) (fasting glucose  $> 6.1$  mmol/l) and impaired glucose tolerance (IGT) (fasting glucose  $> 7$  mmol/l and 2 hour post-challenge 7.8-11.1 mmol/l) are an attempt at recognising these facts. IFG and IGT not only define cohorts at increased risk of cardiovascular disease, but also individuals who may develop progressive deterioration in glucose homeostasis, leading to frank T2DM (Alberti KG *et al*, 1996). IFG and IGT are symptomatic, biochemical classifications, which generally come to light during screening programmes for T2DM (Hoerger TJ *et al*, 2007). However, many people with IFG or IGT will not progress to T2DM but appear to spontaneously revert to normal (Riccardi G *et al*, 1985). Others may progress only very slowly, whereas in some cases the progression may be rapid (Ferrannini E *et al*, 2004).

Although the OGTT has the advantage of being relatively cheap and straightforward to perform as a basic screening test, it is limited by the fact that it is performed during a single two hour period, it is a non-physiological stimulus and individuals show variability in glucose tolerance from day to day. A number of therapeutic interventions will reduce the risk, or at least the pace, of deterioration from IGT and IFG to T2DM (Knowler WC *et al*, *New Eng J Med* 2002) (Chiasson JL *et al*, *Lancet* 2002) (Torgerson JS *et al*, 2004). In order to target interventions to prevent progression to those at greatest risk, we need further information as to which individuals are likely to progress. The documented rate of progression from IGT and IFG to T2DM varies between different studies (Rasmussen SS *et al*, 2008) (Shaw JE *et al*, 1999) (Meigs JB *et al*, 2003) (Heine RJ *et al*, 1996). In general, progression rates are lowest in those that recruit from the general population and highest in those that target 'at-risk' groups. Several abnormalities have been reported to be associated with a greatest risk of progression including age, BMI, fasting and 2 hour plasma glucose concentrations, elevated fasting proinsulin, low 2-hour insulin and fasting triglyceride levels (Alberti KG *et al*, 1996) (Rasmussen SS *et al*, 2008).



Progression to T2DM could be mediated by an increase in insulin resistance, further impairment of insulin secretion or a mixture of both (Jensen CC *et al*, 2002) (Abdul-Ghani MA *et al*, 2006) (Kapitza C *et al*, 2003). As adequate moment-by-moment insulin secretion and action is essential for glycaemic control, one marker of deteriorating carbohydrate homeostasis would be increased fluctuations in blood glucose levels. We therefore propose the use of continuous glucose sensing to quantify the fluctuations.

Continuous subcutaneous glucose monitors (Medtronic- Continuous Glucose monitoring system – CGMS) will measure subcutaneous (interstitial) glucose levels every 10 seconds and then store a smoothed average over 5 minutes, continuously for up to 3 days with a single sensor (Sachendina N *et al*, 2003) (Guerci B *et al*, 2003). The monitors are externally calibrated by the subject who enters the result of his/her home blood glucose testing (using a standard meter) at least 4 times in each 24-hour period. CGMS are accurate and have proved useful in the monitoring of patients with diabetes, including those on insulin pump therapy (Faradji RN *et al*, 2006). It has also been suggested they may be of value in identifying early transplant rejection (Faradji RN *et al*, 2006). They have the advantage of documenting continuous glucose variations throughout the day, rather than during a short OGTT or discontinuous home blood glucose testing.

#### **Inclusion Criteria:**

All people between the ages of 18-80 who have been referred for an oral glucose tolerance test and are found, on that test, to have impaired glucose tolerance and/or impaired fasting glycaemia. Gender, ethnic origin, nationality, religion, belief or sexual orientations are irrelevant in the decision to recruit.

#### **Exclusion Criteria:**

1. Under the age of 18 years. In view of the increased vulnerability of children, they will be excluded from this study. In addition, most people <18 years old will have Type 1 rather than Type 2 diabetes since the prevalence of Type 2 diabetes in children is low.
2. Over the age of 80 years. People become increasingly frail with age and, although we do not wish to exclude any group arbitrarily, a cut-off age of 80 years will ensure people who are vulnerable due to age and frailty are not put at increased risk.
3. Unable or unwilling to give informed consent. We aim to recruit from as wide a range of people as possible. We will explain the research in detail but in plain language and we will provide full patient information sheets. Clearly people who are unwilling to give informed consent will not be recruited. In addition, people who do not have the capacity to give informed consent will be excluded. These will include people with severe cognitive impairment who are unable to retain and assimilate the information provided.
4. Unable or unwilling to comply with research requirements. The study requires that people check their blood glucose levels at home four times a day using a glucose meter over 6 days. Those unwilling to do this will be excluded. In addition, people will need to wear continuous glucose sensor for 6 days and enter their blood glucose results into it. The device is no more complicated to use than a simple mobile telephone, but people who are unable to use it correctly, despite full education, will be excluded from the study. Experience from my clinical practice suggests this is a rare (<5%) problem.
5. Pregnancy. Blood glucose levels are significantly affected by pregnancy and therefore women who are pregnant or plan to become pregnant during the follow-up

period of the study will be excluded. Continuous blood glucose monitoring is perfectly safe during pregnancy and should an unforeseen pregnancy occur in one of the recruited women, they will be excluded from further analysis, but no harm will have been caused.

6. Intercurrent illness with prognosis of  $\leq 2$  years. The aim of this study is to assess the value of glucose monitoring in predicting progression to diabetes over a 3 year period. People who are unlikely to be able to attend for follow up appointments over this period of time will be excluded from the study.
7. Known previous allergic reactions to adhesive plasters.

## **Methods**

### **Recruitment**

1. People attending for a clinically indicated oral glucose tolerance test (OGTT) at the University Hospital of Wales, Cardiff (UHW) will be asked to participate in the study. Eligible candidates will be given a patient information sheet and consented by the Principal Investigator (PI)
2. The PI will obtain the results of the OGTT and contact subjects with impaired fasting glycaemia or impaired glucose tolerance to arrange their attendance at the Diabetes Centre, UHW (visit 1).

### **Statistical Advice**

This project has been discussed with Professor Newcombe, Cardiff University School of Medicine and a proposed sample size of 50 subjects with IFG/IGT is considered justifiable. This is based on the assumption that 30% will have been diagnosed as having definite diabetes by 2 years and that half the remaining subjects, i.e 35% will have reverted to normal. A comparison of those who become diabetic with those who revert to normal would have a power of 80% to detect a shift of exactly 1 standard deviation in the mean value for any parameter, such as the peak glucose or AUC, using a test at the conventional two-sided alpha level.

### **VISIT 1**

1. The PI will check that participants have understood the patient information sheet and that signed consent has been obtained.
2. The PI will document a brief medical history, including family history of diabetes, risk factors for diabetes and current medication.
3. Participants will be taught home blood glucose monitoring and will be given a glucose meter and sufficient strips to test at least 4 times a day for the following 6 days.
4. Participants will be fitted with a continuous subcutaneous glucose monitor (CGMS, Medtronic) and educated in its use. They will be given a written reminder of how the monitor works together with a telephone number to call should it become disconnected or appear to malfunction.
5. 10 ml of blood will be taken from a peripheral vein to check HbA1c, analogous markers of glycaemic control, auto- antibodies and serum lipids
6. Participants will be asked to return on day 3 for Visit 2.

### **VISIT 2**

1. The PI will check that the participants have recorded at least 4 home blood glucose levels each day during the previous 3 days and resolve any questions regarding the use of CGMS.
2. The glucose sensor fitted on Visit 1 will be replaced with a new sensor.

3. Participants will be asked to continue to check their home blood glucose levels at least 4 times a day for the following 3 days.
4. Participants will be asked to return 3 days later for Visit 3.

### **VISIT 3**

1. The PI will check that the participants have recorded at least 4 home blood glucose levels during the previous 3 days and resolve any questions that have arisen out of the use of the CGMS.
2. The CGMS sensor and monitor will be removed.
3. Participants will be reminded that they will be given an appointment for a repeat oral glucose tolerance test in 12 months time.

### **FOLLOW UP OGTT at 12 MONTHS – VISIT 4**

1. 6-8 weeks prior to the 12 month anniversary of the first OGTT, participants will be contacted, inviting them to telephone the PI to make an appointment for a repeat OGTT.
2. Two reminders will be sent to participants. Those that do not respond will be presumed to have withdrawn consent.
3. At OGTT, a total of 20 ml of blood will also be taken from a peripheral vein via a canunlae (2.5 ml blood every 15 minutes, for 120 minutes in total) to check HbA1c, analogous markers of glycaemic control, auto- antibodies and serum lipids.
4. The PI will obtain the results of the 12-month OGTT and contact the participants with the results. Those that have progressed to a diagnosis of diabetes will have their follow-up discussed with their general practitioners.
5. Procedures as on Visit 1. Repeat CGM study and will be given an appointment for Visit 5.

### **VISIT 5 and VISIT 6**

1. Procedures as Visit 2 and Visit 3

### **FOLLOW UP AT OGTT at 36 MONTHS – VISIT 7**

1. 6-8 weeks prior to the 36 month anniversary of the second OGTT, participants will be contacted in writing, inviting them to telephone the PI to make an appointment for a repeat OGTT.
2. Two reminders will be sent to participants. Those that do not respond will be presumed to have withdrawn consent.
3. At OGTT, a total of 20 ml of blood will be taken from a peripheral vein via a canunlae (2.5 ml blood every 15 minutes for 120 minutes in total) to check HbA1c, analogous markers of glycaemic control, auto- antibodies and serum lipids.
4. The PI will obtain the results of the 36-month OGTT and contact the participants with the result. Those that have progressed to a diagnosis of diabetes will have their follow-up discussed with their general practitioners.
6. Procedures as on Visit 1. Repeat CGM study and will be given an appointment for Visit 8.

### **VISIT 8 and VISIT 9**

7. Procedures as Visit 2 and Visit 3

### **APPENDIX – References**

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## **PATIENT INFORMATION LEAFLET**

### **Information about Research (ETHICS)**

**STUDY TITLE: Continuous glucose monitoring for the prediction of Type 2 diabetes mellitus**

**Version 2011/1, 7<sup>th</sup> September 2011**

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

#### **What is the purpose of the study?**

The aim of this study is to find out whether using a device to measure the blood glucose levels of people continuously over several days helps identify those who will go on to develop Type 2 diabetes.

#### **Why have I been invited?**

You have come along today for an oral glucose tolerance test. This has been arranged for you by a doctor because they think you might have diabetes or you might be at risk of developing diabetes in the future. The results of the test today will be sent to the doctor who arranged it. The test may show one of three things:

1. The test may be completely normal
2. The test may show you have diabetes
3. The test may show you do not have diabetes at present, but the blood sugar level is higher than a completely normal value. This is called 'impaired fasting glycaemia' or 'impaired glucose tolerance'.

We are interested in identifying people in the third group. Normally, the results of your blood tests would be kept confidential and only seen by doctors treating you. We are asking for your consent for our research team to look at your results and contact you again if they fall into this group of impaired fasting glycaemia or impaired glucose tolerance.

We are asking everyone who has a glucose tolerance test at this hospital to help us with this research, except children, the very elderly, pregnant women or people who are very unwell for other reasons.

#### **Do I have to take part?**

It is entirely up to you to decide whether you want to take part. We will describe the study and go through the information sheet, which we will then give you. We will then ask you to sign a consent form to allow us to look at the results of the glucose tolerance test that you are having today. If you do not wish to sign this form or take part, we will not look at your results or use them for research purposes. If you do consent to us

looking at your results, we will contact you again if they suggest you may be suitable for the research study. We will then describe the study again to you and, if you wish to take part, we will then ask you to sign a second consent form to show you have agreed. You are free to withdraw at any time, without giving a reason or explaining yourself. This would not affect the standard of care you receive.

### **What will happen to me if I take part?**

If you agree to take part, the results of your glucose tolerance test today will be reviewed by a member of the research team. If the test is completely normal, or if it shows that you have diabetes, the results will be sent to the doctor who arranged your test, and you will hear nothing further about this research. If your glucose tolerance test shows you have 'impaired glucose tolerance' or impaired fasting glycaemia', the results will be sent to the doctor who arranged your test and, in addition, you will be invited to participate in the next stage of the research project. You can, of course, decide not to take part at that stage or at any time later.

**Before you leave the hospital today, you will be asked to sign a Consent Form. This will give us permission to look at the results of your glucose tolerance test and to contact you again about the research project.**

**If you do take part,** you will be asked to attend the Diabetes Centre at the University Hospital of Wales where we will teach you how to check your own blood glucose at home by pricking your finger and placing a spot of blood onto a stick that fits into a small meter. The meter will record the result in its memory. We will ask you to check your blood glucose at least 4 times a day for the following 6 days.

You will also be fitted with a continuous glucose monitor. This involves us placing a small piece of plastic (an electrode) just under the skin in your tummy. There is a sharp scratch, less painful than a blood test, but no other discomfort. This electrode is connected to a monitor, about half the size of a mobile telephone, which we will ask you to keep with you continuously for 3 days. We will show you how the monitor works as you will need to enter the results of your own blood glucose meter results at least 4 times a day in a written diary. You will be given a telephone number to call should the monitor become dislodged whilst you are at home. The monitor is designed to allow you to carry on with your normal work and life whilst it is connected and we would encourage you to just carry on with your normal activities.

In addition, a total of 20 ml of blood will be taken from a peripheral vein to check HbA1c, analogous markers of glycaemic control, auto- antibodies and serum lipids. After 3 days, we will ask you to return to the Diabetes Centre so that we can replace the electrode. We will then want to continue using the monitor for another 3 days before coming back so that we can disconnect it.

People with impaired glucose tolerance or impaired fasting glycaemia are at increased risk of developing diabetes in the future. You will be given some general advice as to how to reduce the risk when you come to the Diabetes Centre and this can be reinforced by your own General Practitioner who will be informed of your results.

In order to check whether diabetes has developed, it is common practice to repeat the glucose tolerance test in 12 months' time and again the year after if necessary. We will arrange for this to be done and following these repeat glucose tolerance tests, we will ask you to use the continuous glucose again for a 6 day period. In addition, a total of 20

ml of blood will be taken from a peripheral vein via a cannulae (2.5 ml blood every 15 minutes) to check physiological blood markers of diabetes (HbA1c, analogous markers of glycaemic control, auto- antibodies and serum lipids).

Each visit to the Diabetes Centre will take 2-3 hours. We are unable to compensate you for your loss of earnings, but we will reimburse the costs of parking at the hospital.

### **What will I have to do?**

#### ***You will:***

Have to agree that we can look at the results of your glucose tolerance test to help us decide if you could take part in the monitoring study.

Attend the Diabetes Centre at the University Hospital of Wales for up to 3 hours to be taught home blood glucose monitoring and have the continuous glucose sensor fitted.

Check your blood glucose levels at home using a pin-prick device and a meter at least 4 times a day for 6 days and enter the results into a paper diary.

Wear the continuous glucose monitor at home for 6 days (returning to the Diabetes Centre on day 3 to have the electrode changed).

Have a repeat glucose tolerance test and repeat the use of the glucose monitor in 12 months and 24 months' time.

Have a blood test to check HbA1c, analogous markers of glycaemic control, auto-antibodies and serum lipids initially, 12 and 24 months later respectively.

### **What is the device being used?**

We will use a continuous glucose monitor manufactured by Medtronic. We routinely use this device in people with diabetes, although it is currently not used to help predict people are at risk of developing diabetes in the future.

### **What are the alternatives?**

At the moment, people who are found to have an abnormal glucose tolerance test are just given general lifestyle advice and have the test repeated every year in case they develop diabetes. If you decide not to take part in this research, you will be offered this standard follow-up.

### **What are the possible disadvantages, side-effects and risks of taking part?**

The major disadvantage of taking part is the inconvenience of having to attend the Diabetes Centre, checking your blood glucose by a pin-prick 4 times a day, being attached to the glucose monitor for 6 days and having a blood test. Very rarely people are allergic to the plaster we use to fix the electrode to the skin. If you develop any itching or redness around the plaster, you will be asked to simply remove it. **There is a small risk of infection (less than 1 chance in 1000) at the site of the glucose monitor. If this were to occur, it would be treated with antibiotics.**

### **What are the possible benefits to take part?**

Set against the disadvantages, it may be valuable to learn blood glucose testing since you will be asked to do this in the future should you develop diabetes. Some people also find that the blood glucose results help encourage them to avoid foods that clearly put their sugar levels up.

We cannot promise the study will help you personally, but the information we get from this study will help improve the treatment of people with abnormal glucose tolerance tests. In particular, it may be that the wearing of a continuous glucose monitor will help us predict which people are at the greatest risk of developing diabetes and this will allow us to use intensive lifestyle changes or drugs to reduce the risk.



## CONSENT FORM

**Title of Project: Continuous glucose monitoring for the prediction of Type 2 diabetes mellitus**

**Name of Researcher: Dr John Alcolado**

**Stage 1 [Consent to look at your results for research purposes and contact you again]**

- 1) I confirm that I have read and understand the information sheet dated 10 June 2009 (version 2009/2) and 7 September 2011 (Version 2011/1) for the above study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.

Please initial box

- 2) I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

Please initial box

- 3) I understand that the relevant sections of my medical notes and data included during the study, including the results of my oral glucose intolerance tests may be looked at by individuals involved in this research, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

Please initial box

- 4) I agree to my GP being informed of my participation in the study.

Please initial box

- 5) I agree to have the result of my glucose tolerance test looked at for research. If my results are abnormal, I agree to the researchers contacting me about my recruitment into the study of continuous glucose monitoring. I understand that I am free to decide not to take part in the study at that stage or at any point in the future.

Please initial box

\_\_\_\_\_  
Name of patient                      Date                      Signature

\_\_\_\_\_  
Name of person  
obtaining consent                      Date                      Signature

## **GP INFORMATION LETTER (10<sup>TH</sup> June 2009, Version 2009/1)**

<GP NAME>

<GP ADDRESS>

<DATE>

Dear Dr <GP NAME>

<PATIENT NAME, PATIENT DOB, PATIENT ADDRESS>

### **Study Title: Continuous glucose monitoring for the prediction of Type 2 diabetes mellitus**

I am writing to inform you that the patient named above has consented to take part in a study on continuous glucose monitoring for the prediction of Type 2 diabetes mellitus. Your patient has recently had a glucose tolerance test performed at this hospital and was found to have either impaired glucose tolerance or impaired fasting glycaemia. You should have already received the results of this test from the laboratory, but a copy is enclosed for your information. The aim of the study is to see whether the results of continuous glucose monitoring will help predict which patients go on to develop Type 2 diabetes mellitus. As part of this study, your patient has been taught home blood glucose monitoring and will wear a continuous glucose monitor for a period of 6 days. We will arrange a follow-up glucose tolerance testing in 12 and 24 months' time. The study does not require the patient to take any trial medication. A copy of the Patient Information Leaflet is enclosed for your information.

If you require any further information, please do not hesitate to contact me.

Yours sincerely,

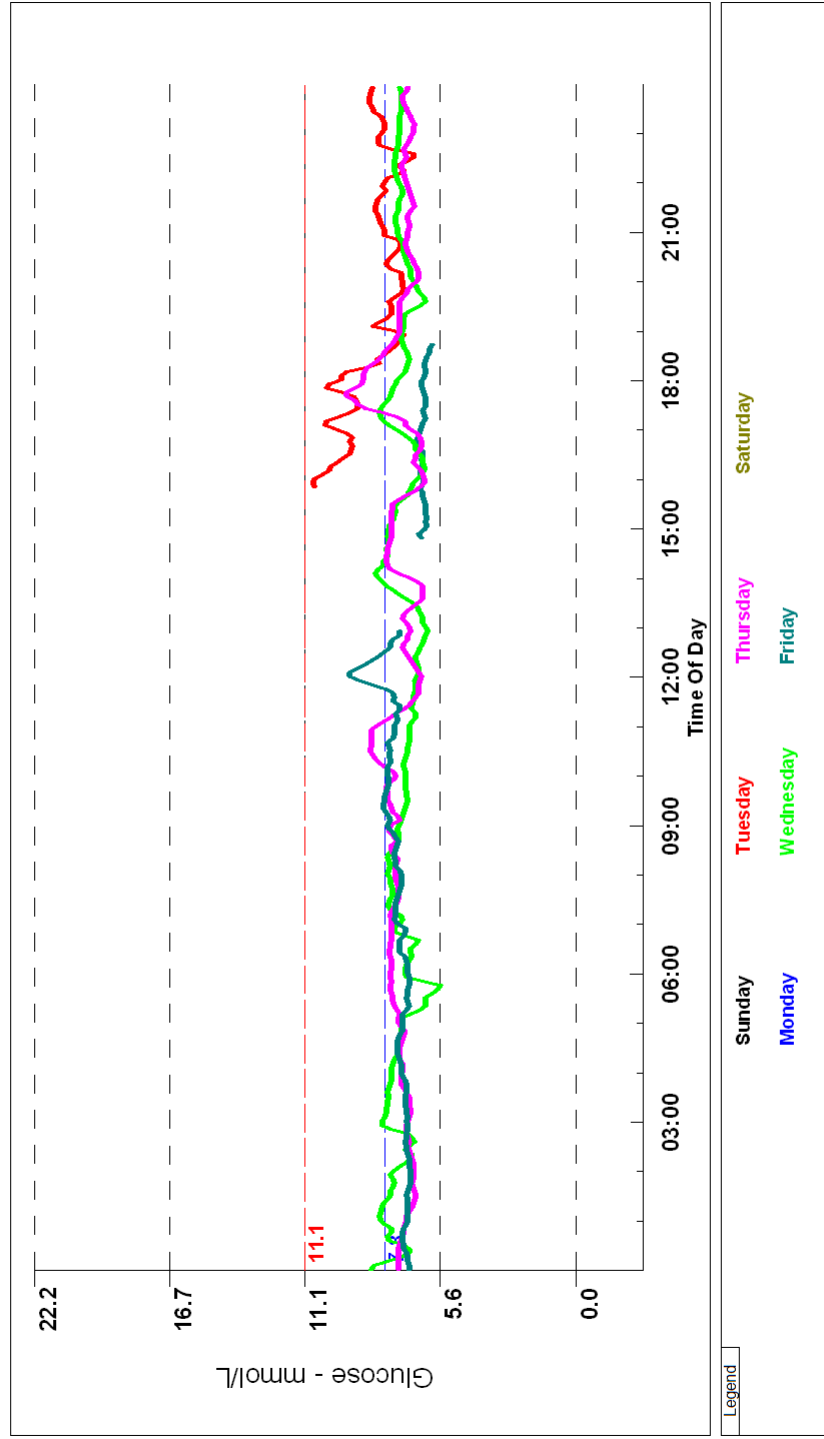
**John Alcolado**  
**Clinical Reader in Medicine**  
**Consultant Physician**

## **APPENDIX 2 – CGM PROFILES: LEAST VARIABILITY**

Sensor Modal Day

Patient:  
ID: ipro-003a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

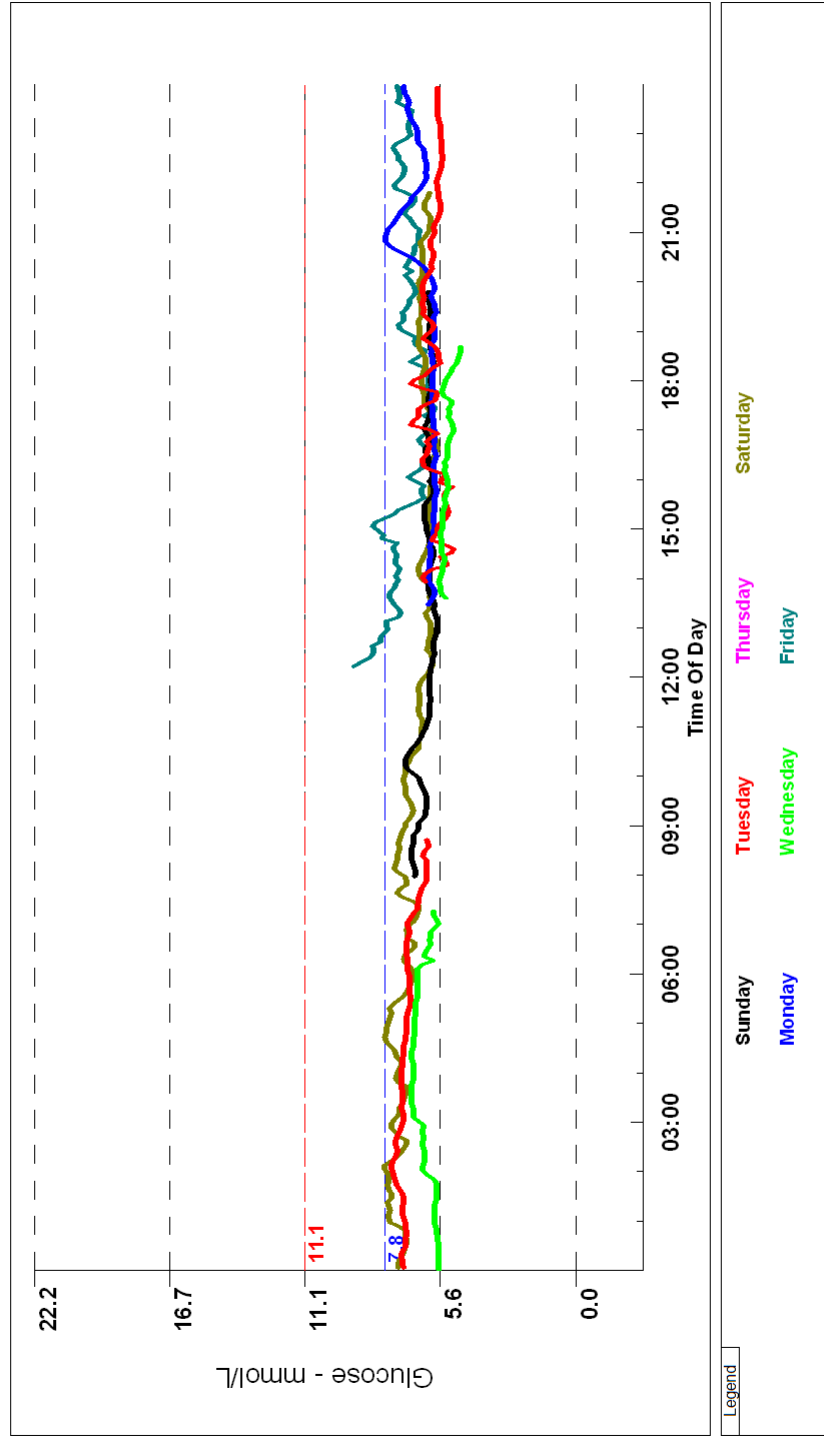


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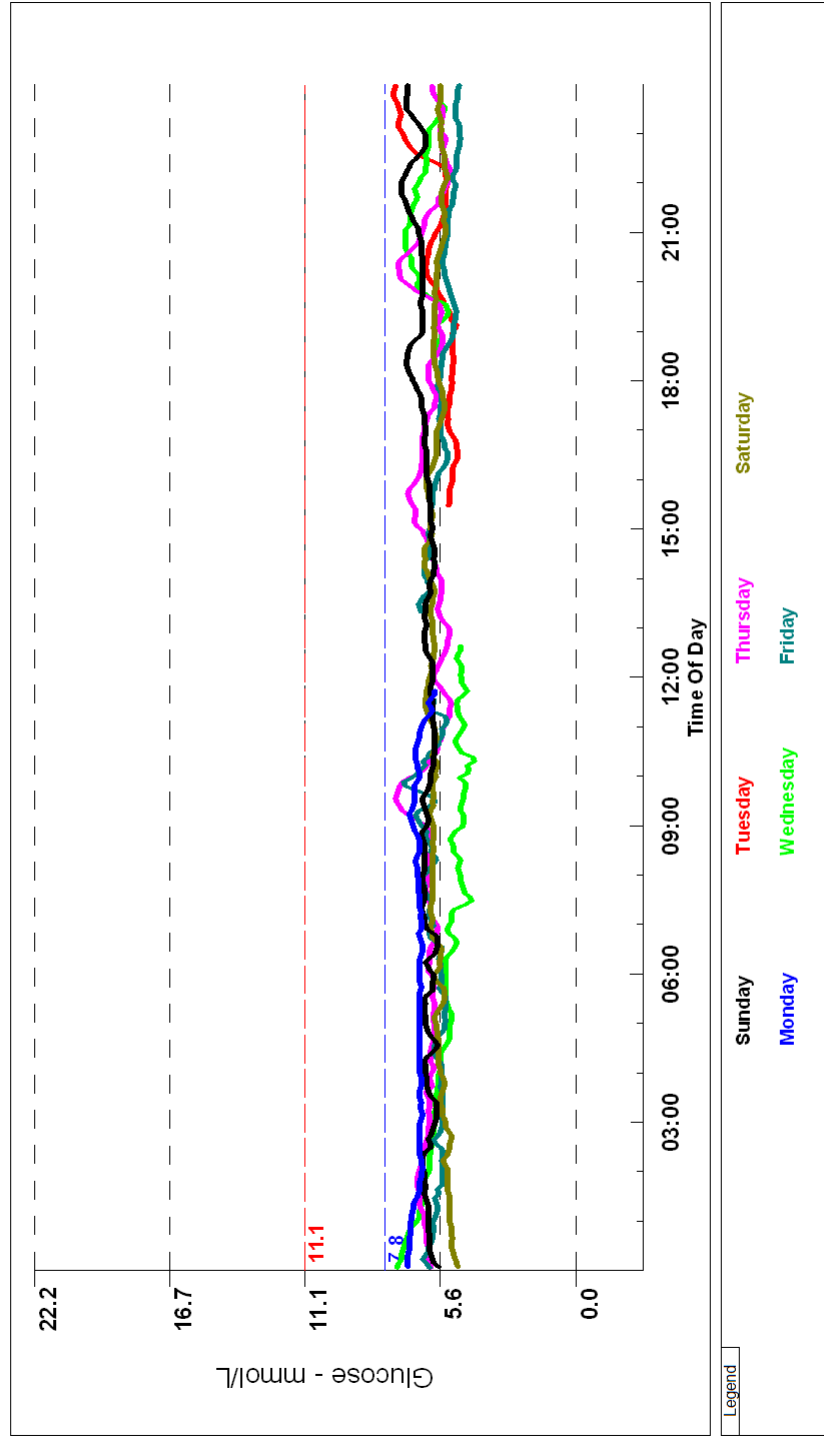
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Sensor Modal Day

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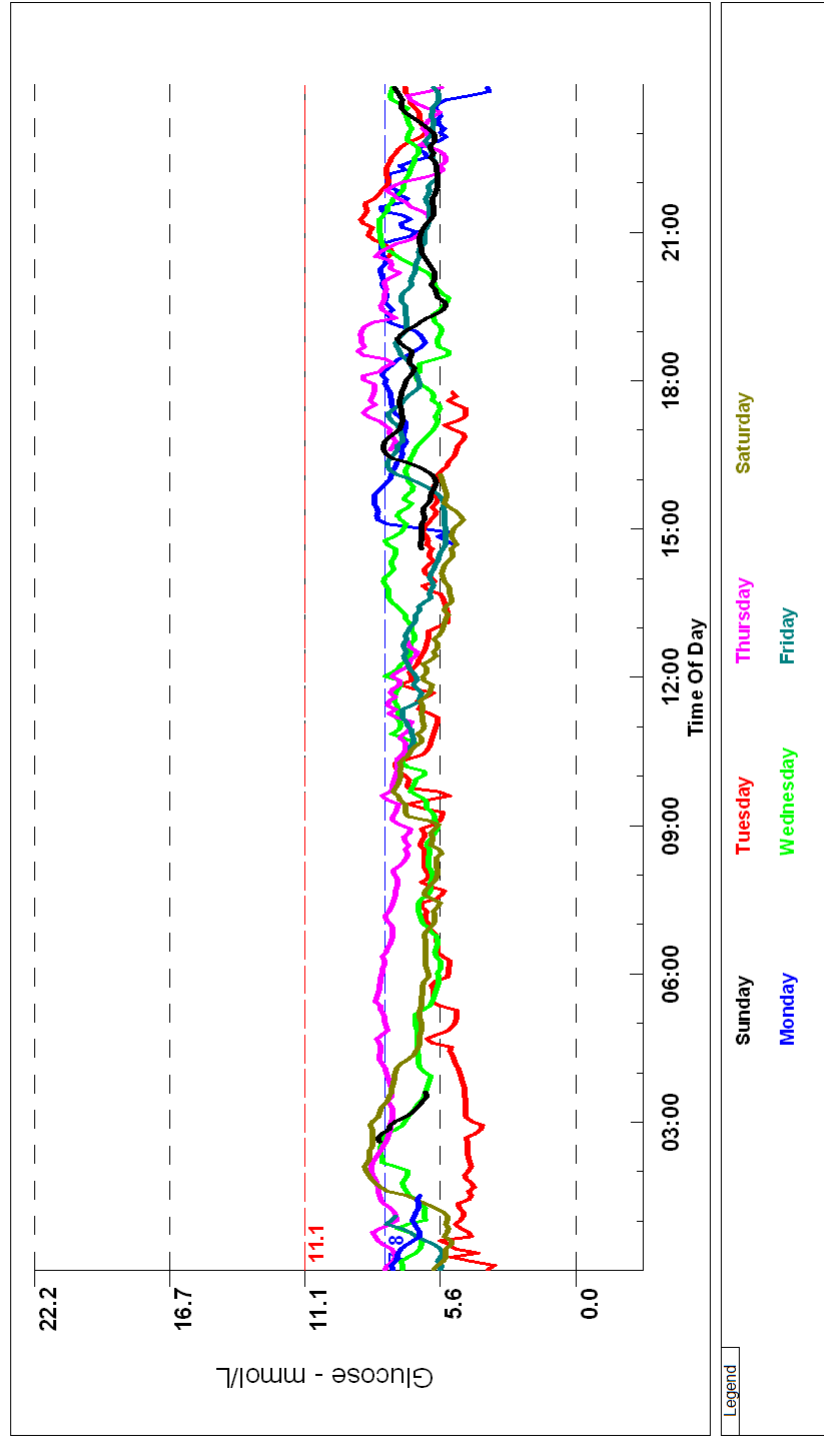
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CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-007a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

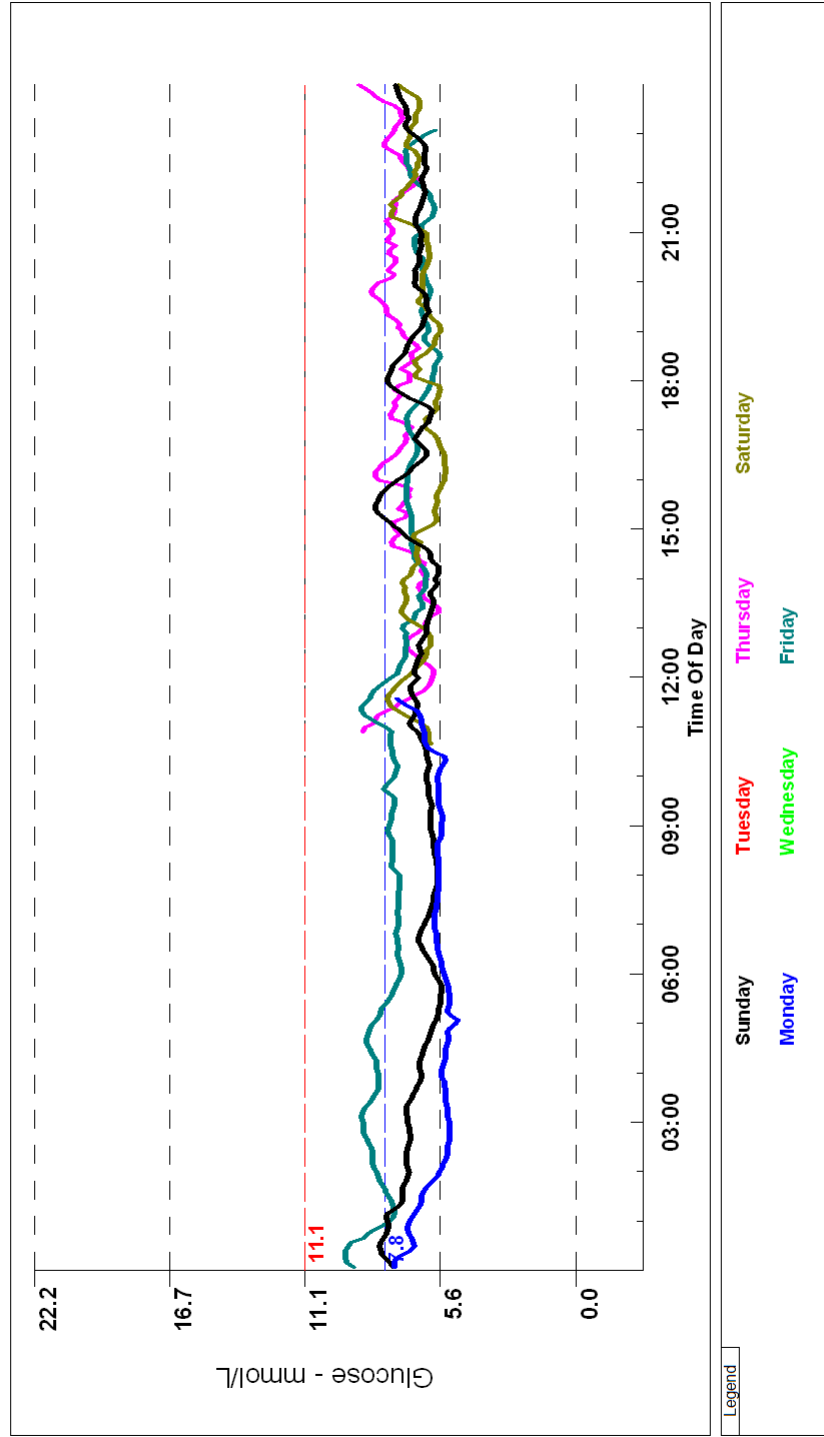




Sensor Modal Day

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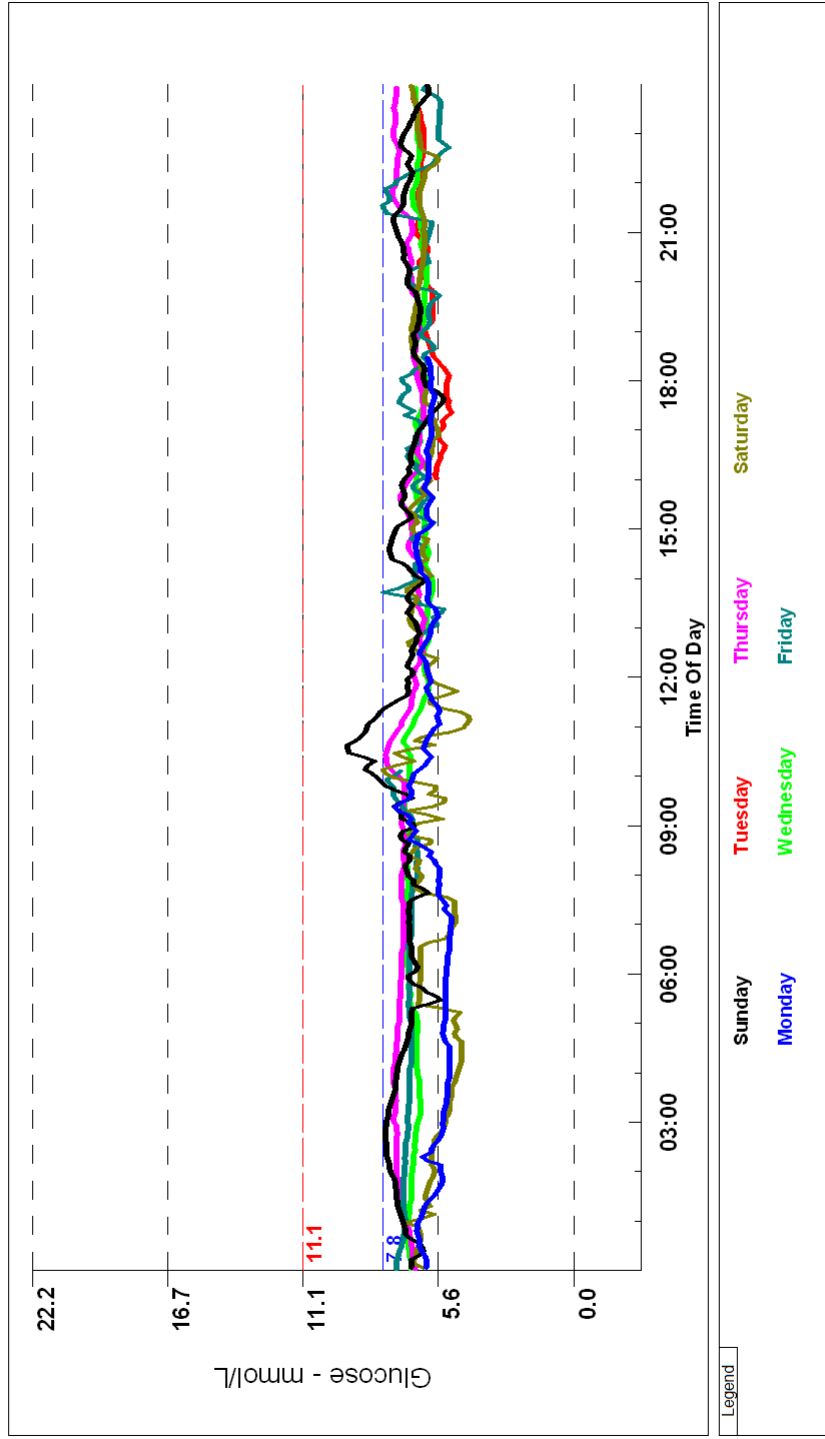
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CGMS iPro 2.2A



Sensor Modal Day

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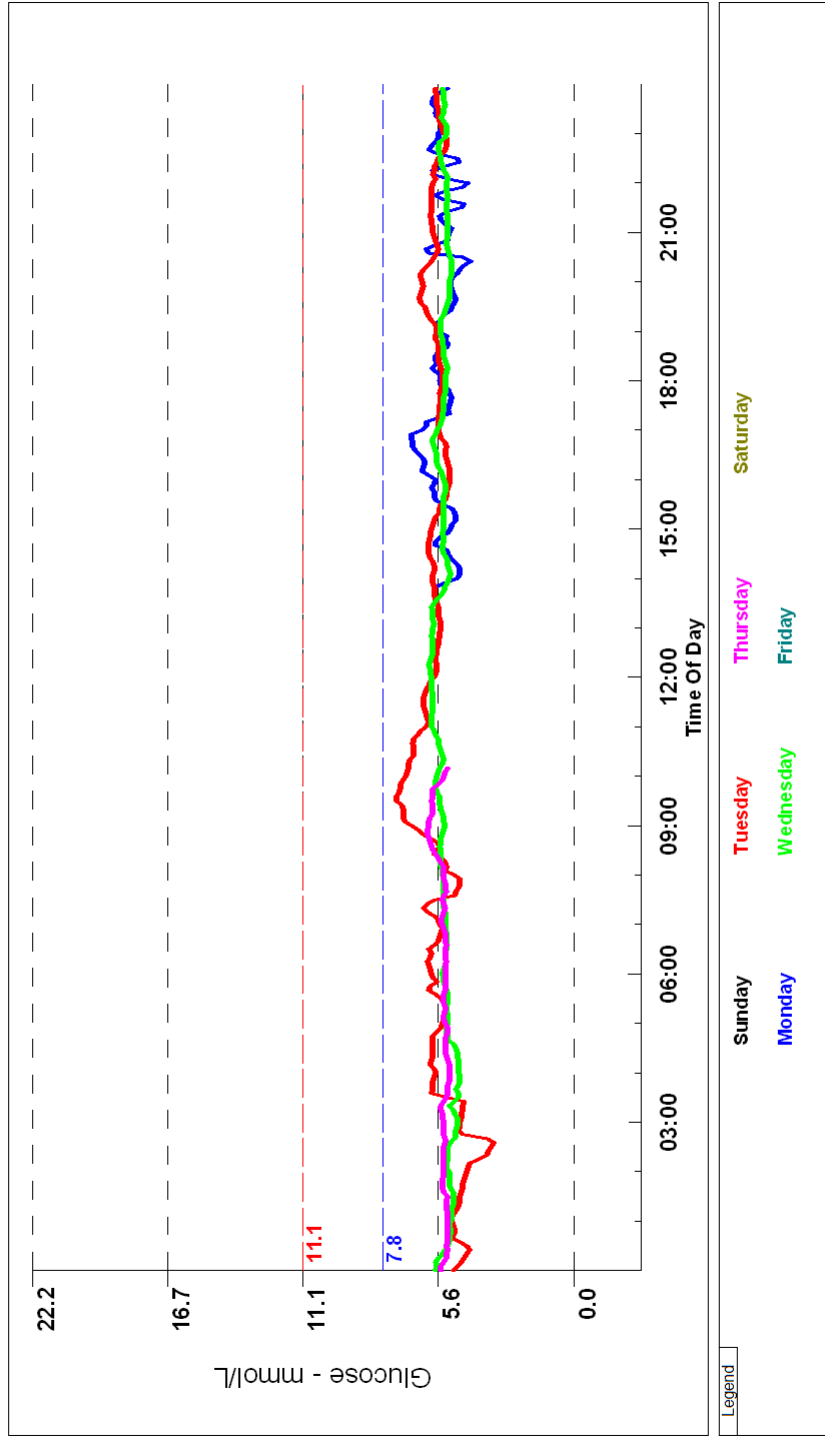
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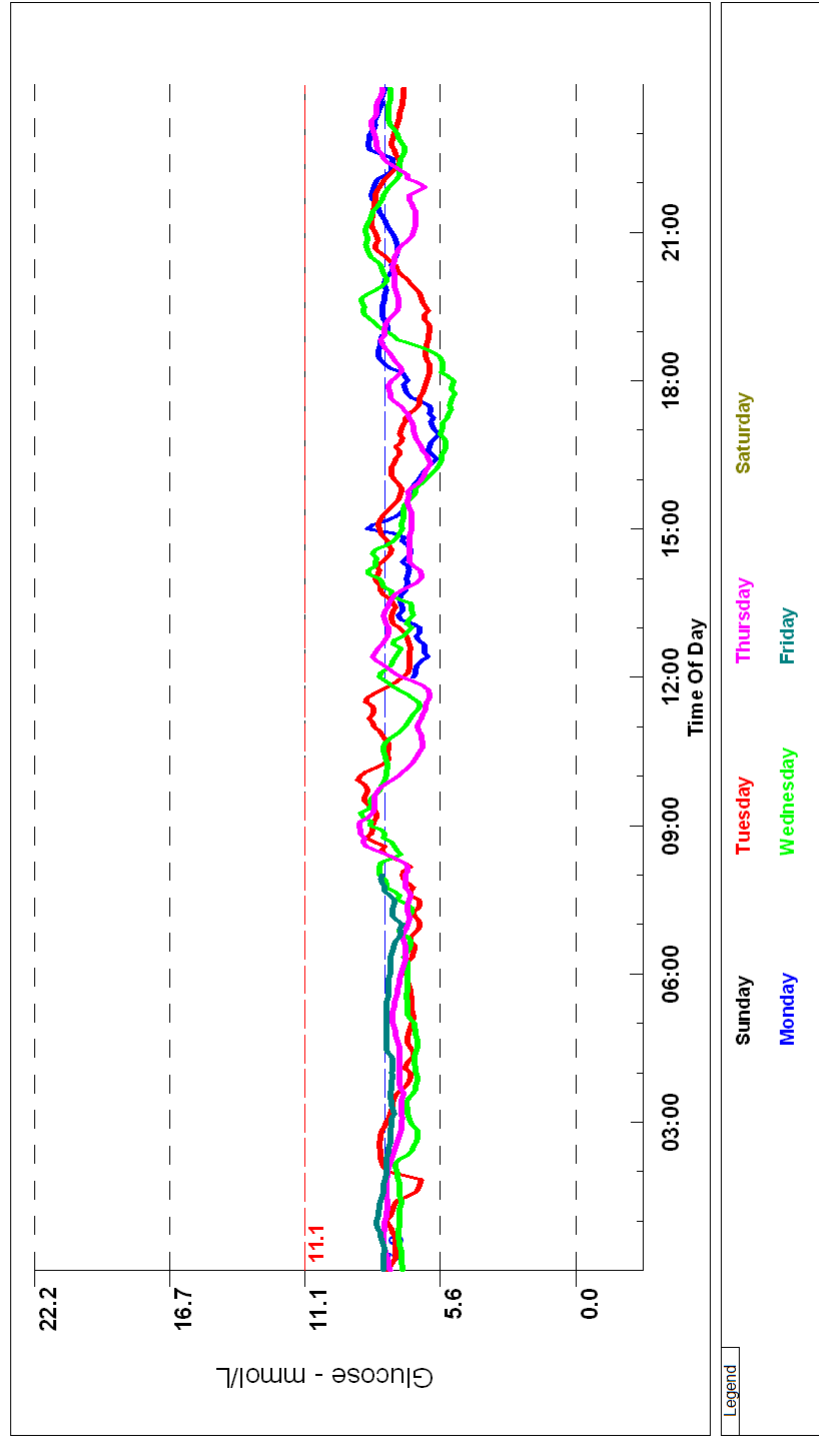
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Sensor Modal Day

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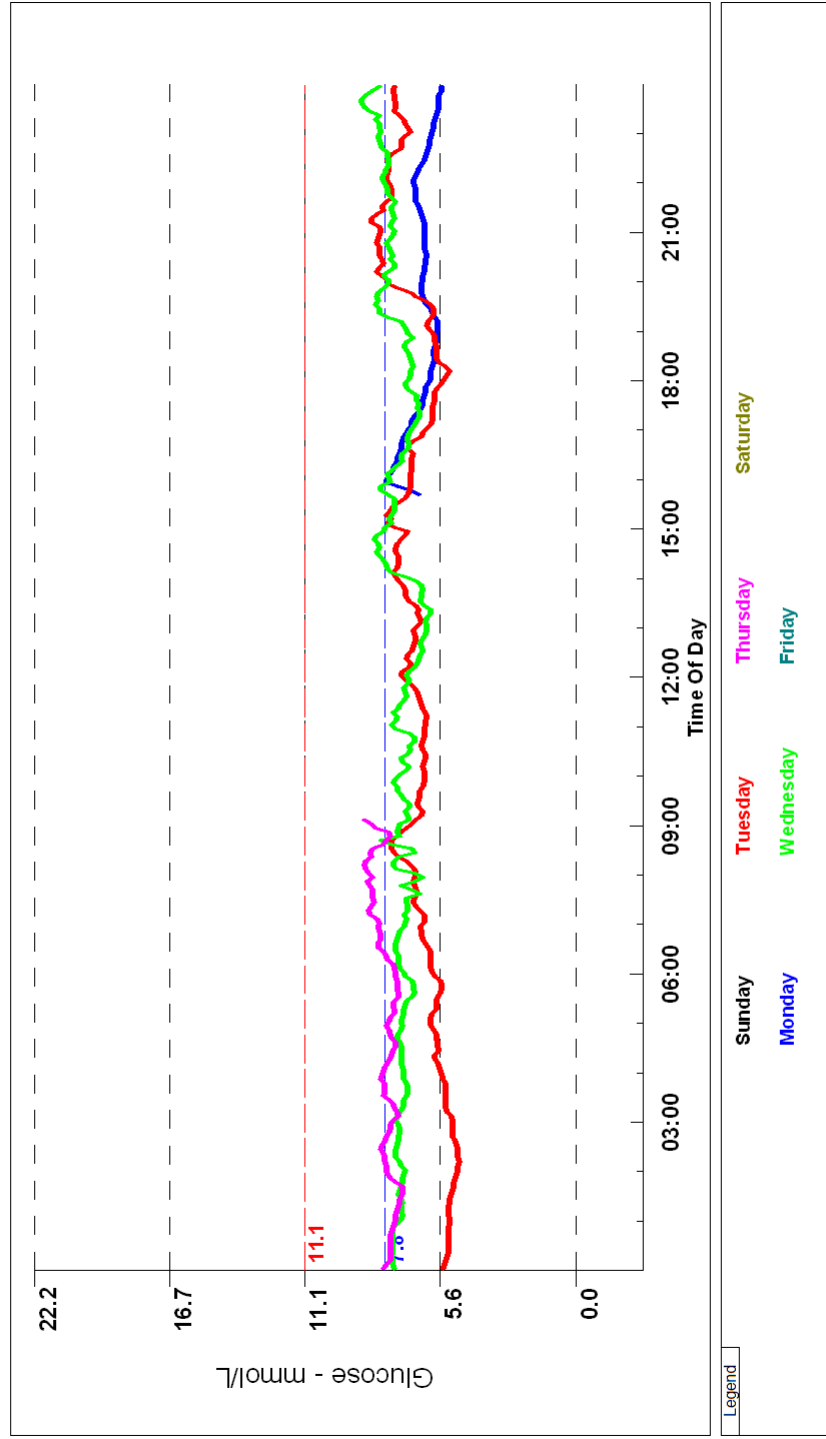
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Sensor Modal Day

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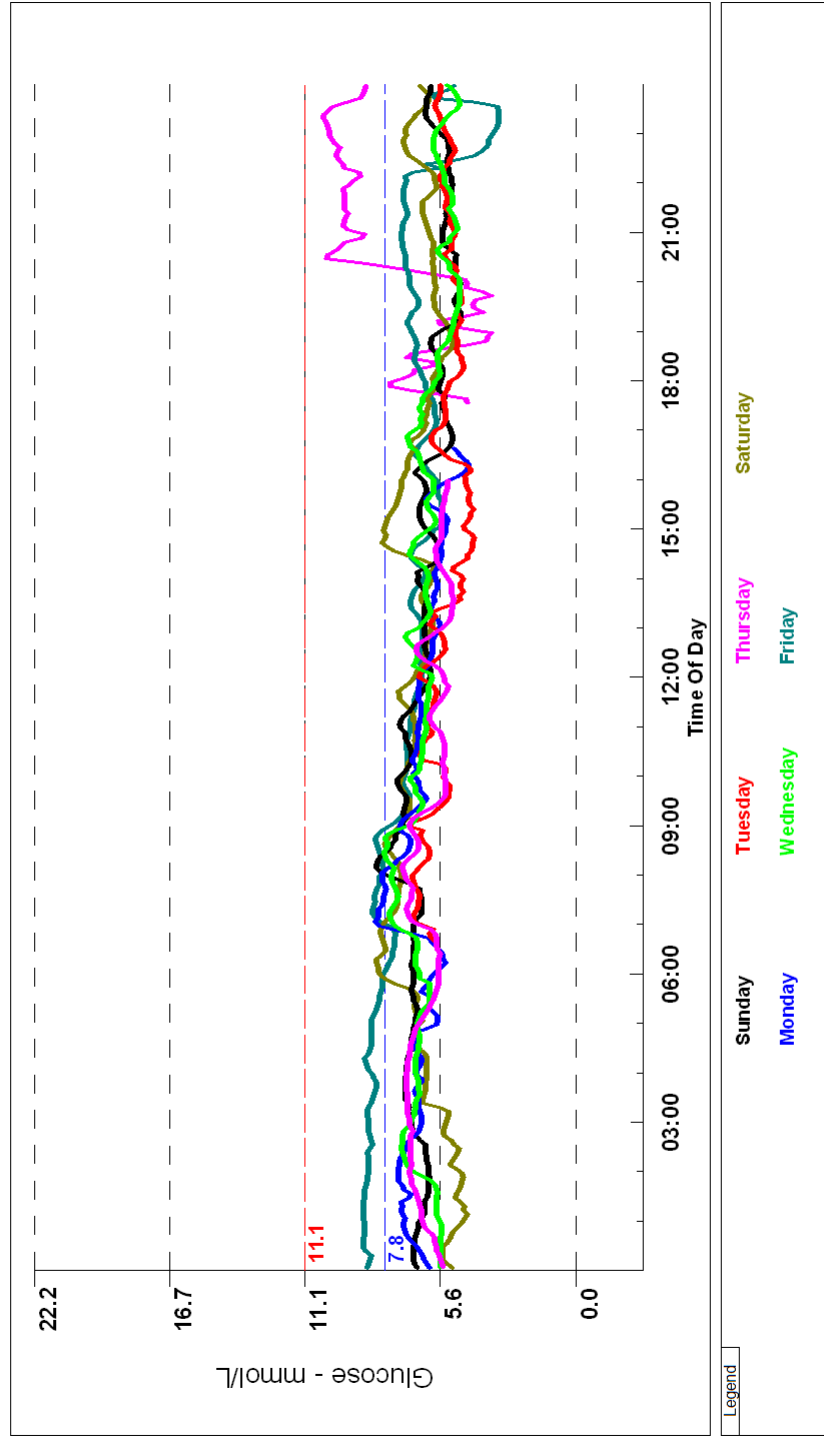
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Sensor Modal Day

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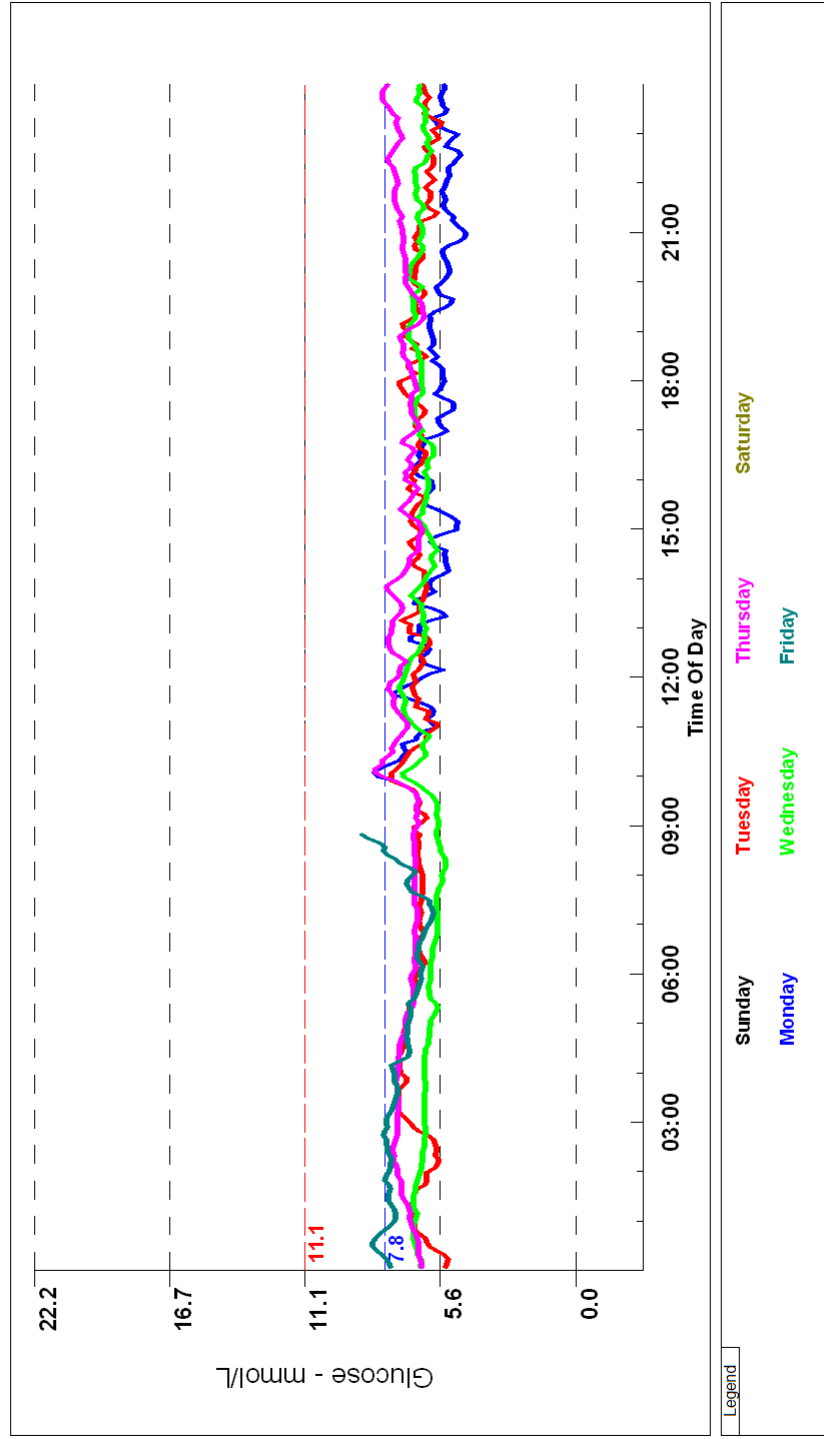
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Sensor Modal Day

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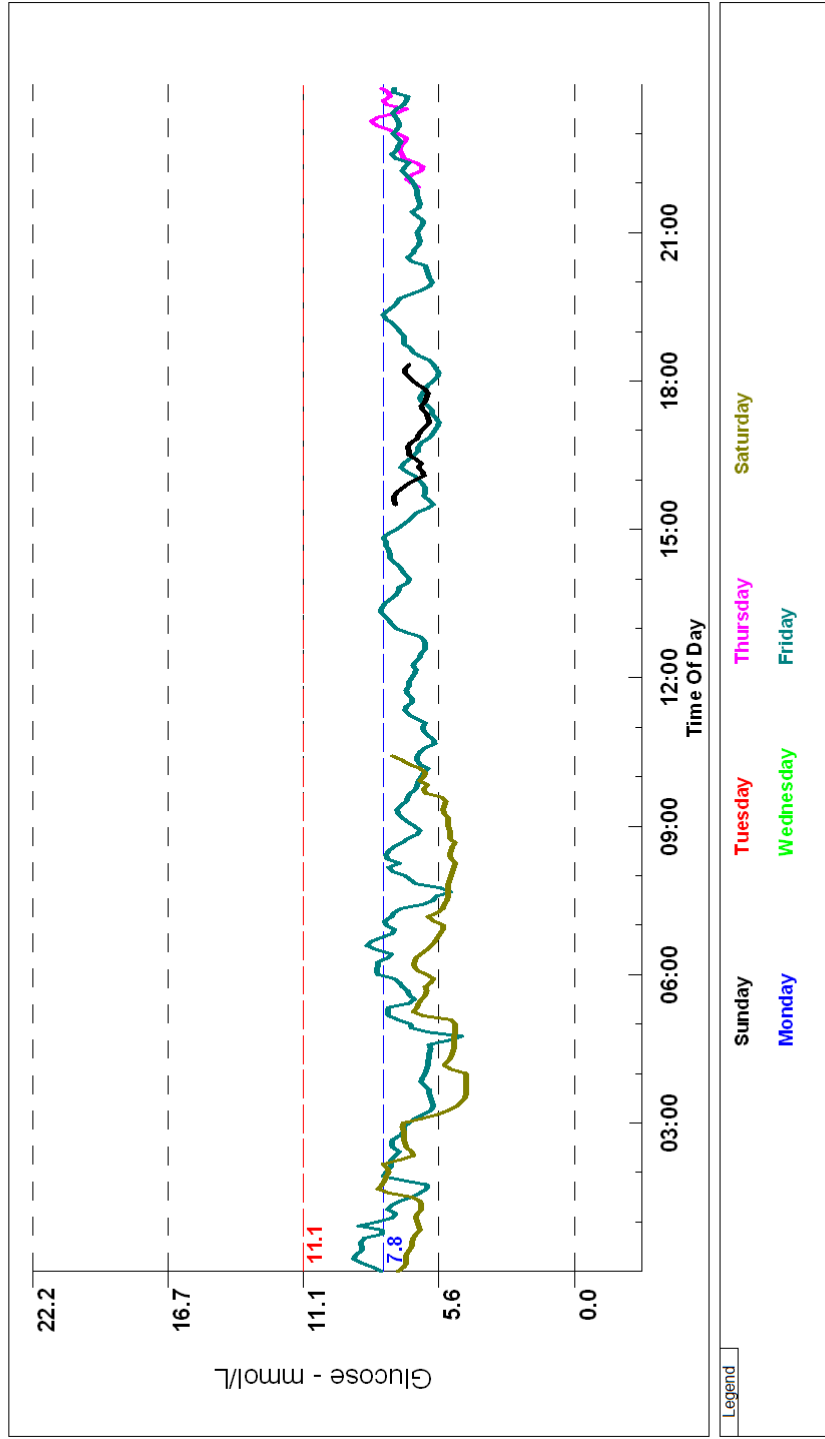


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Sensor Modal Day

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Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

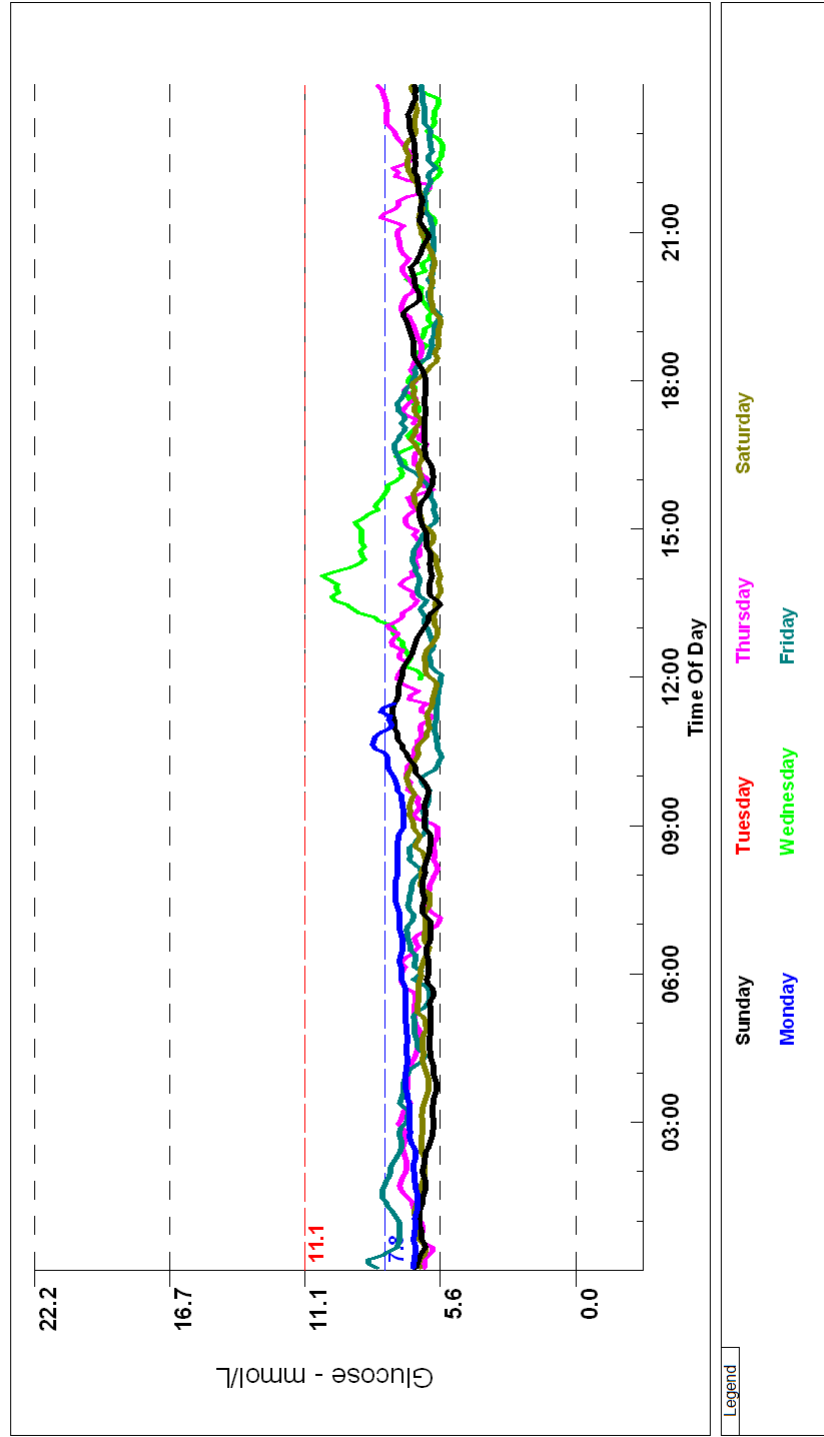




Sensor Modal Day

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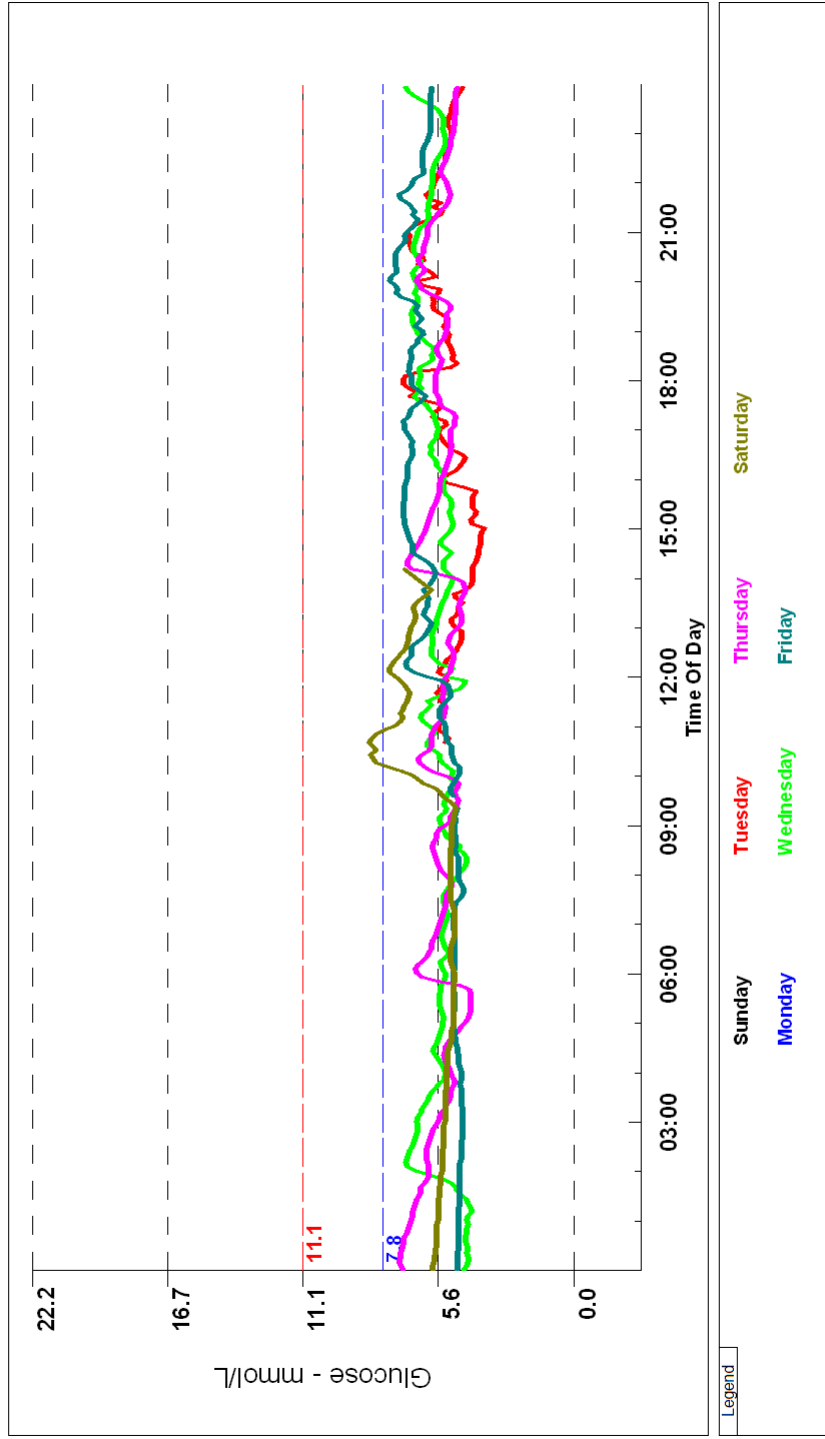
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CGMS iPro 2.2A



Sensor Modal Day

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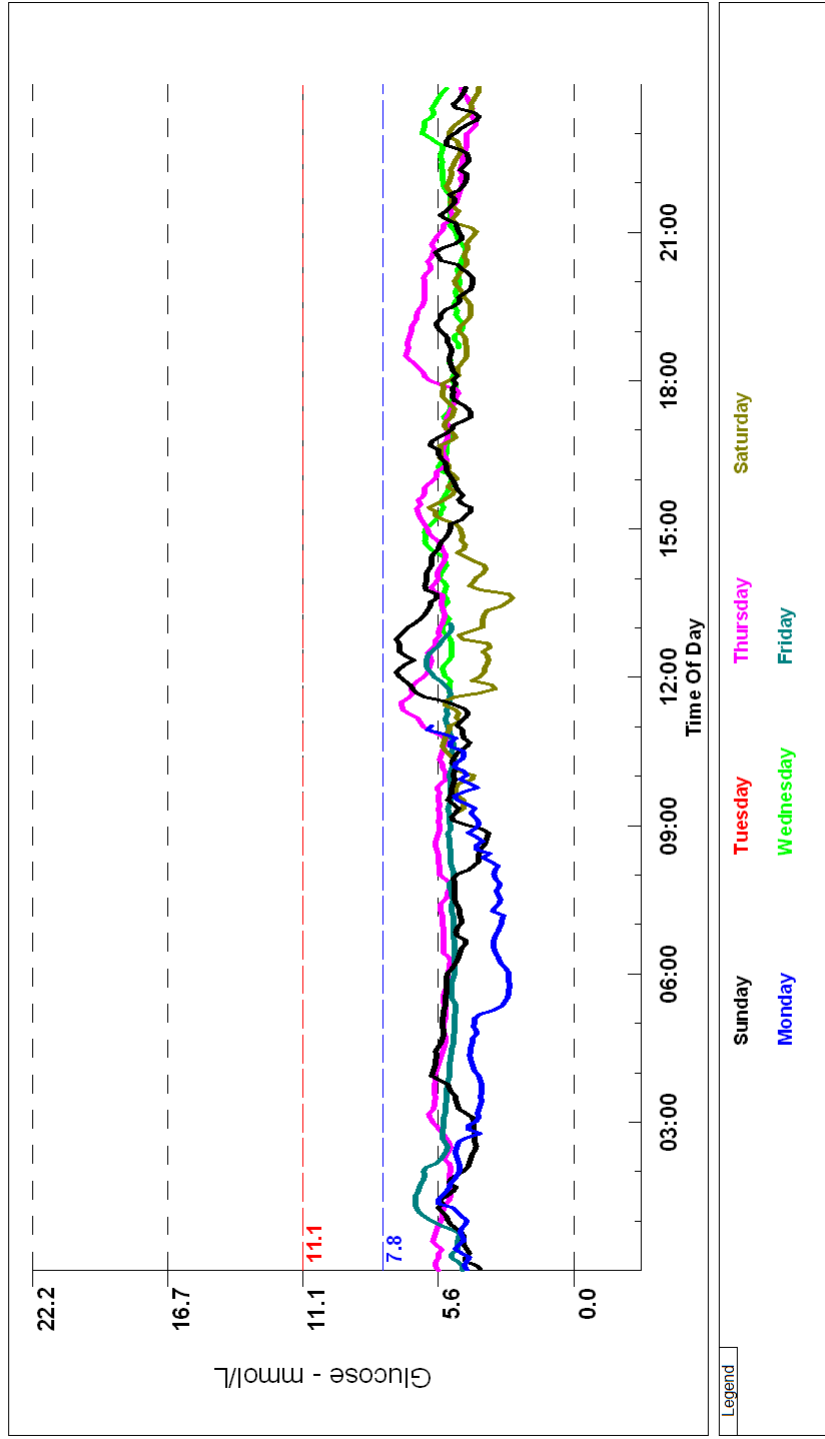
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-029a

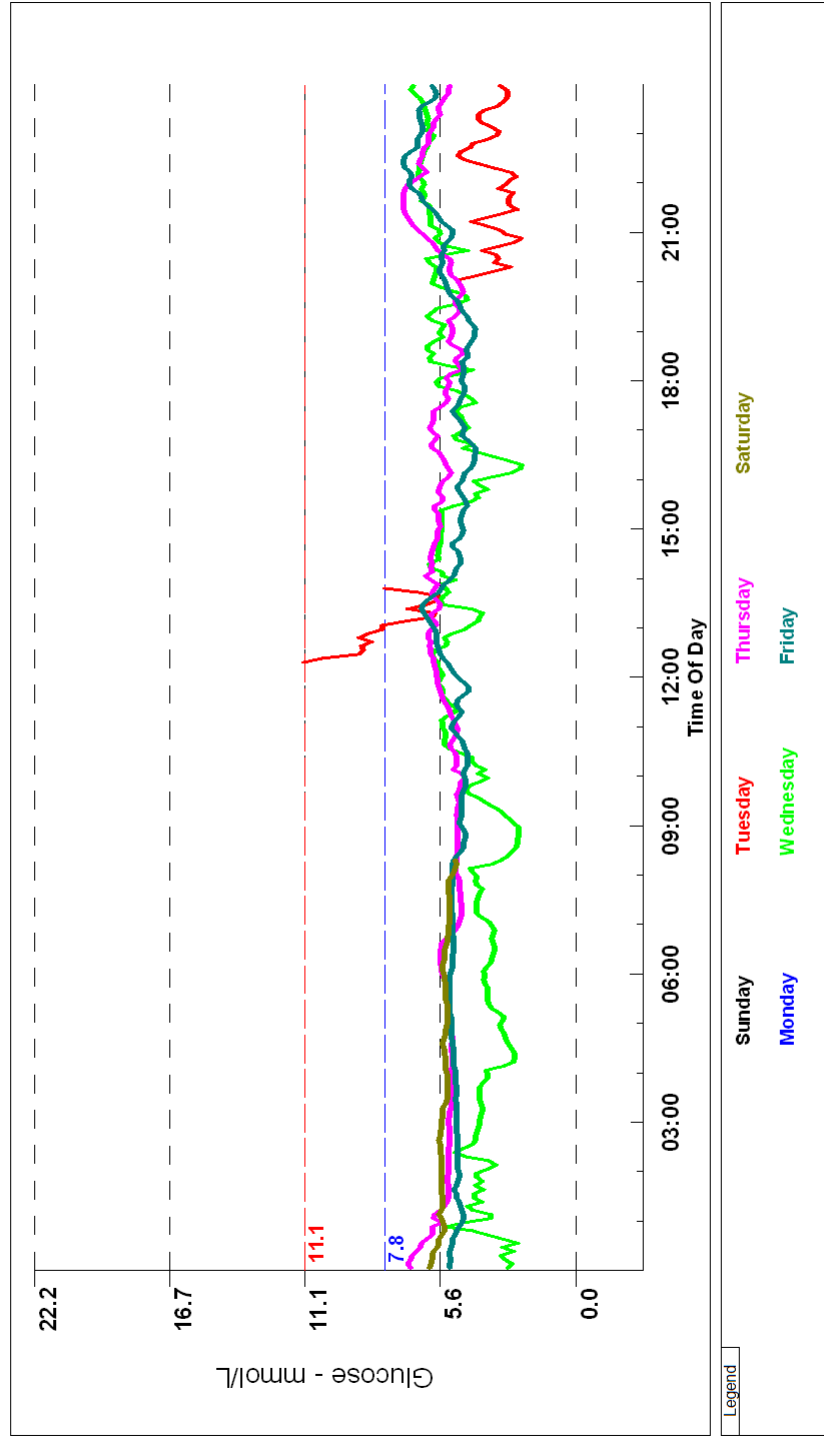
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-029b

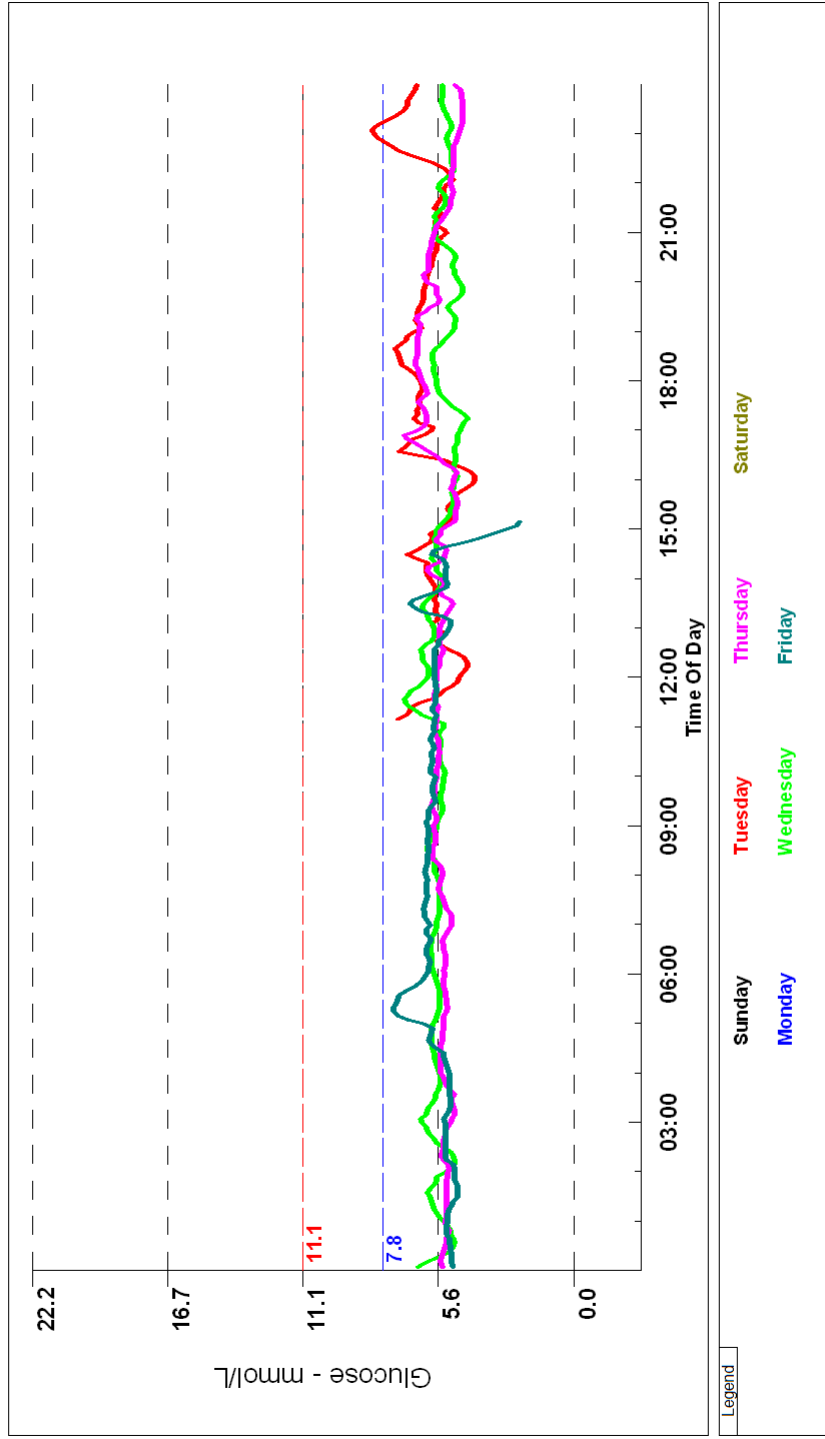
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-030b

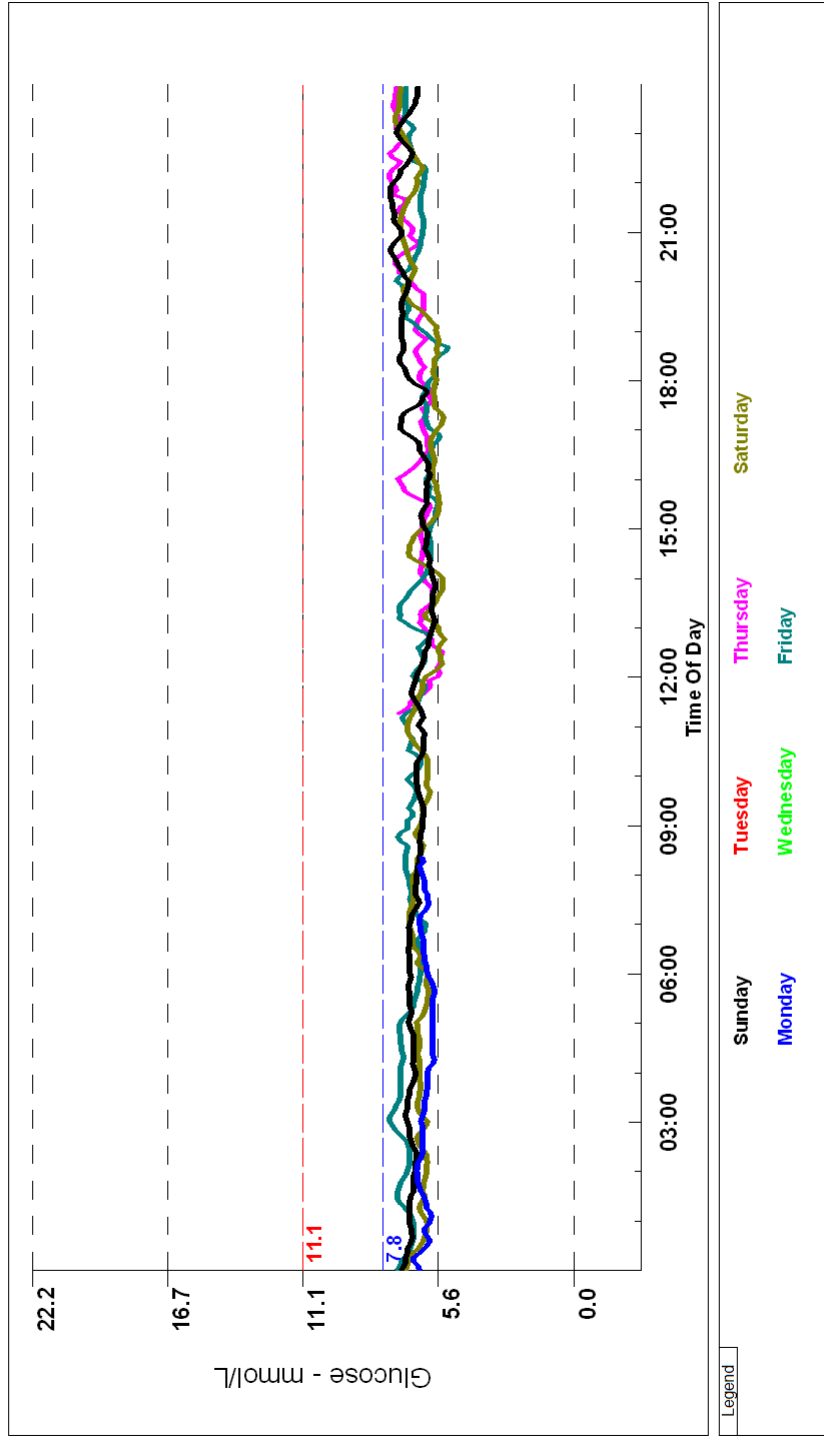
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-043b

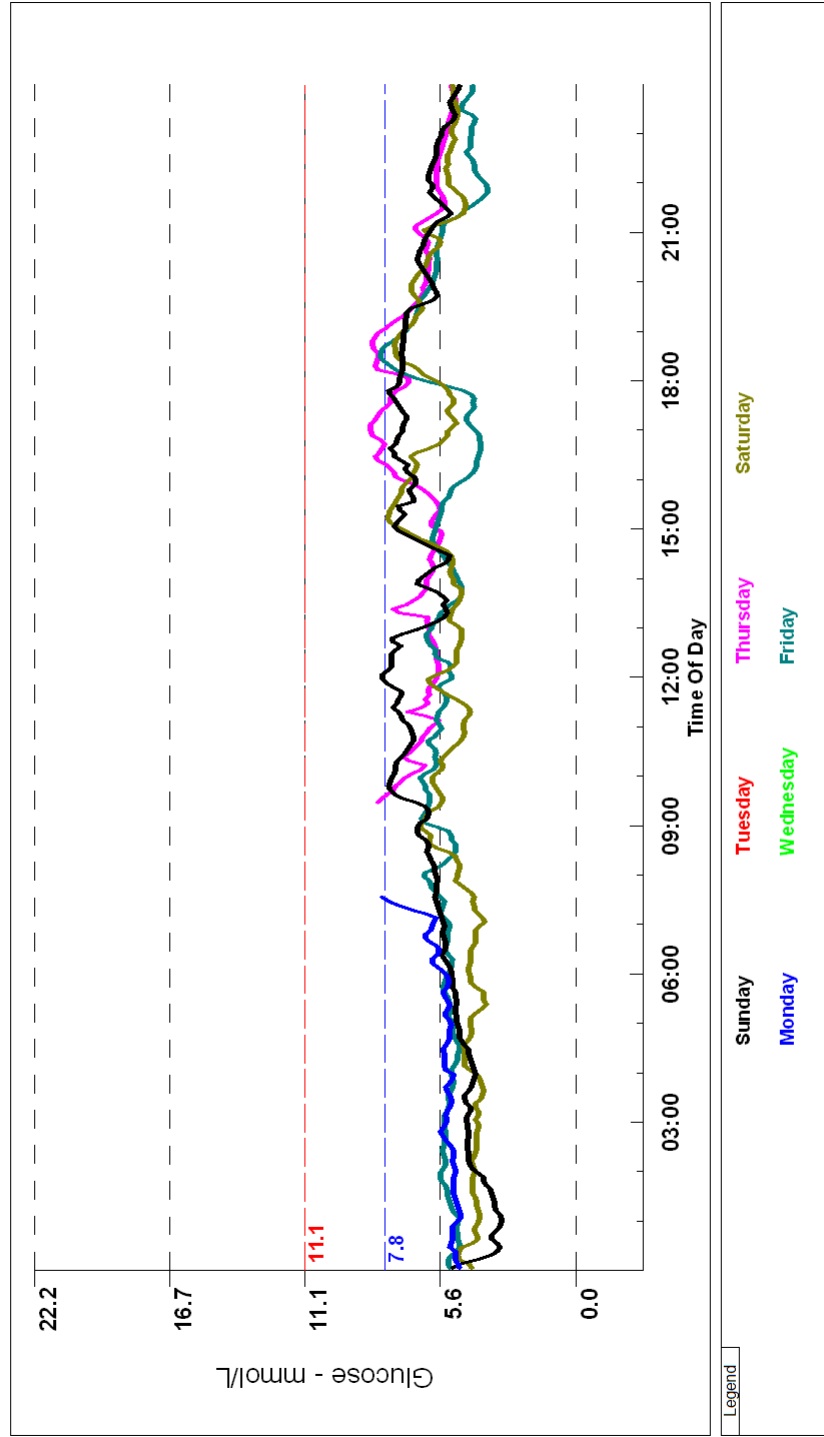
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-047b

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



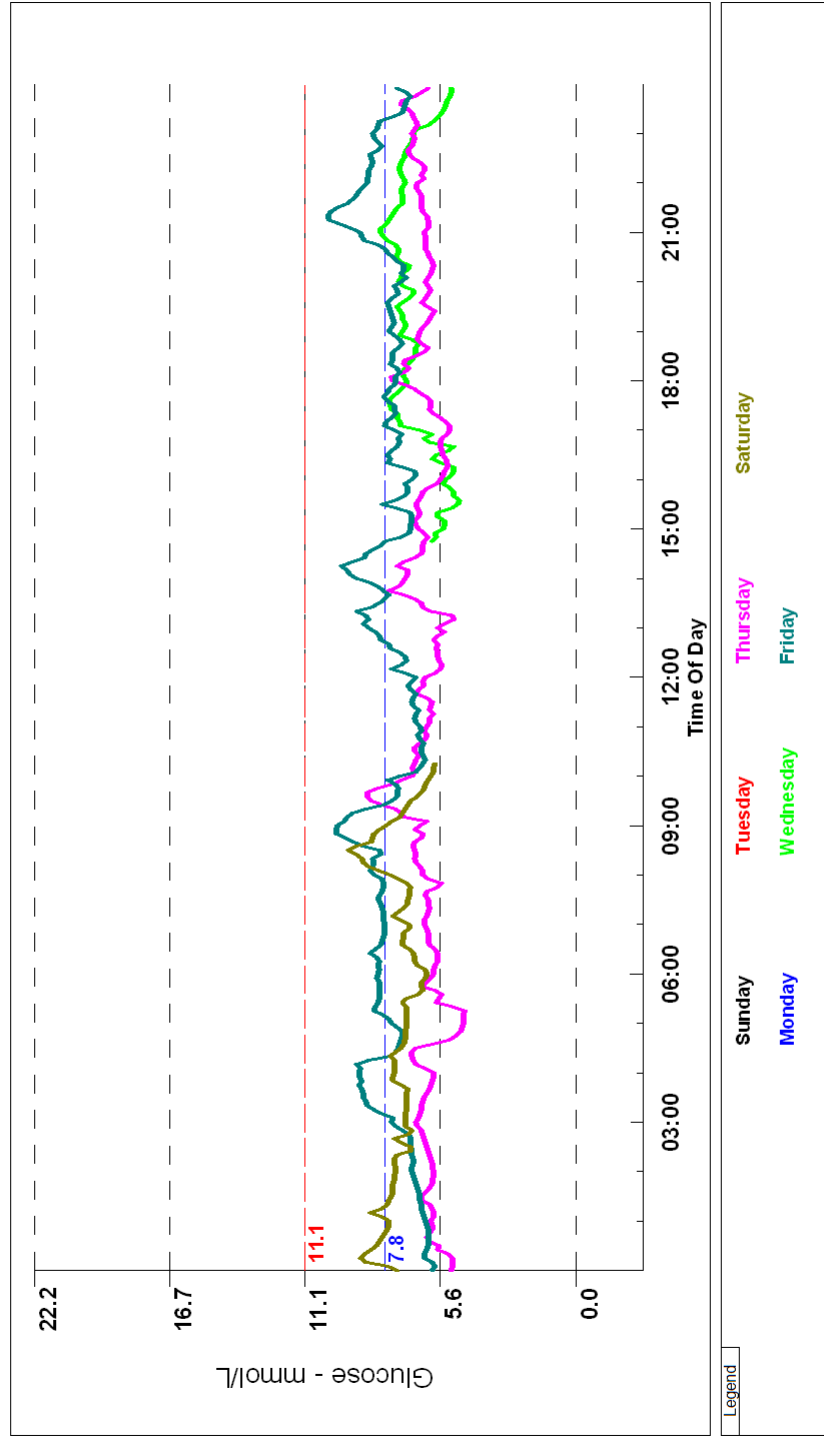
## **APPENDIX 3 – CGM PROFILES: MEDIUM VARIABILITY**



Sensor Modal Day

Patient:  
ID: ipro-002B

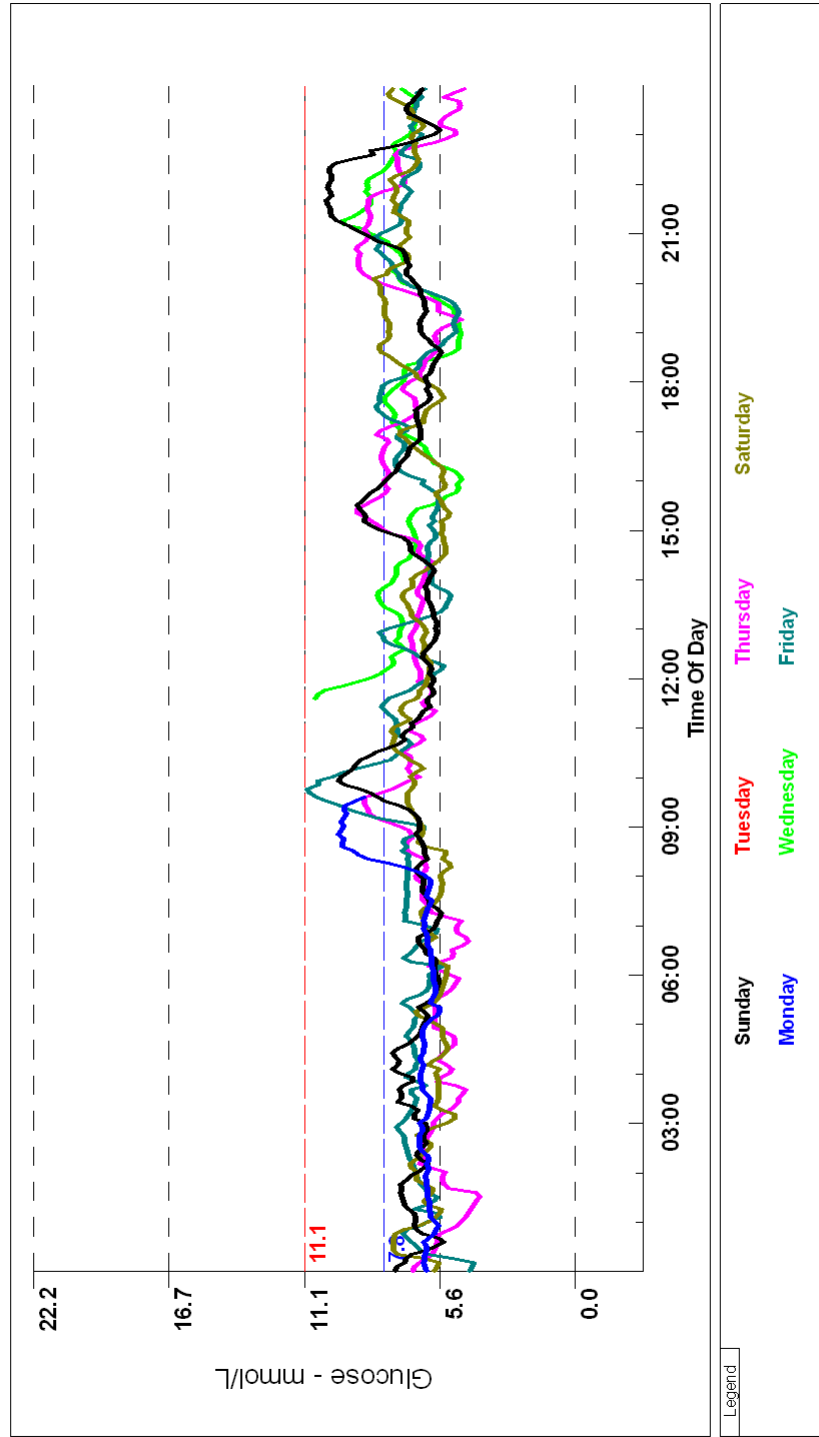
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-005b

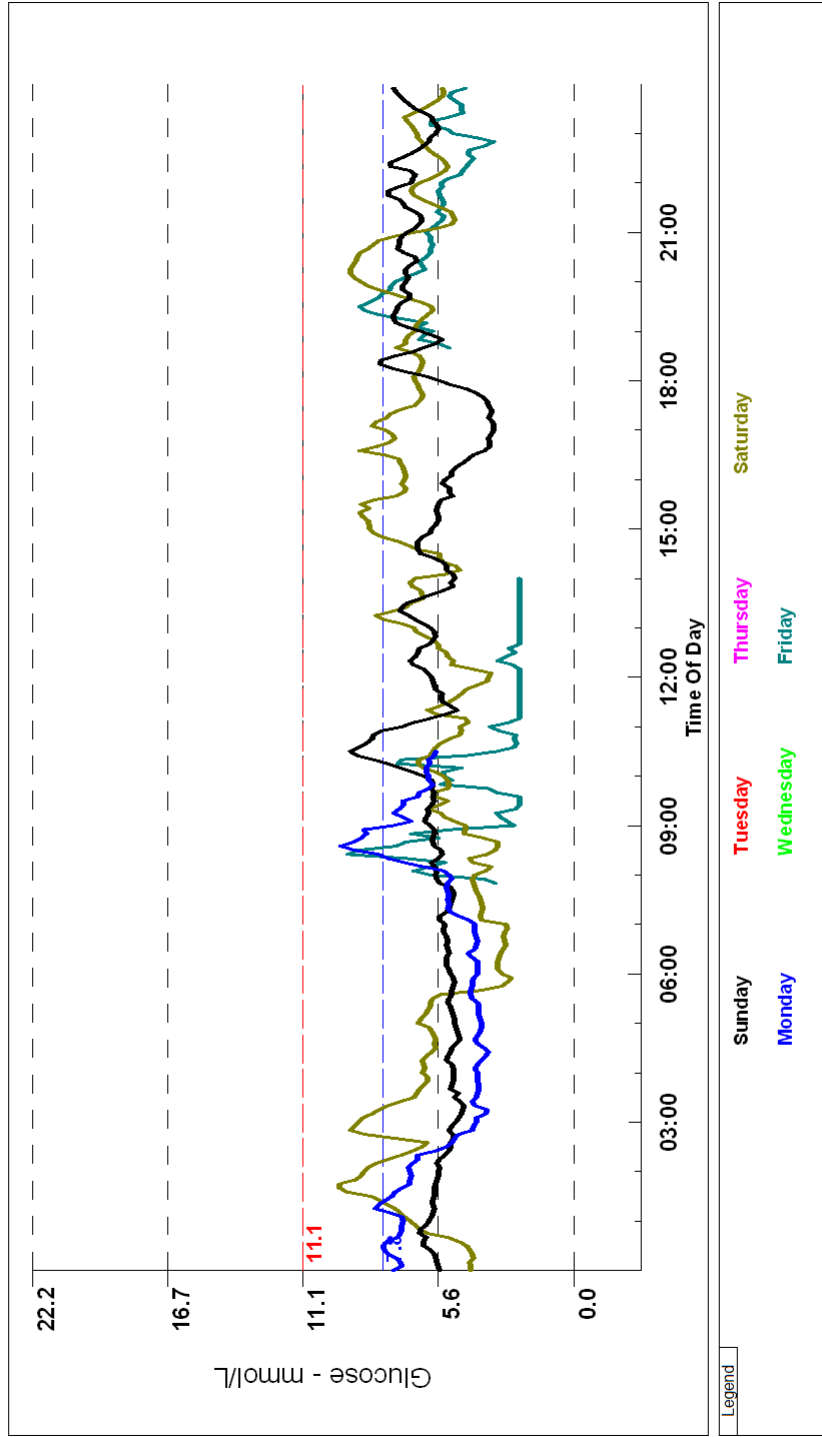
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-009aa

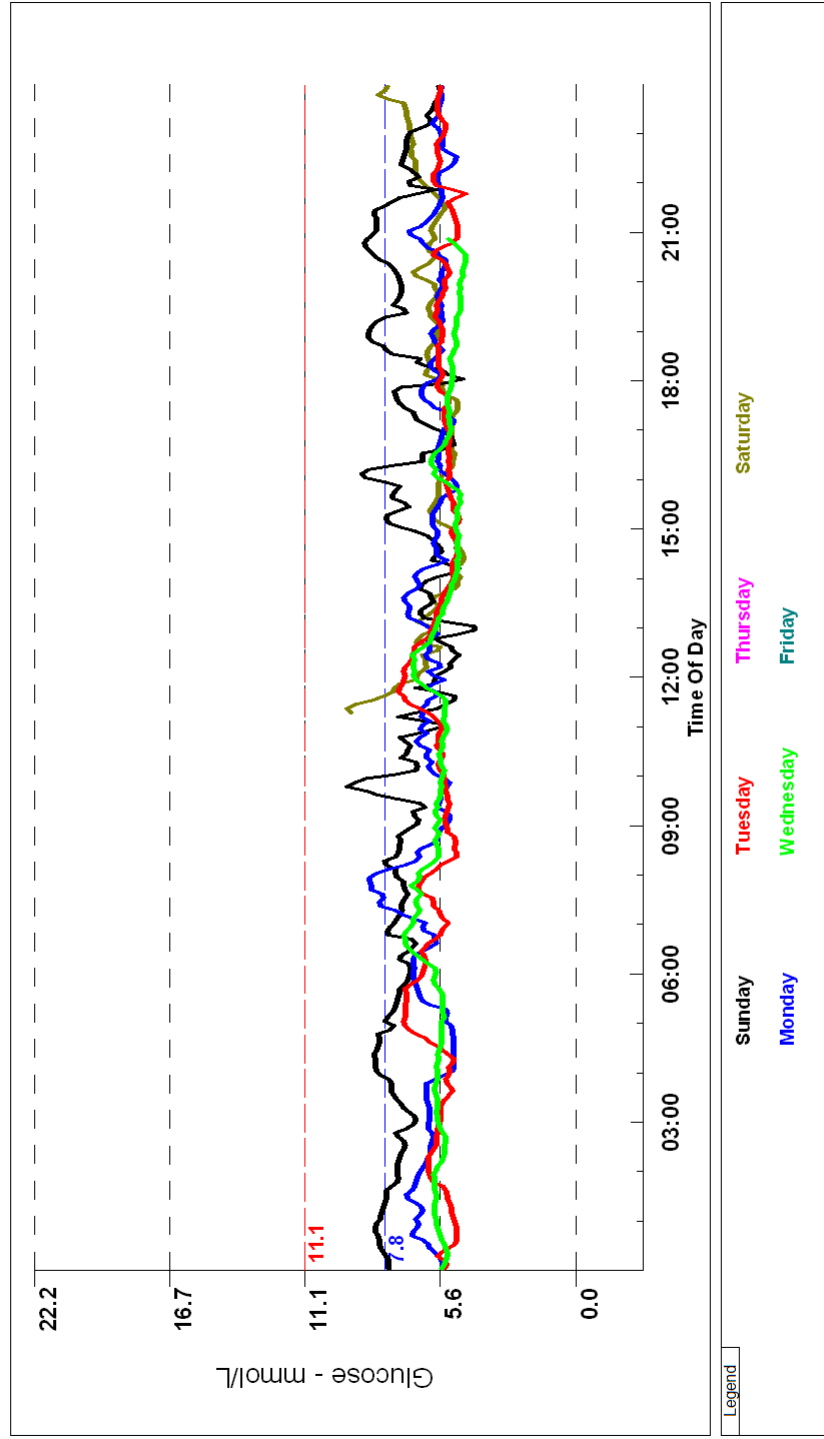
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-009b

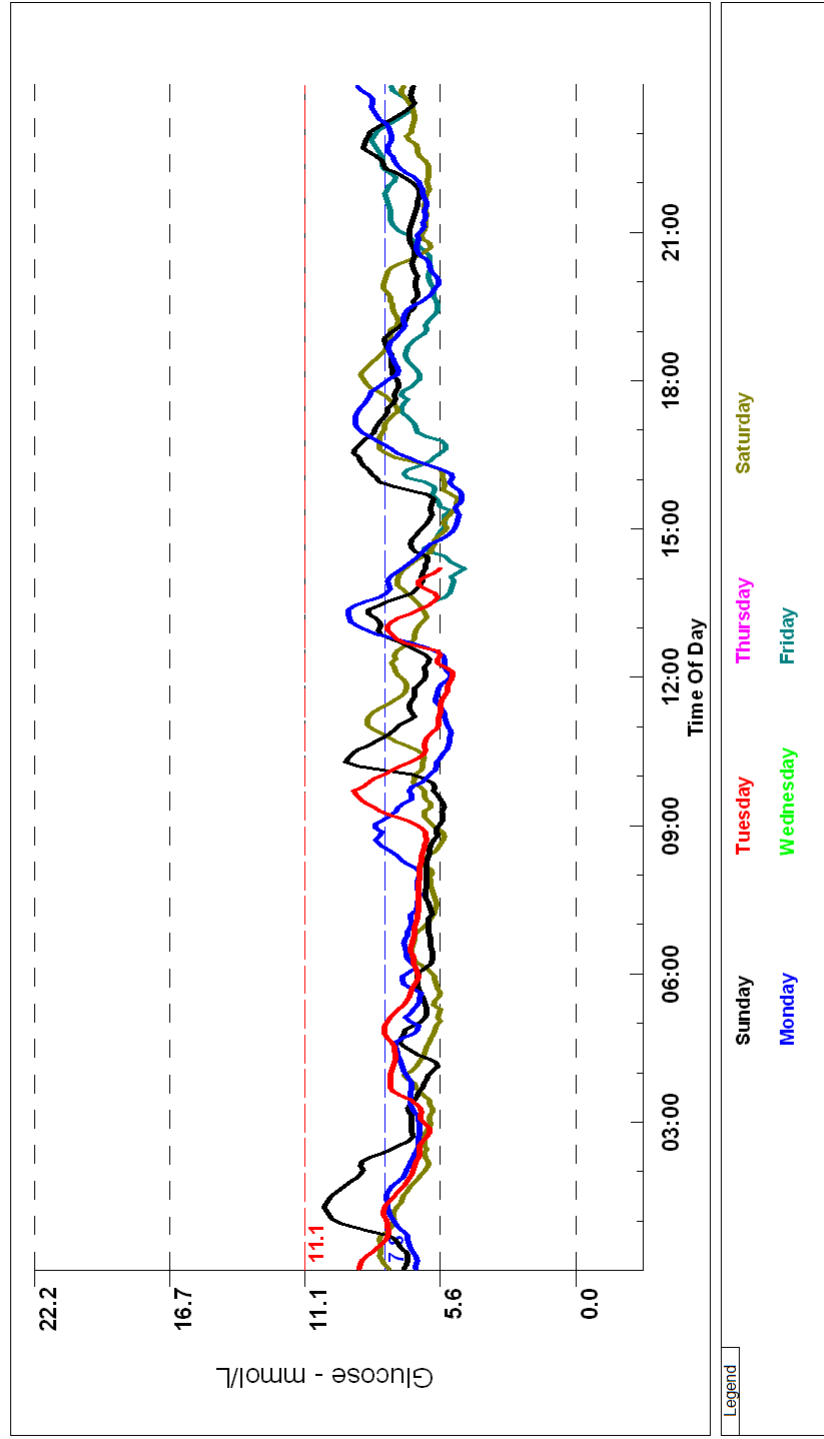
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-015a

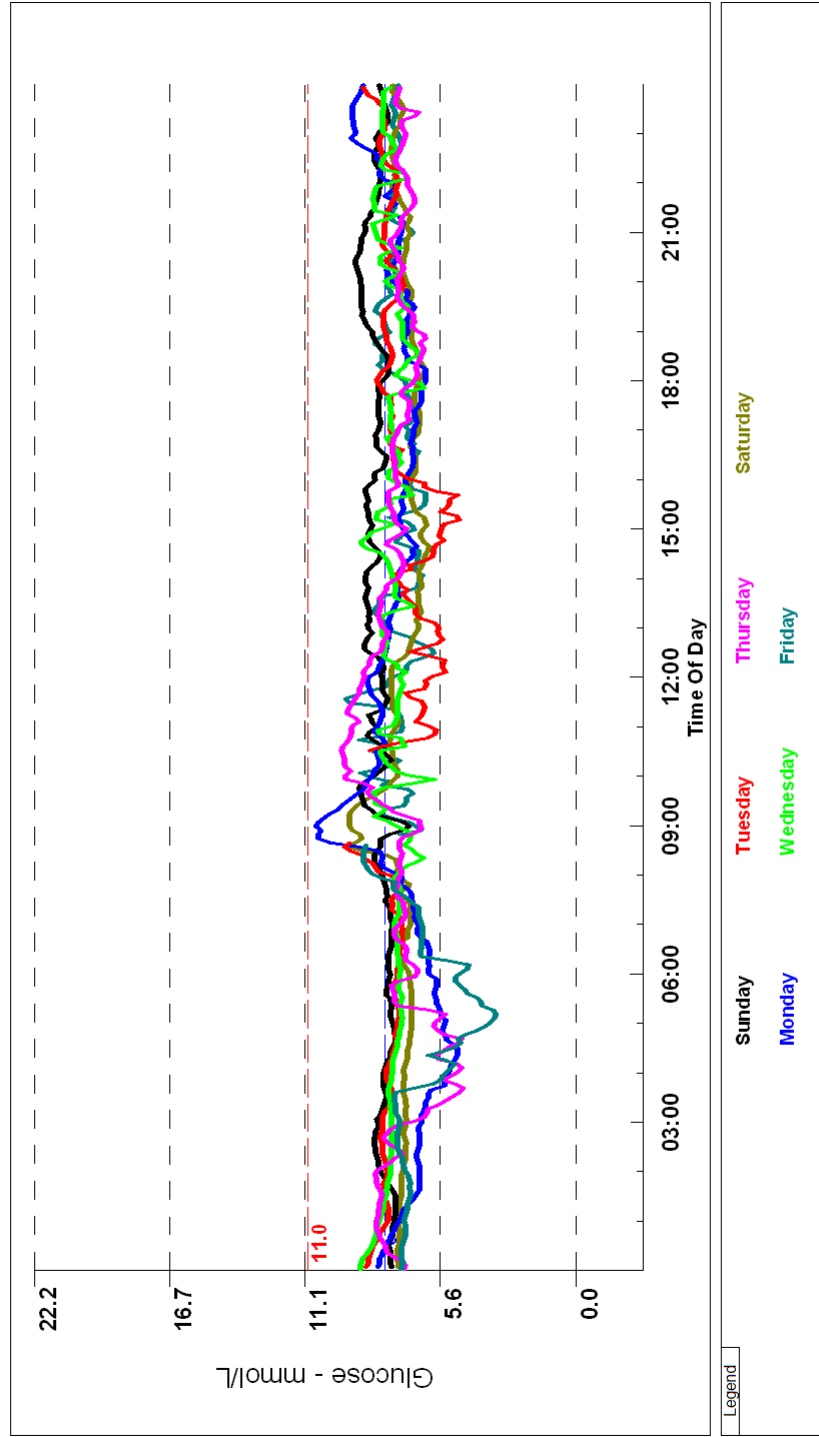
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-016a

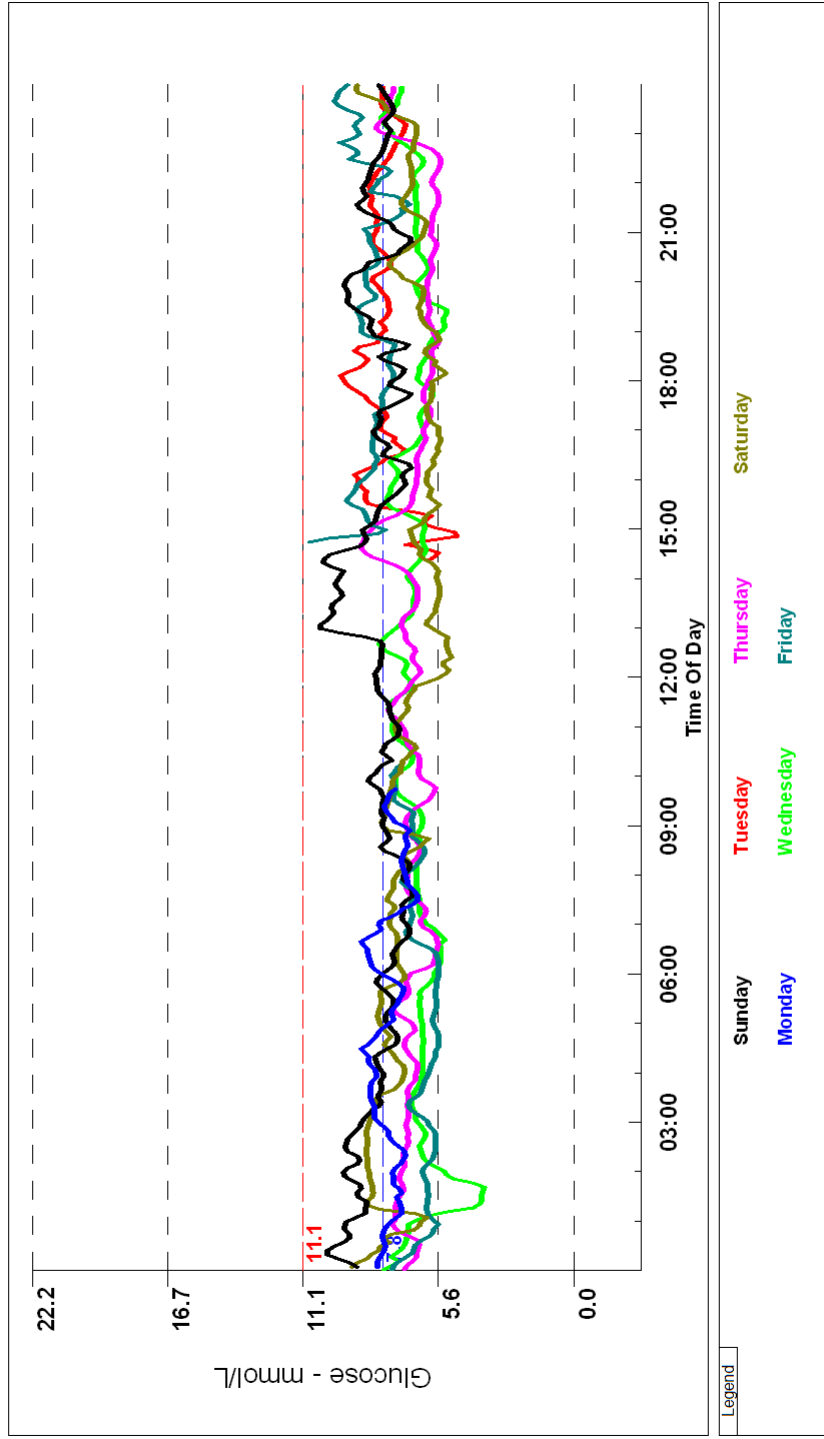
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-017a

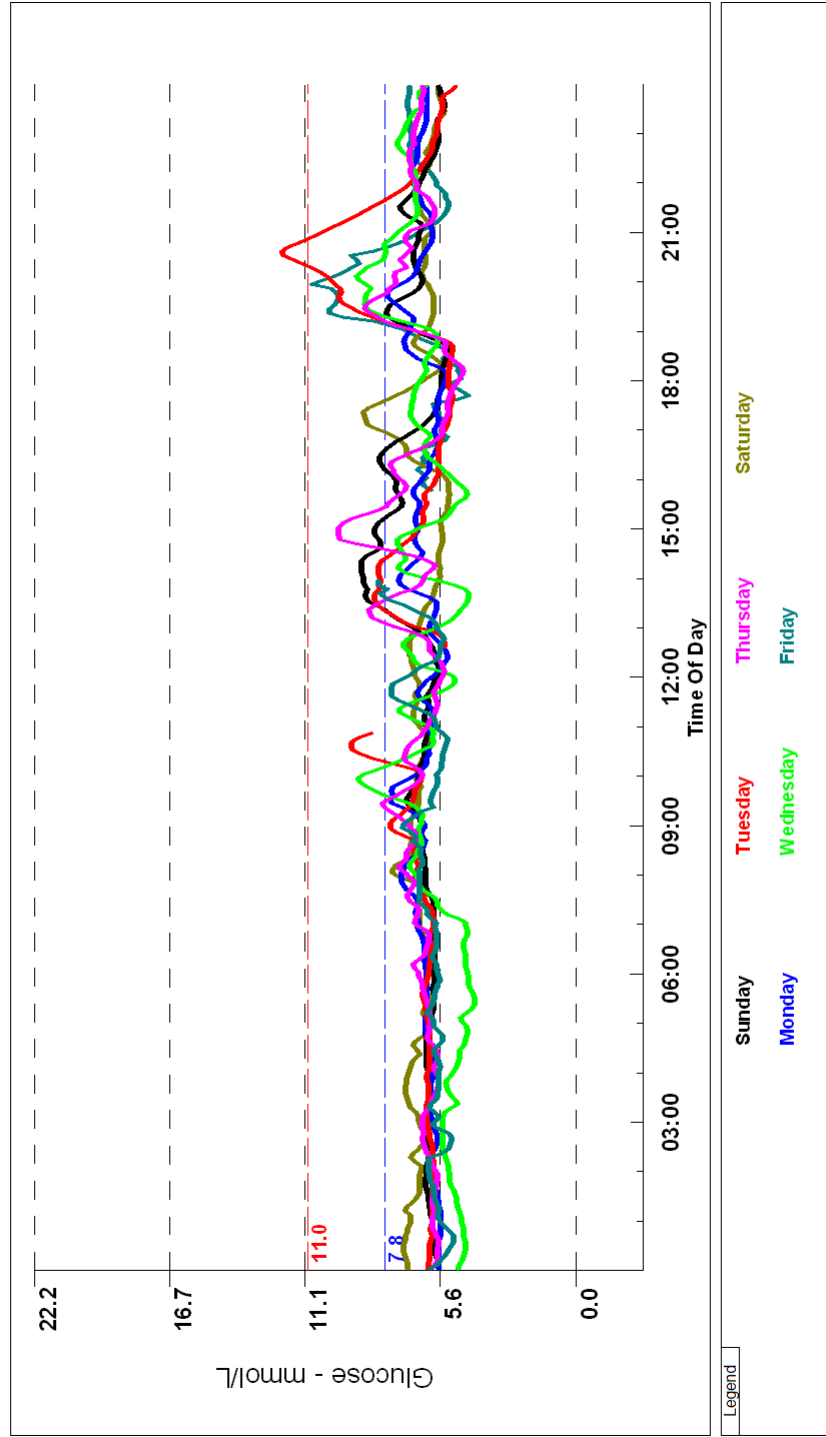
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-018a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

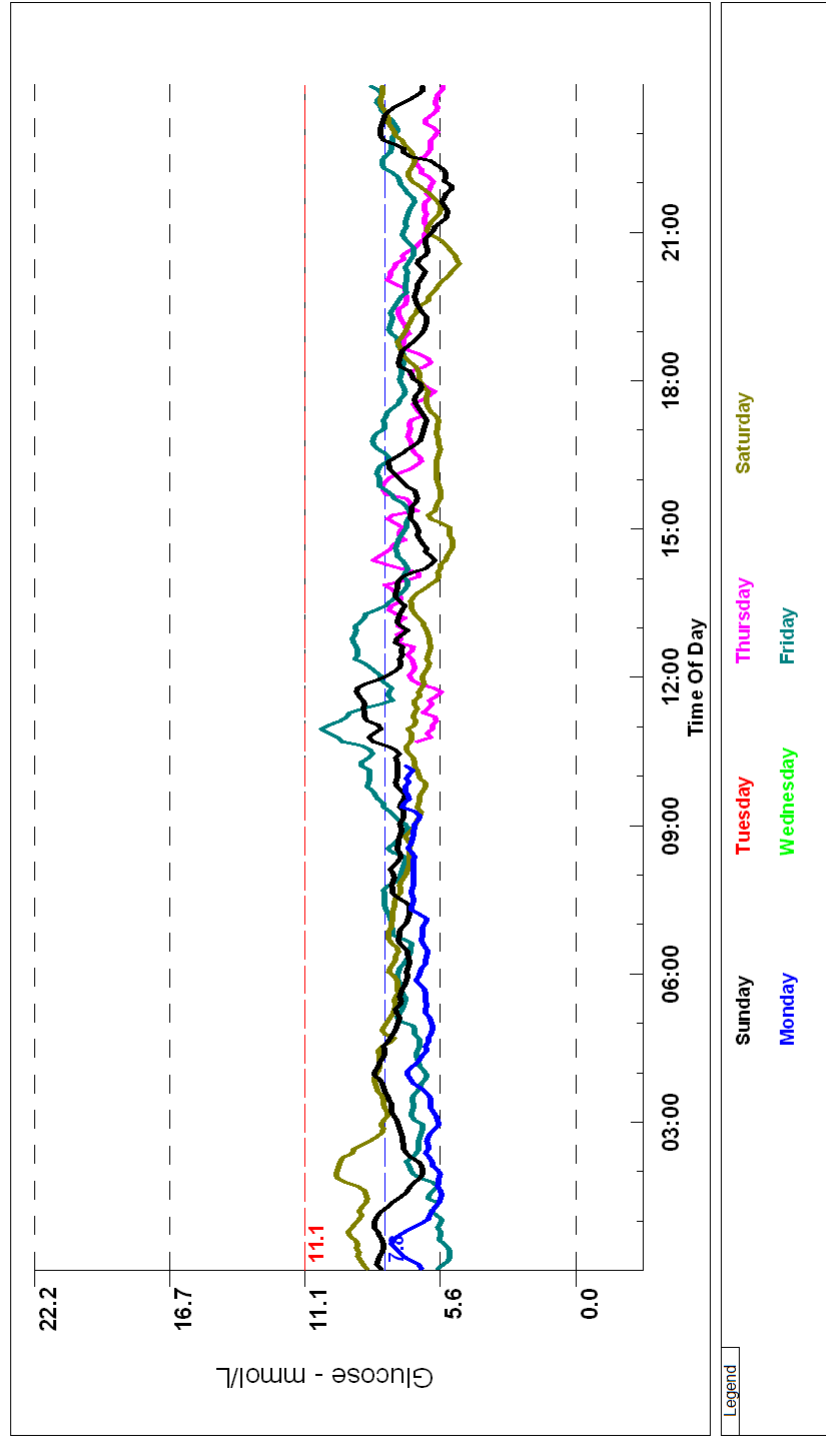




Sensor Modal Day

Patient:  
ID: ipro-020b

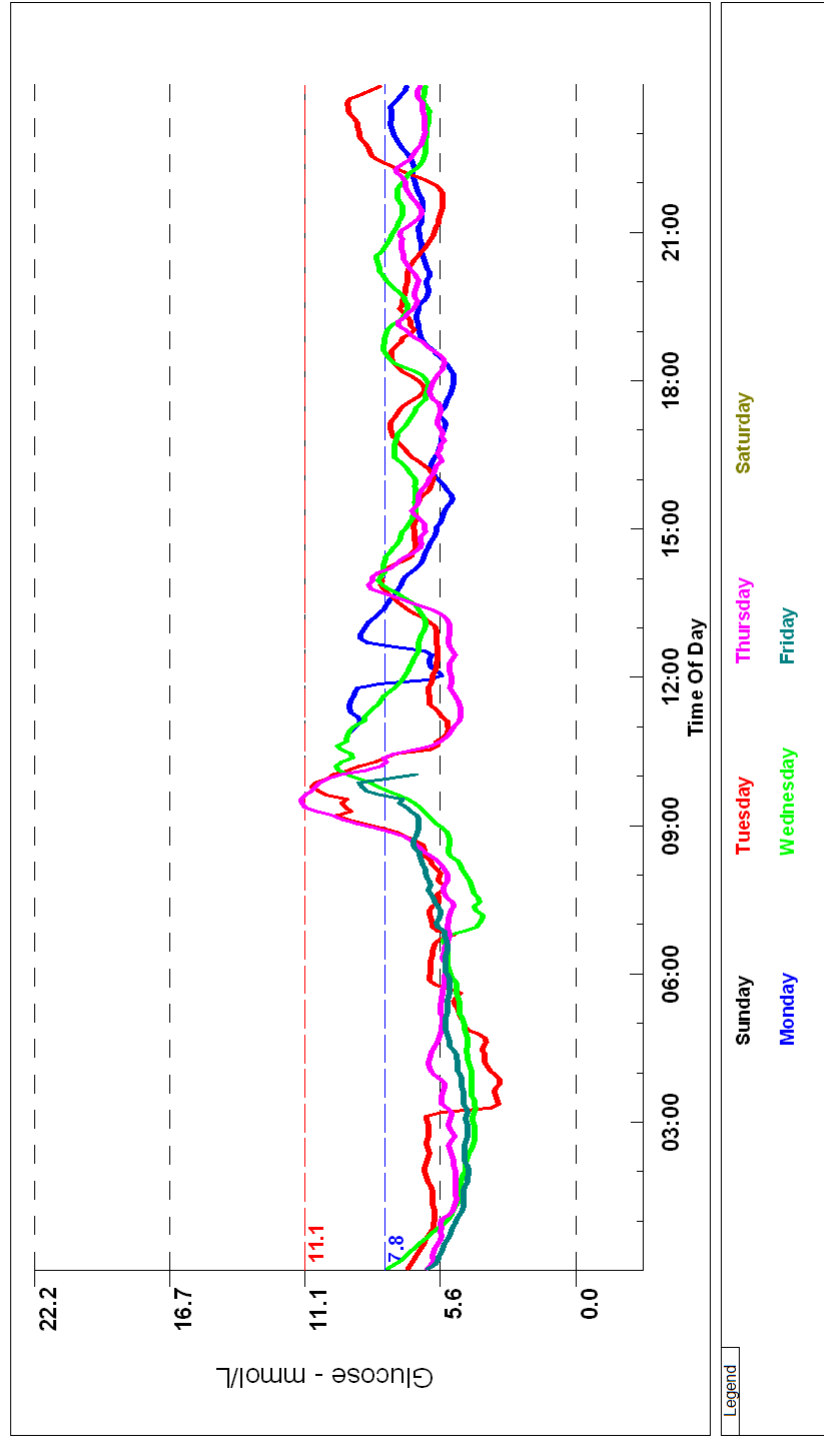
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-021b

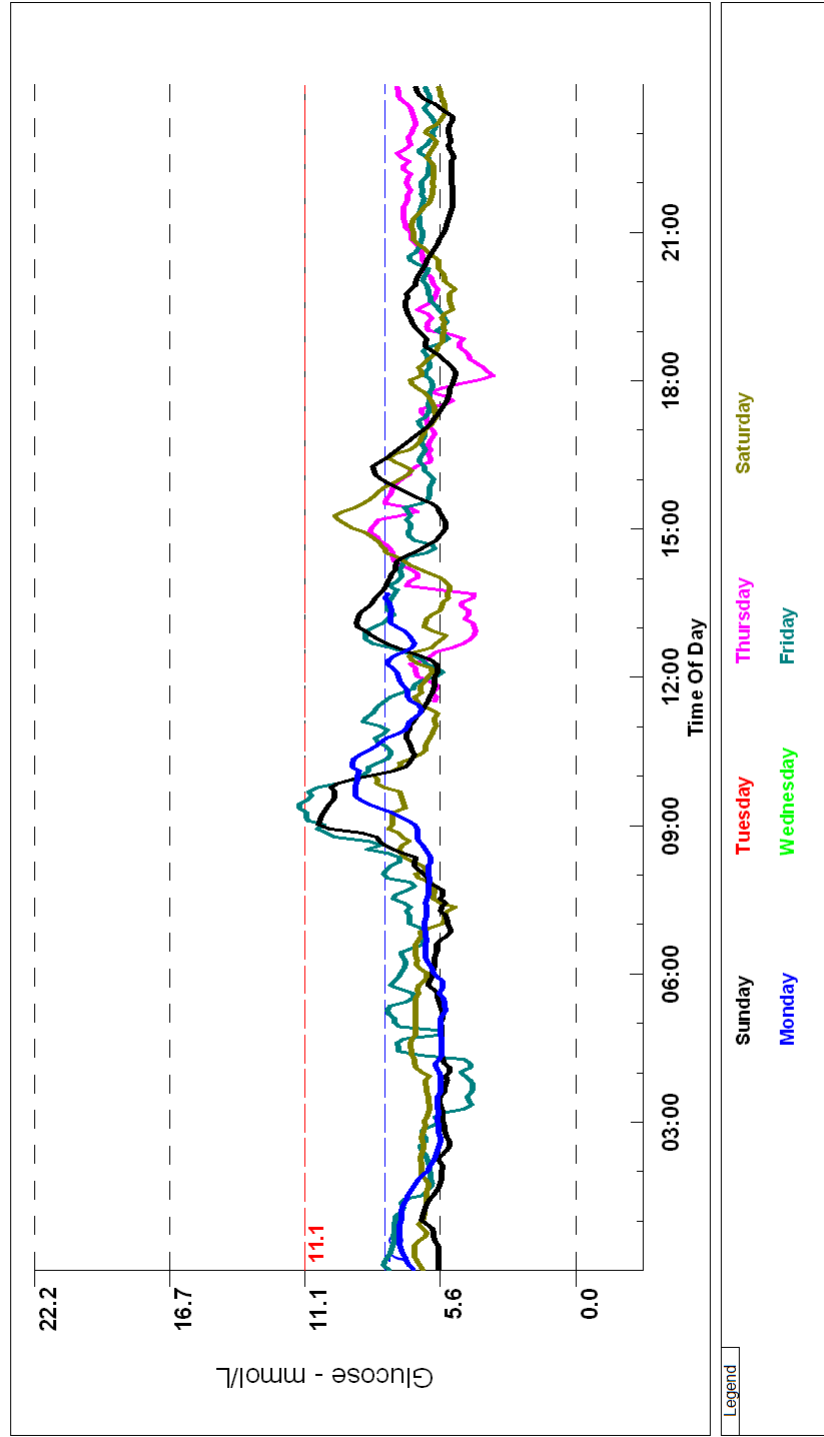
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-022b

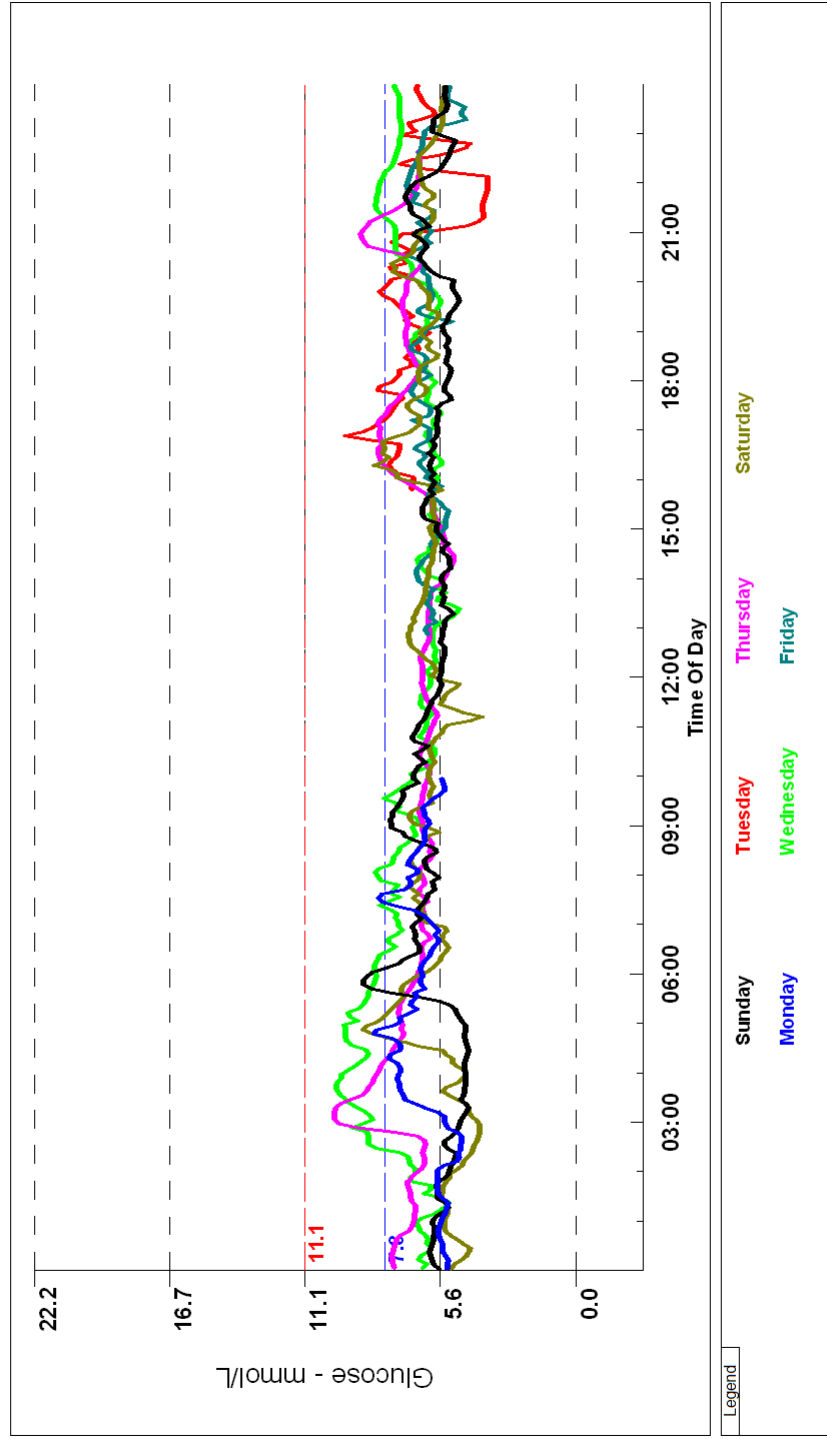
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-023a

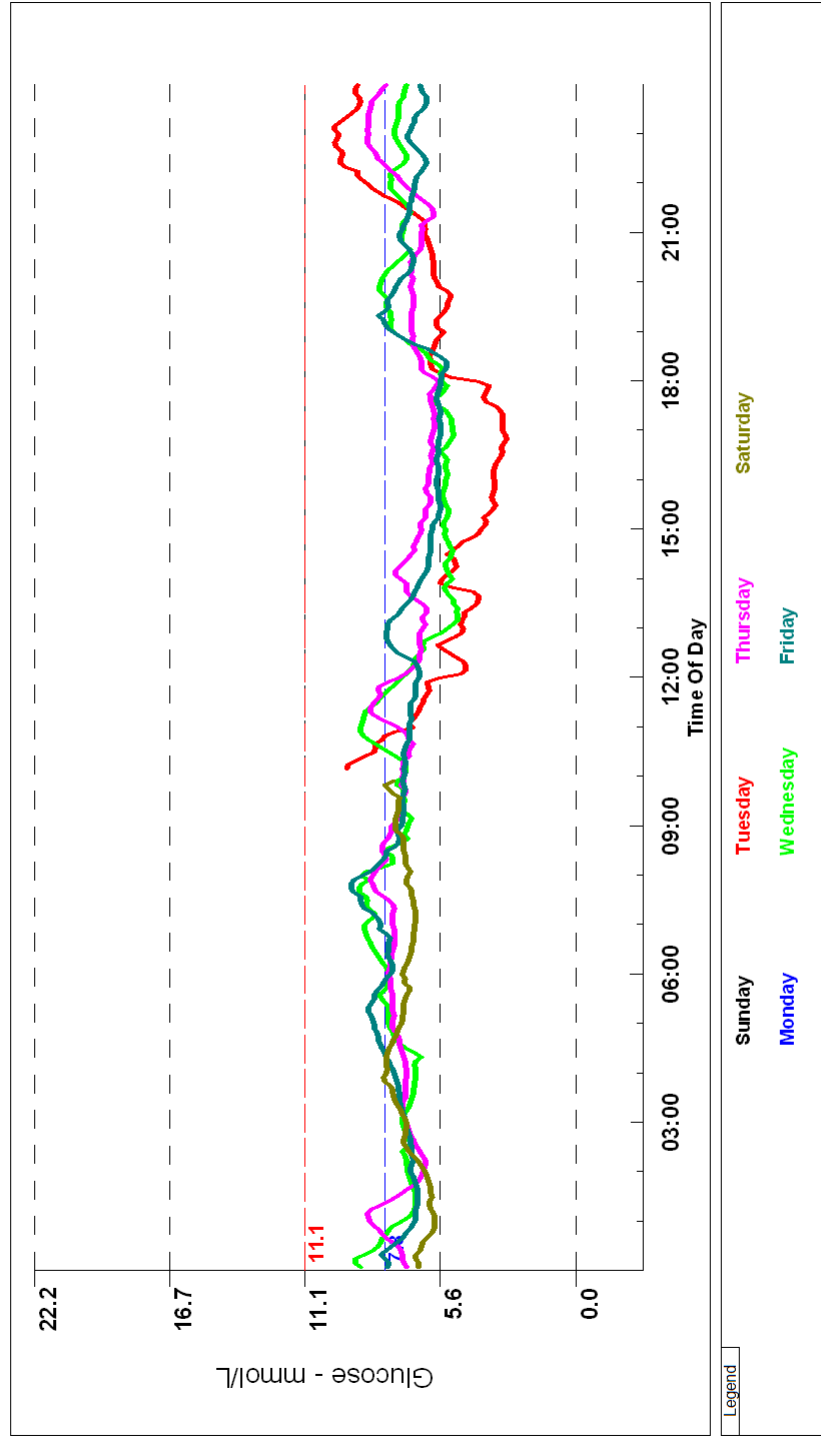
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-024b

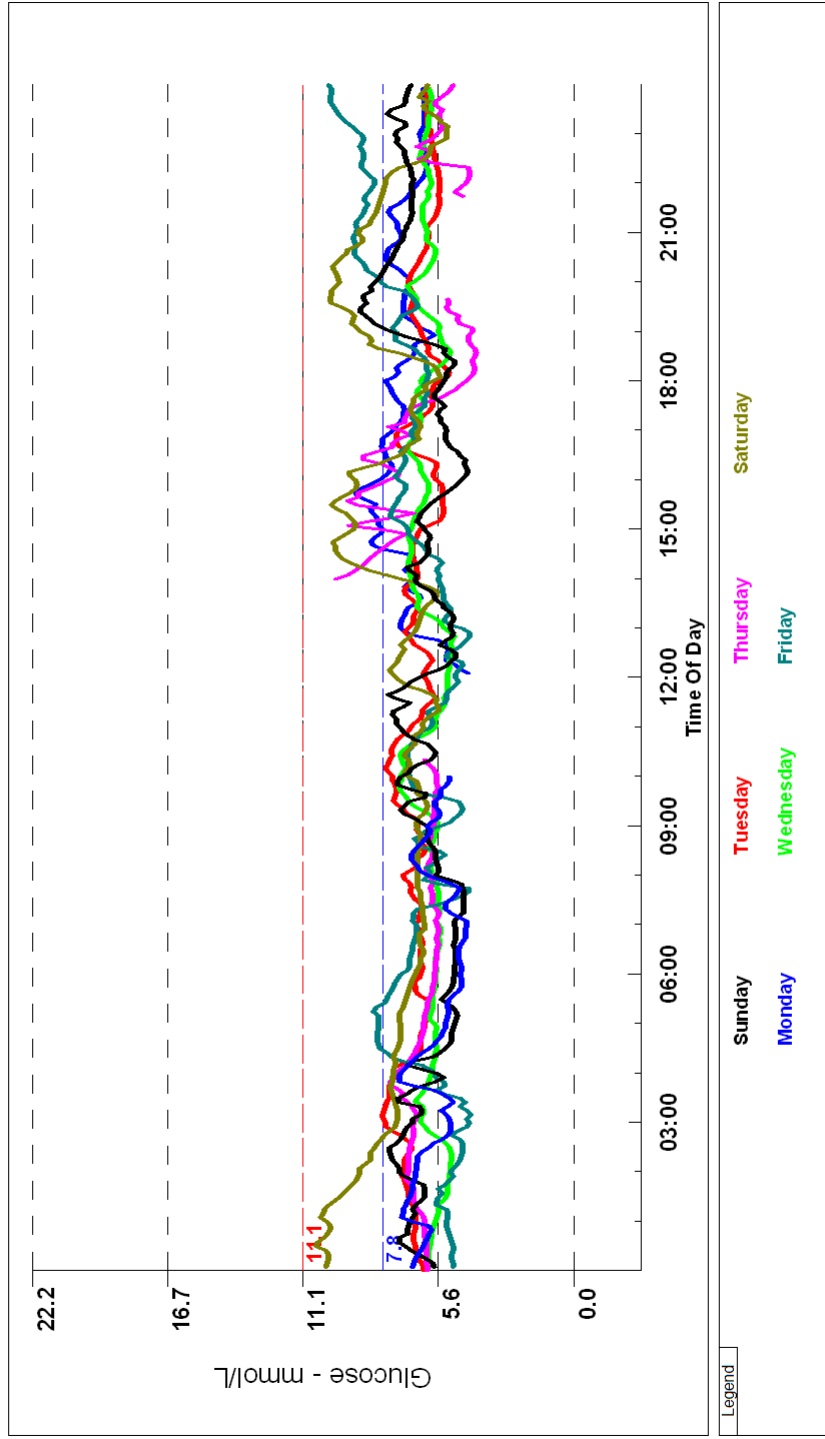
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-025a

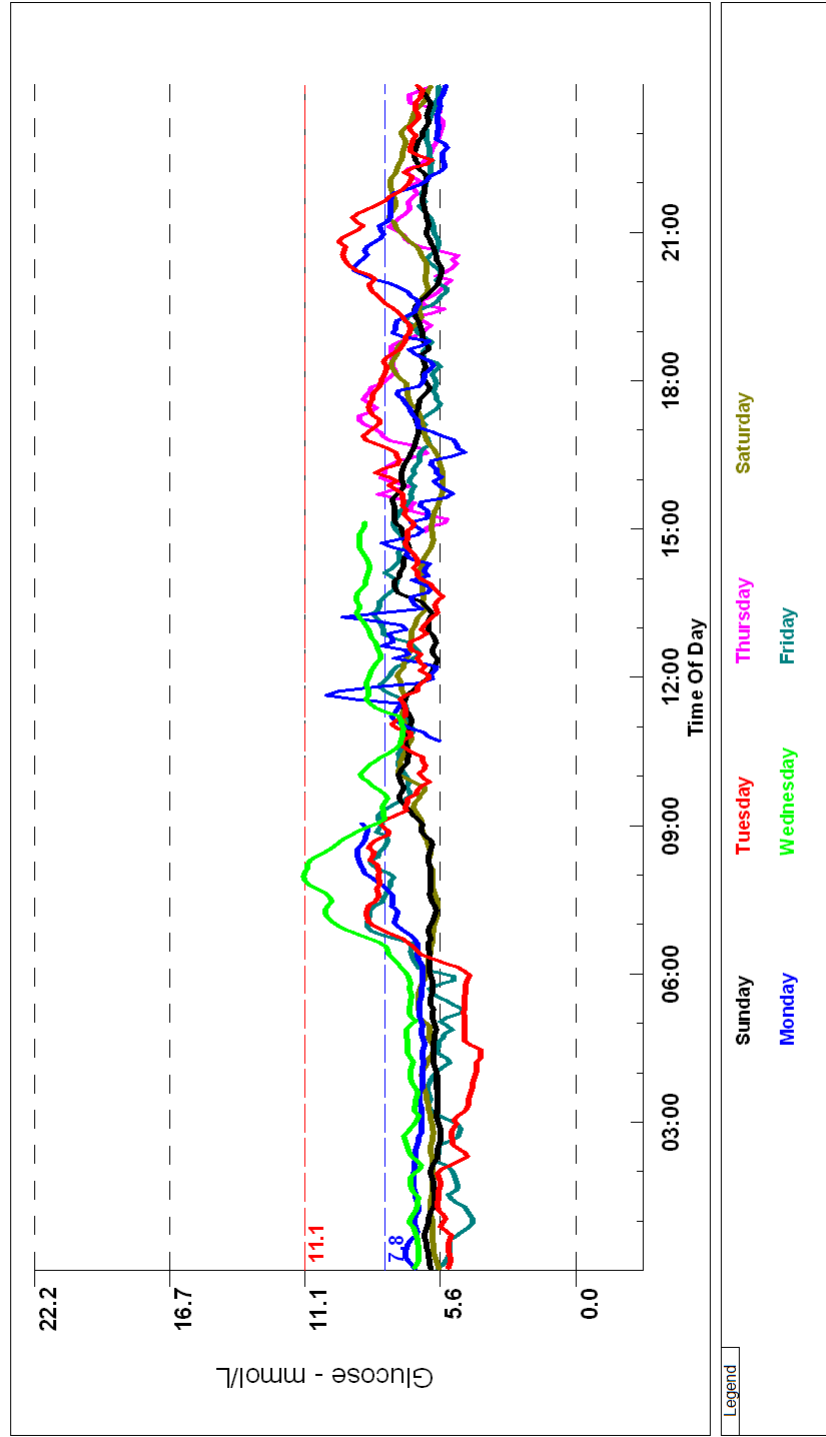
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-026a

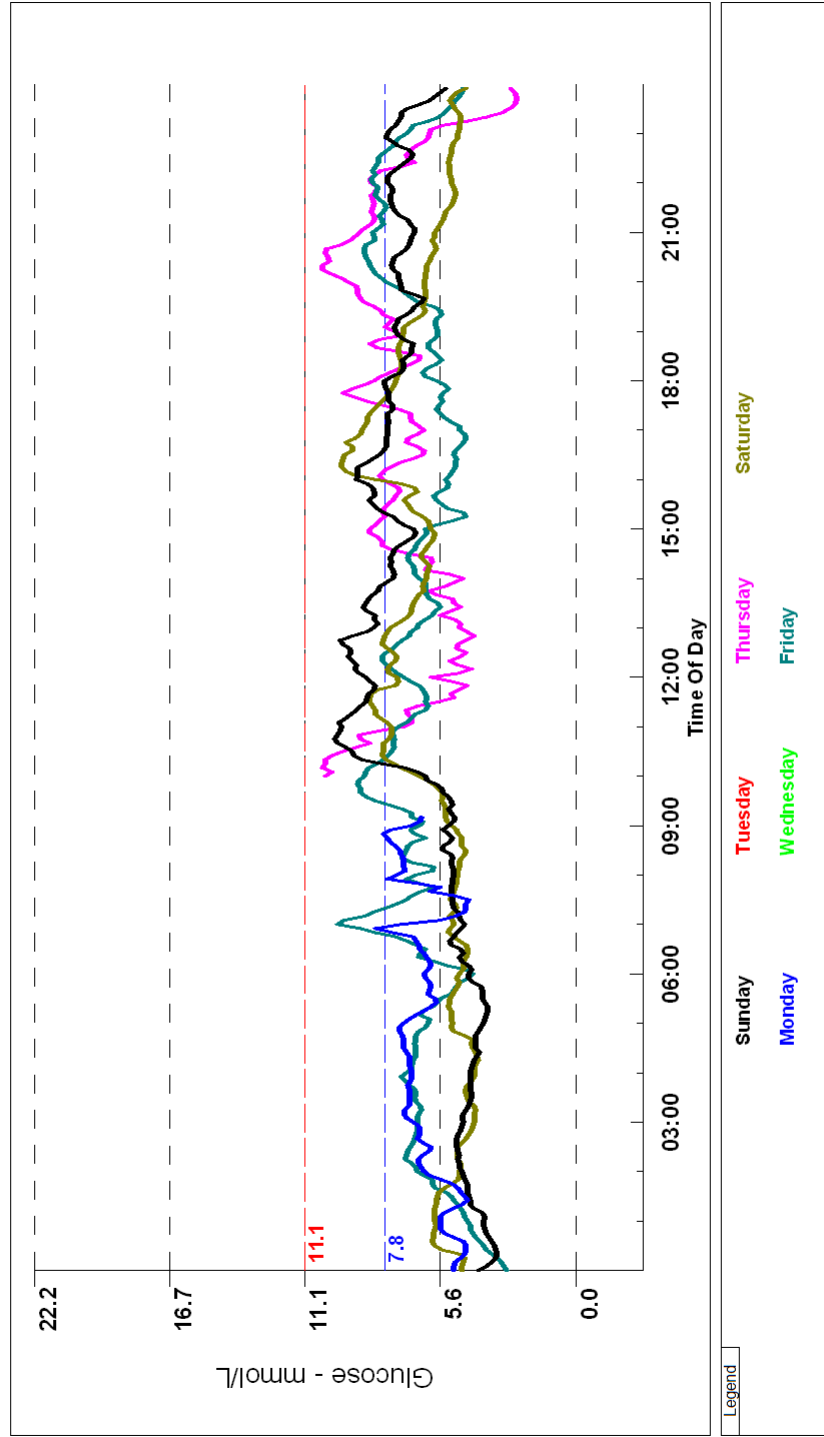
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-026b

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

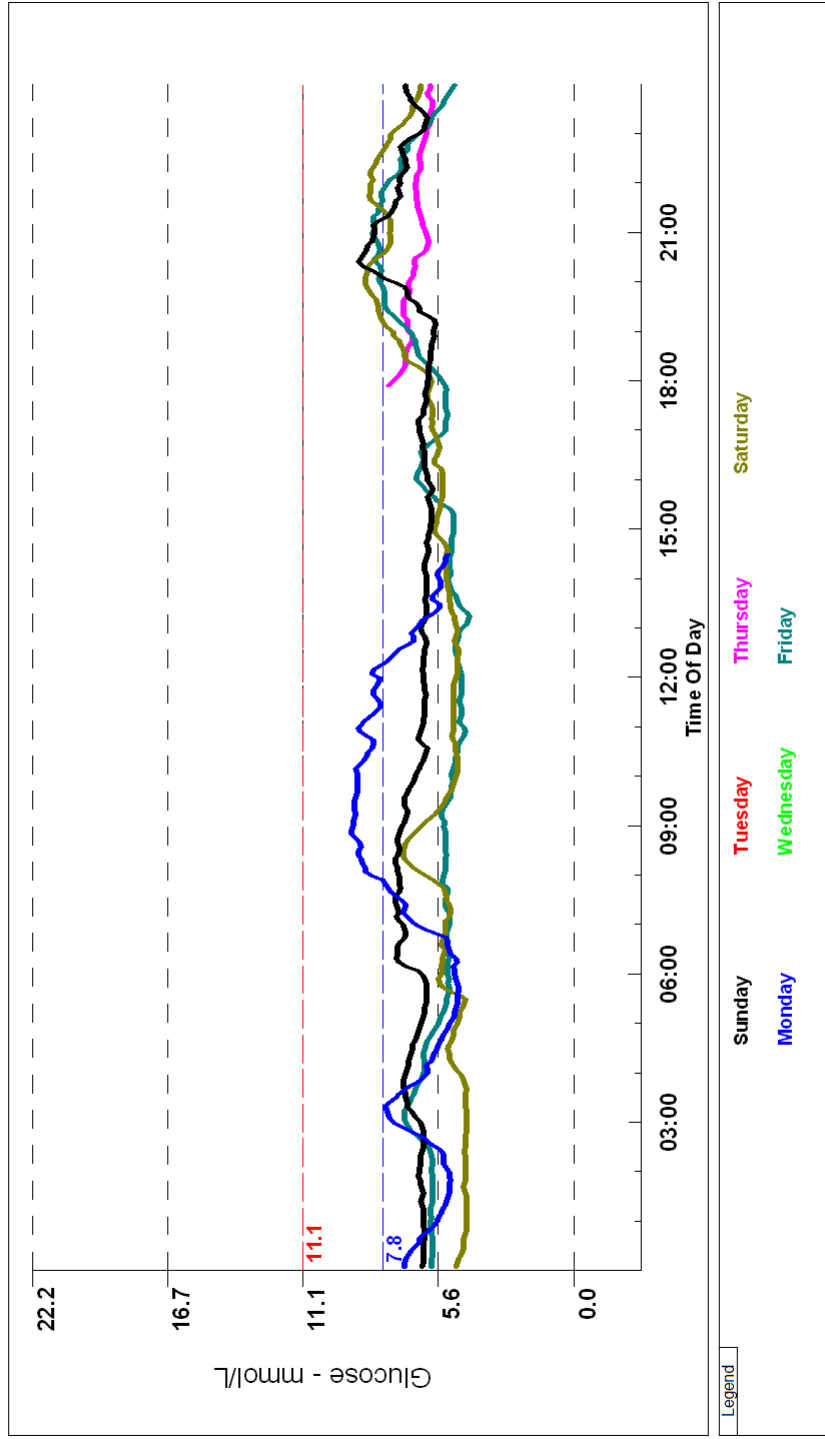




Sensor Modal Day

Patient:  
ID: ipro-027a

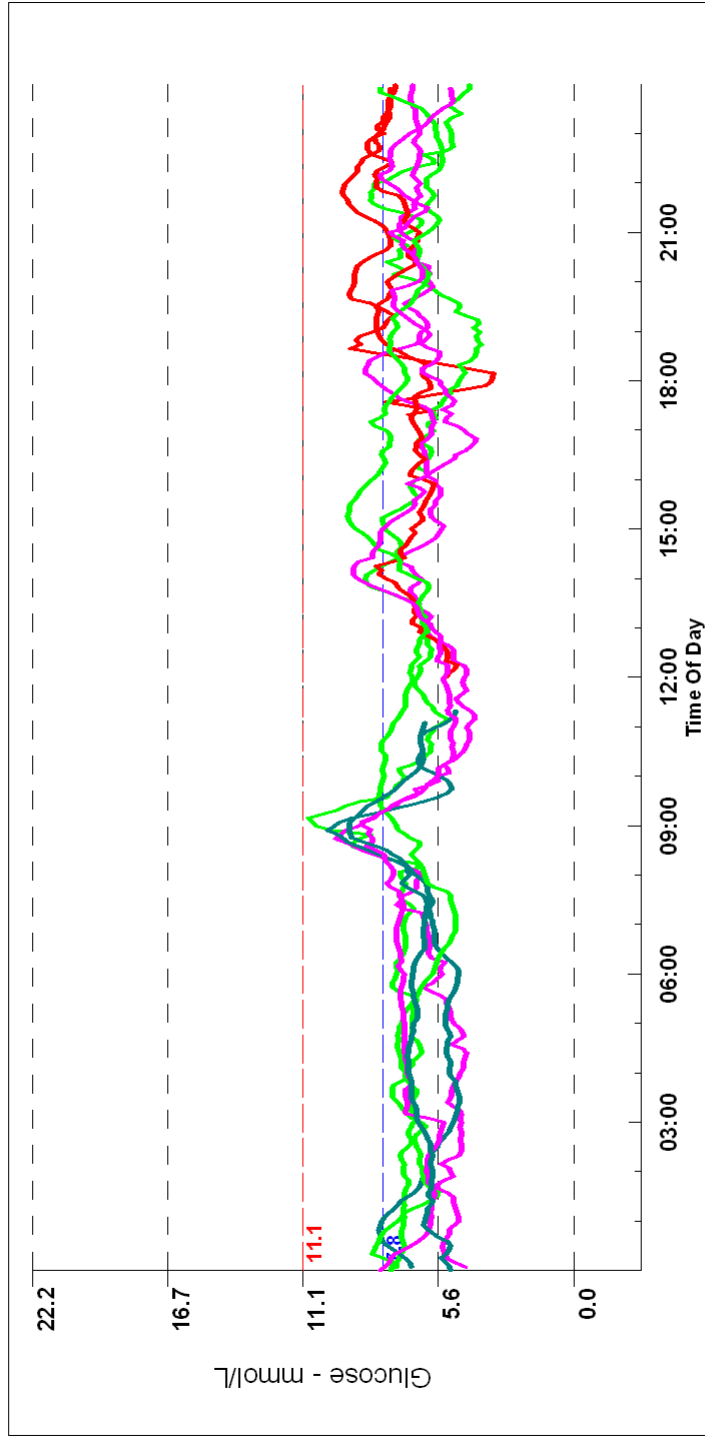
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-030a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



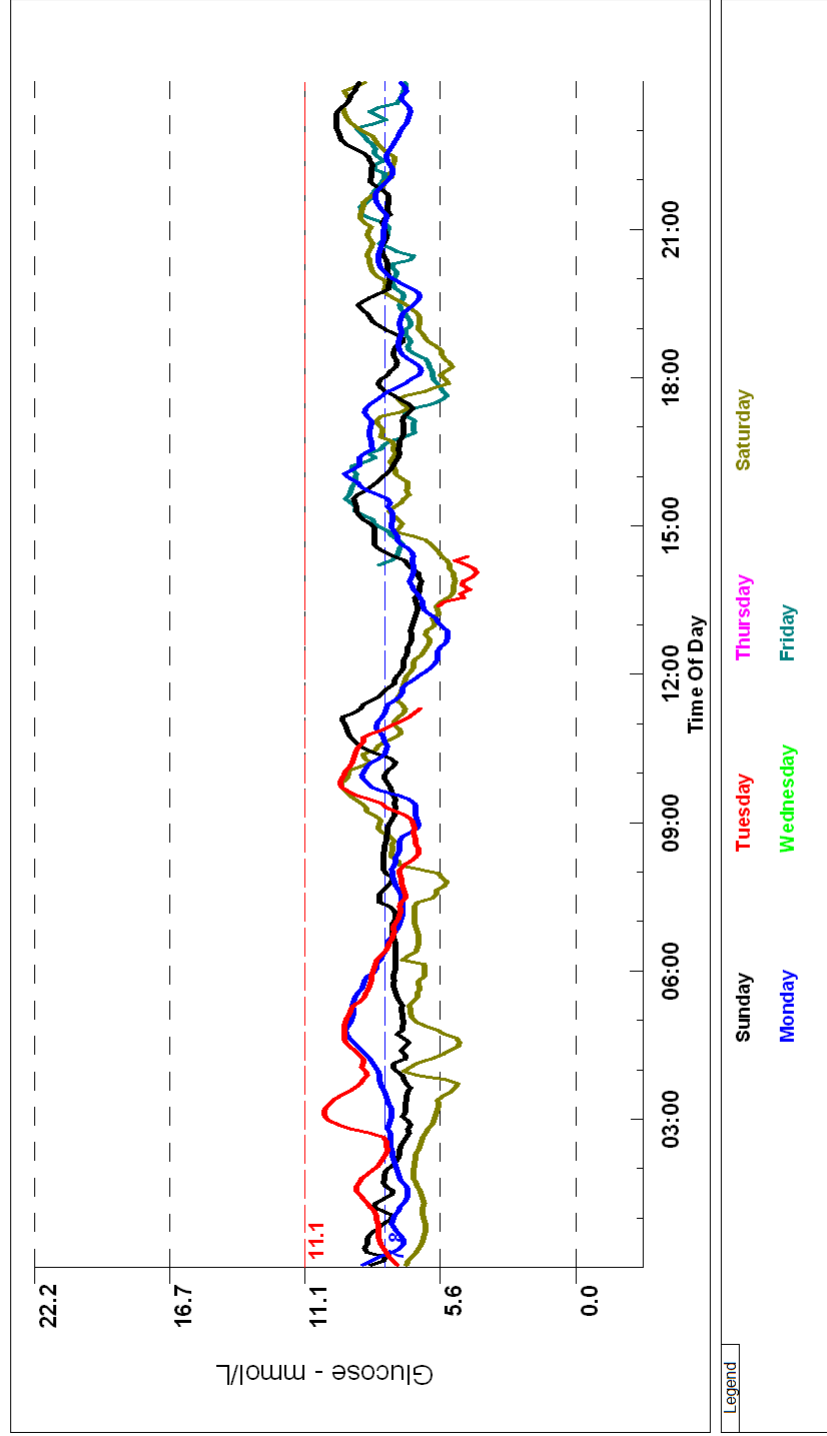
Legend

Sunday  
Monday  
Tuesday  
Wednesday  
Thursday  
Friday  
Saturday

Sensor Modal Day

Patient:  
ID: ipro-032a

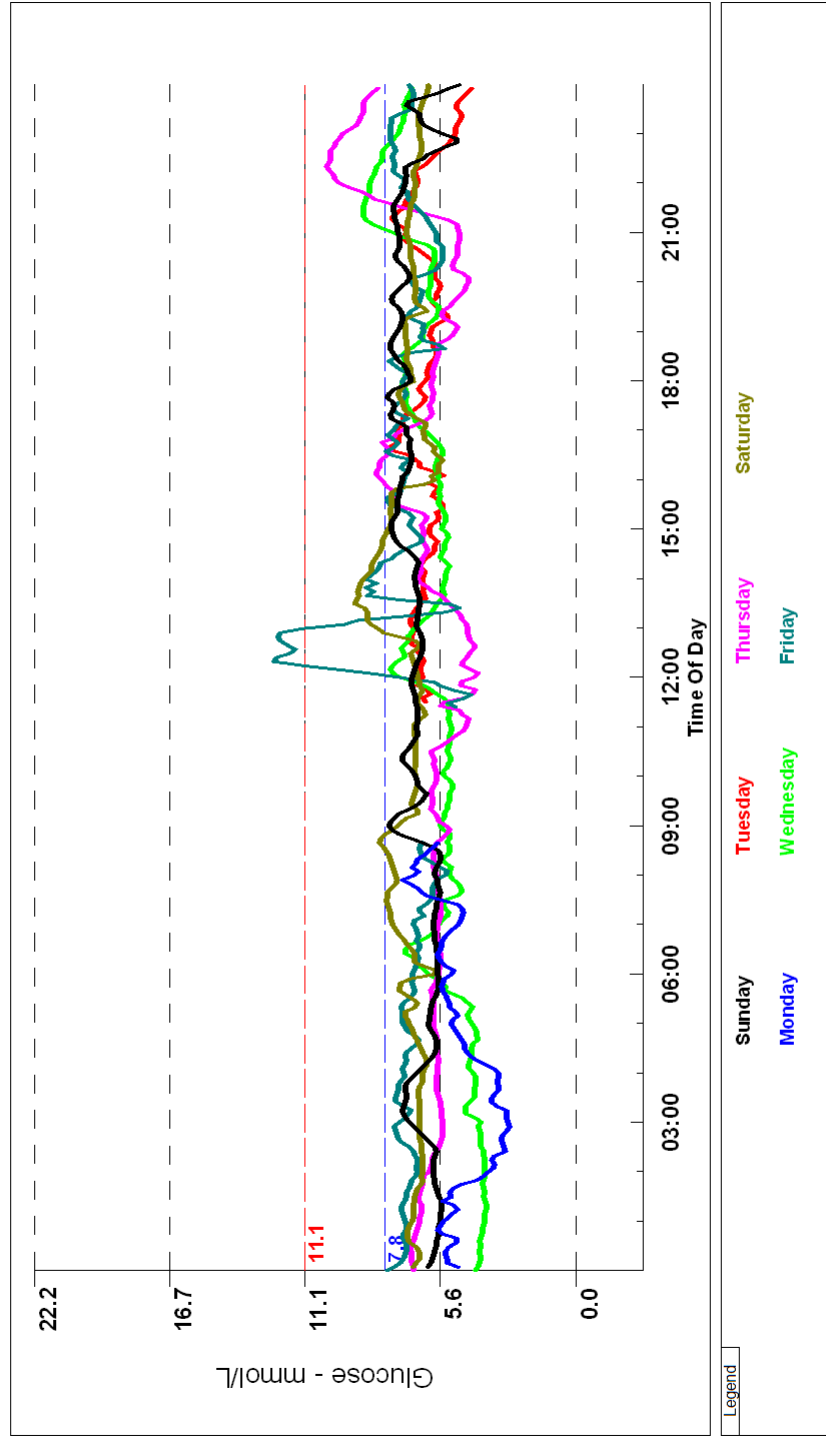
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-033a

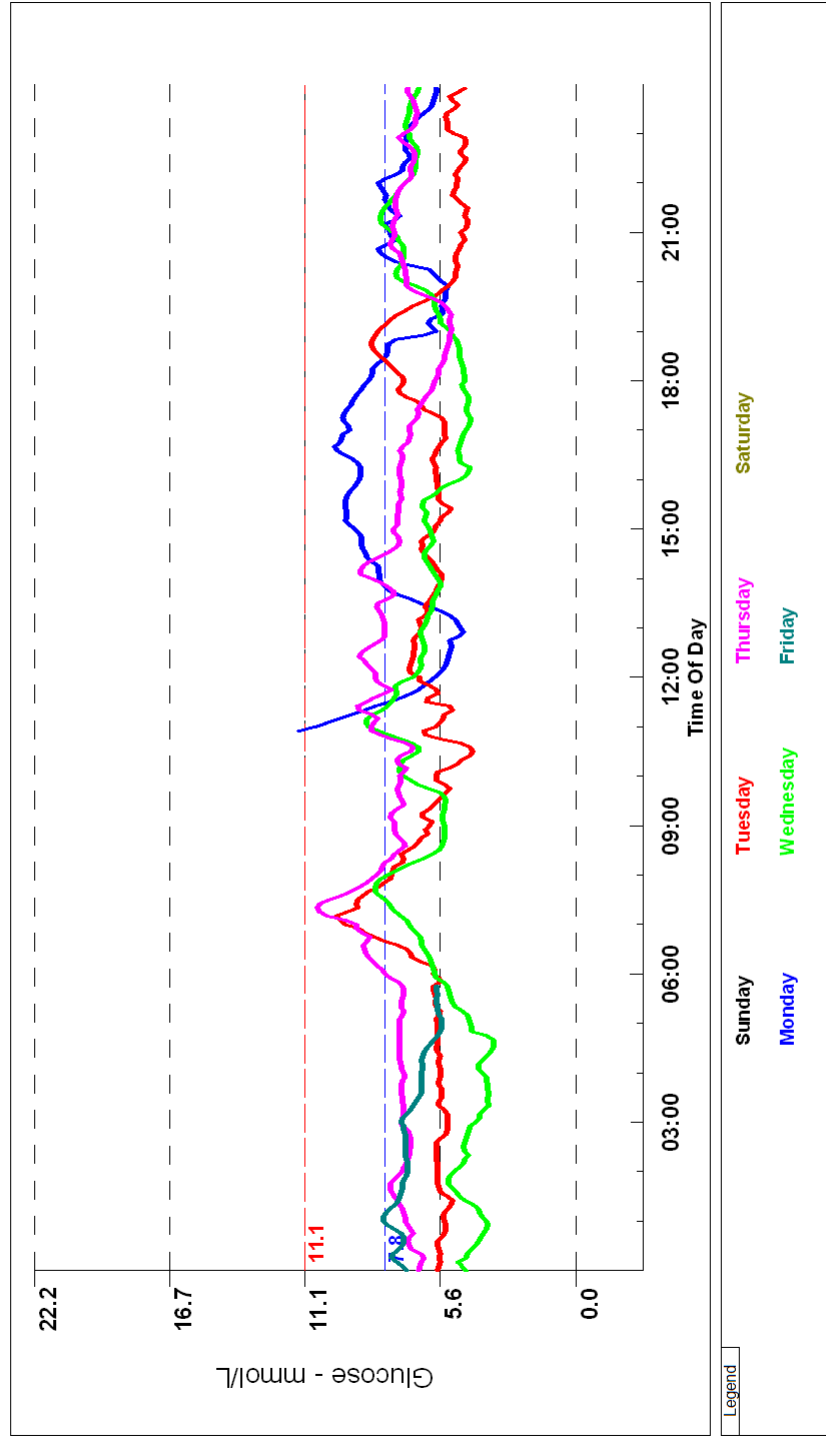
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-034b

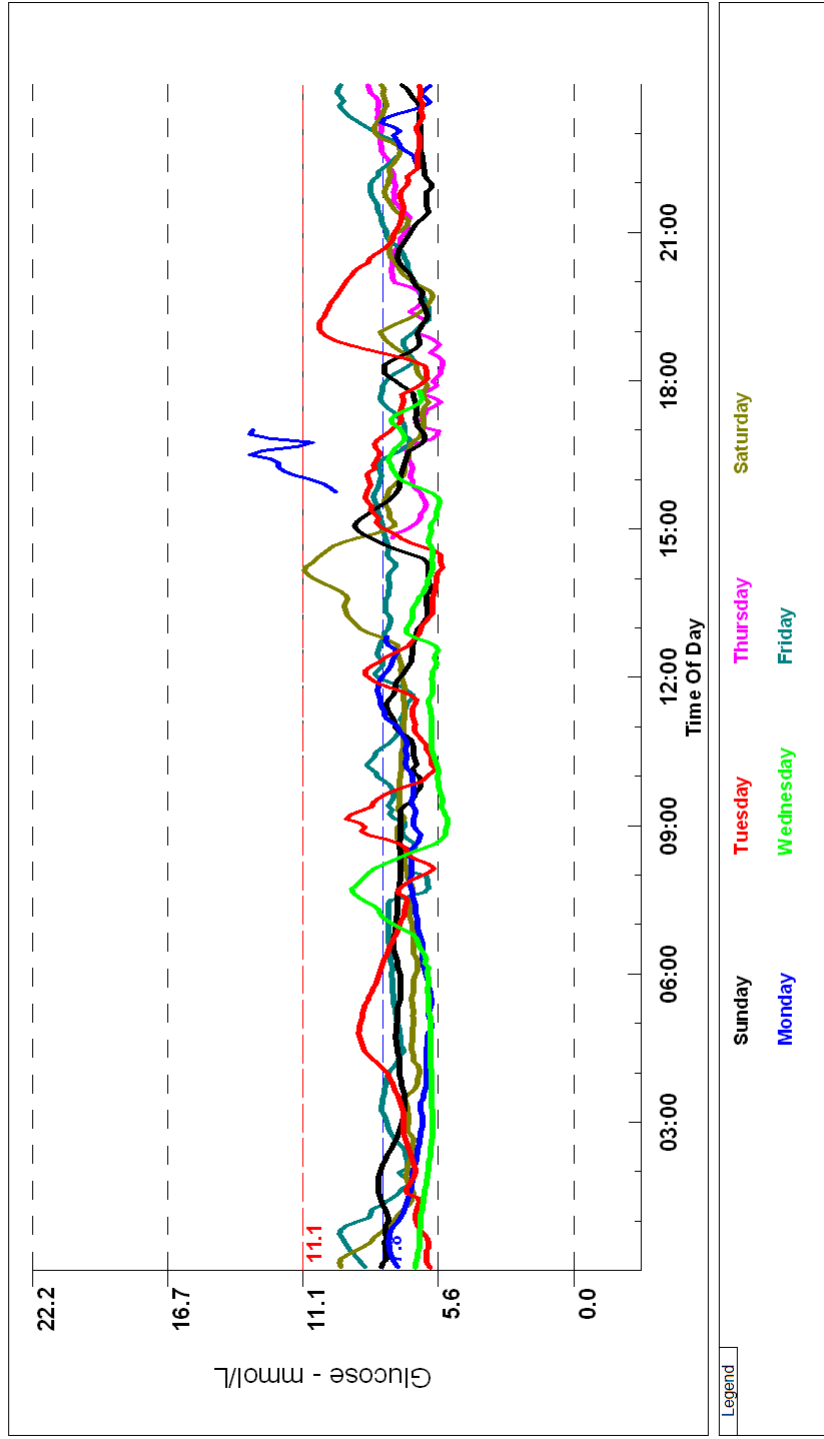
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-035a

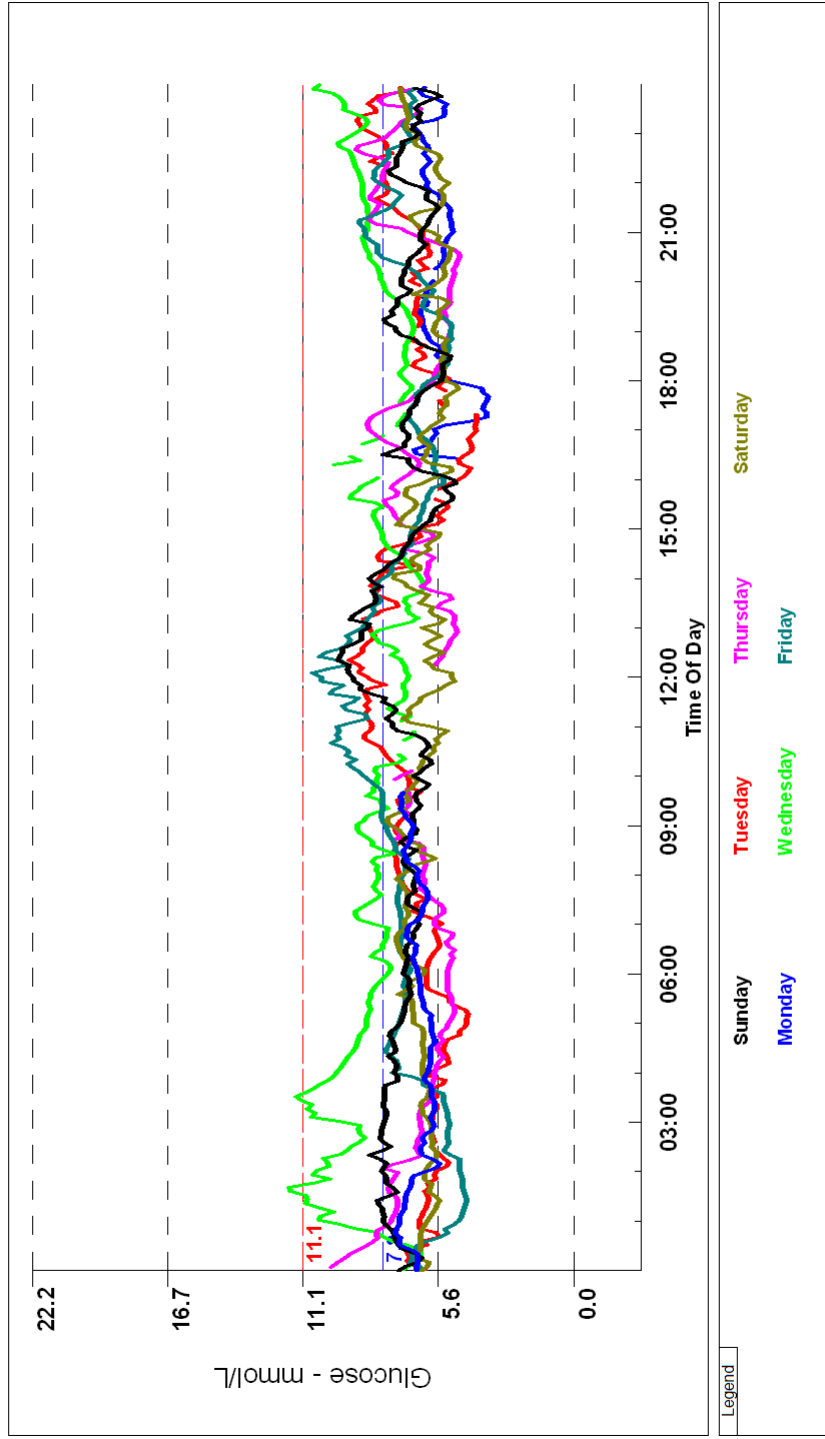
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-036a

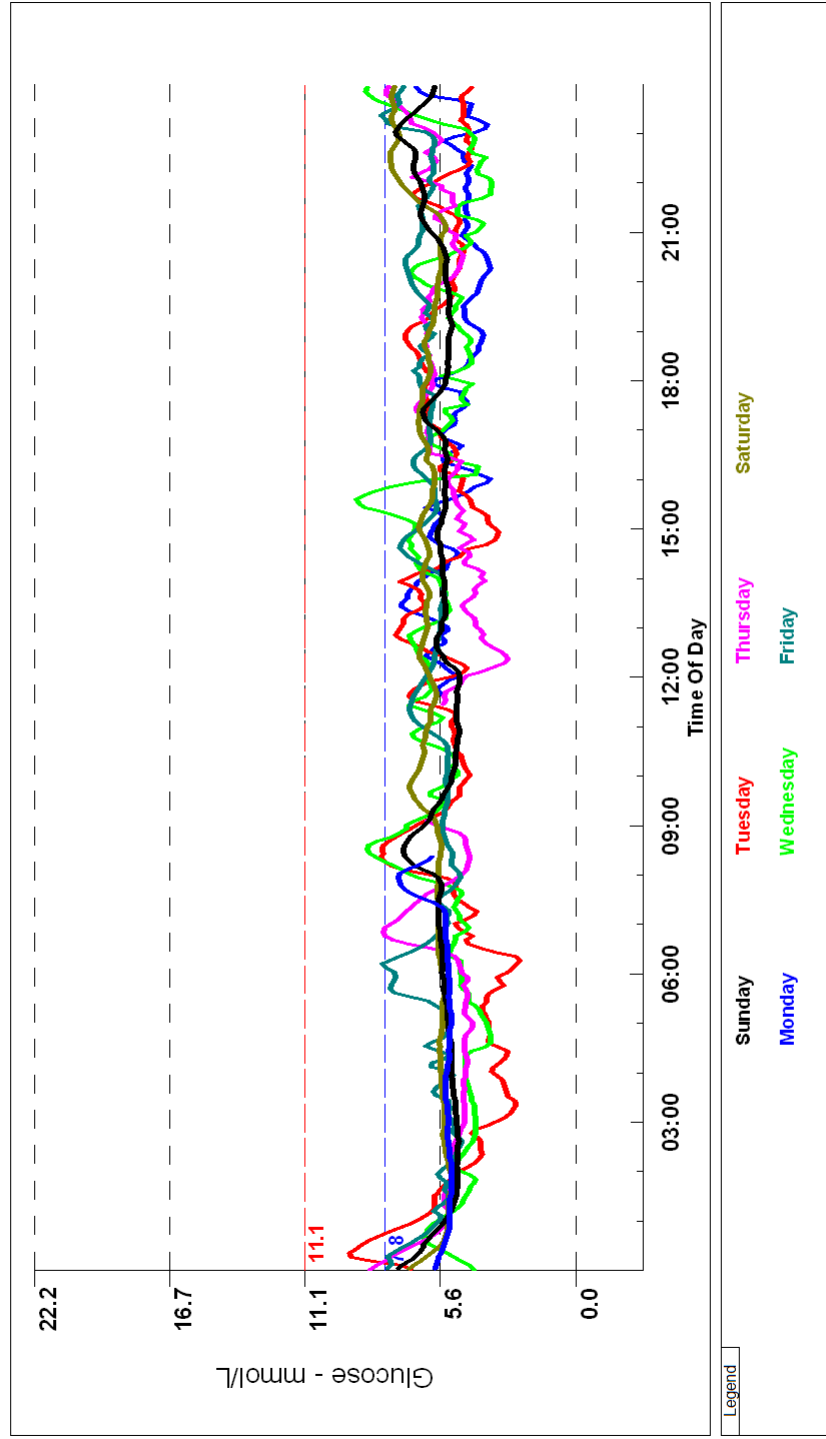
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-037a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

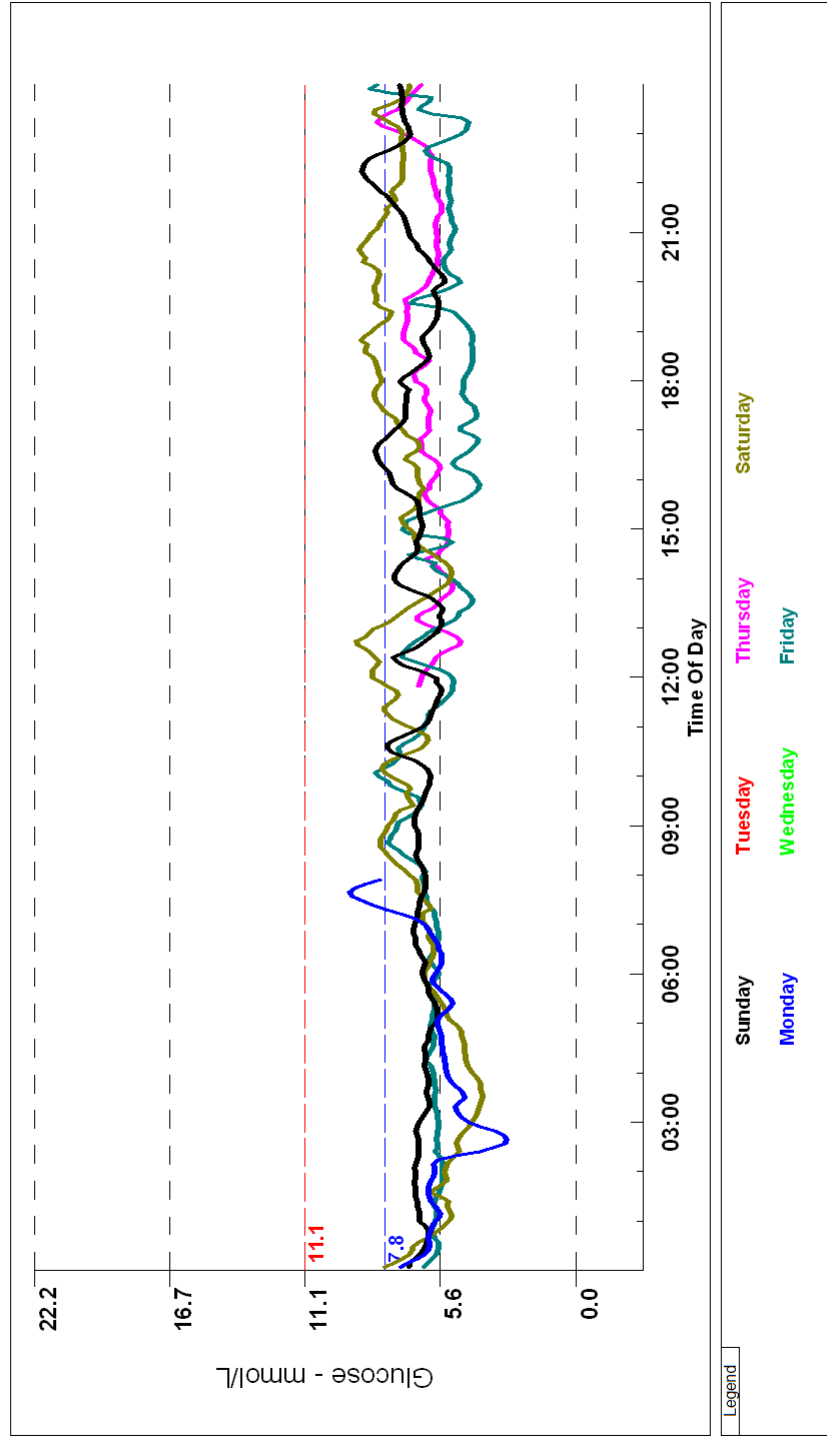




Sensor Modal Day

Patient:  
ID: ipro-037b

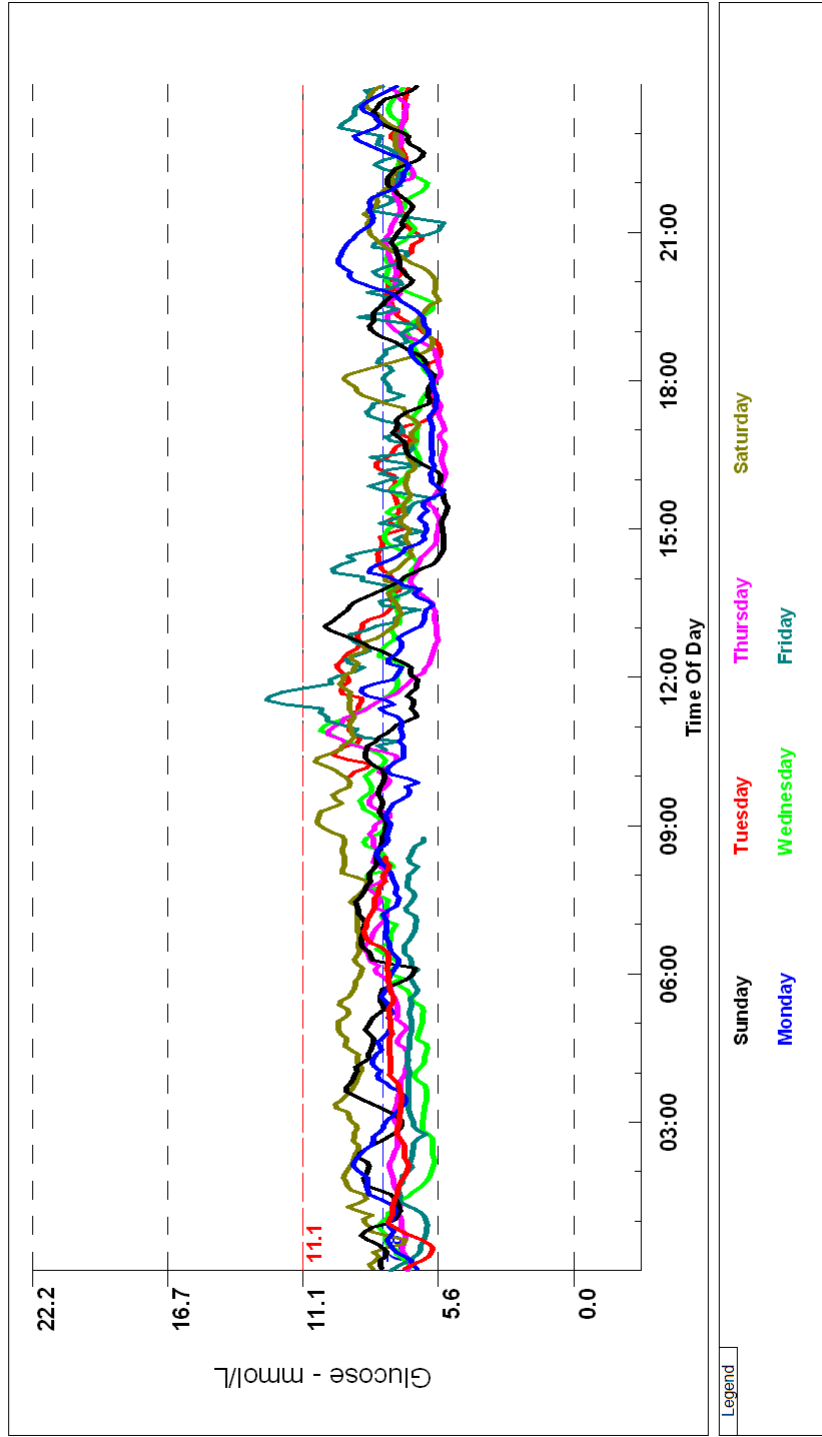
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-038a

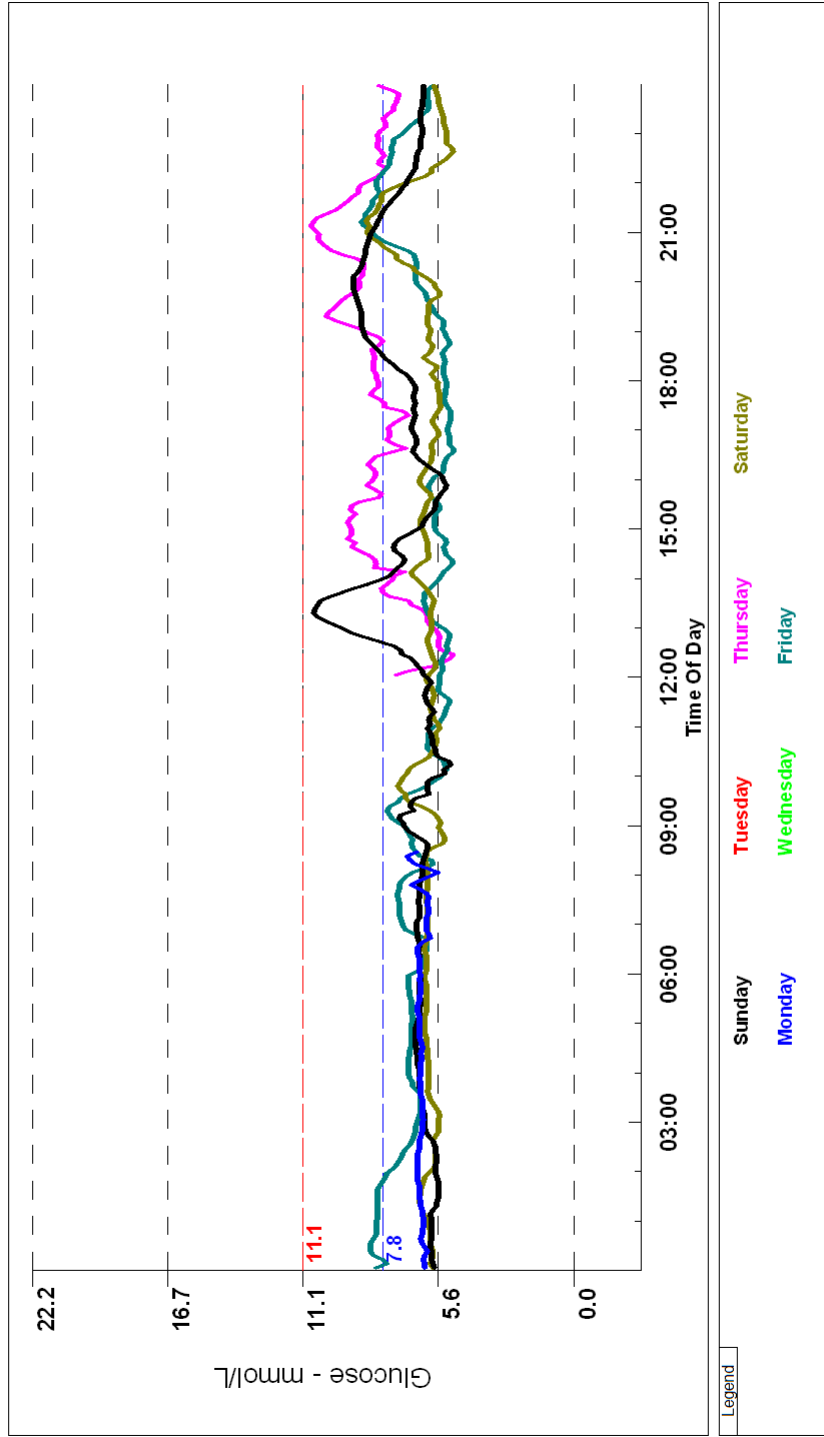
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-039b

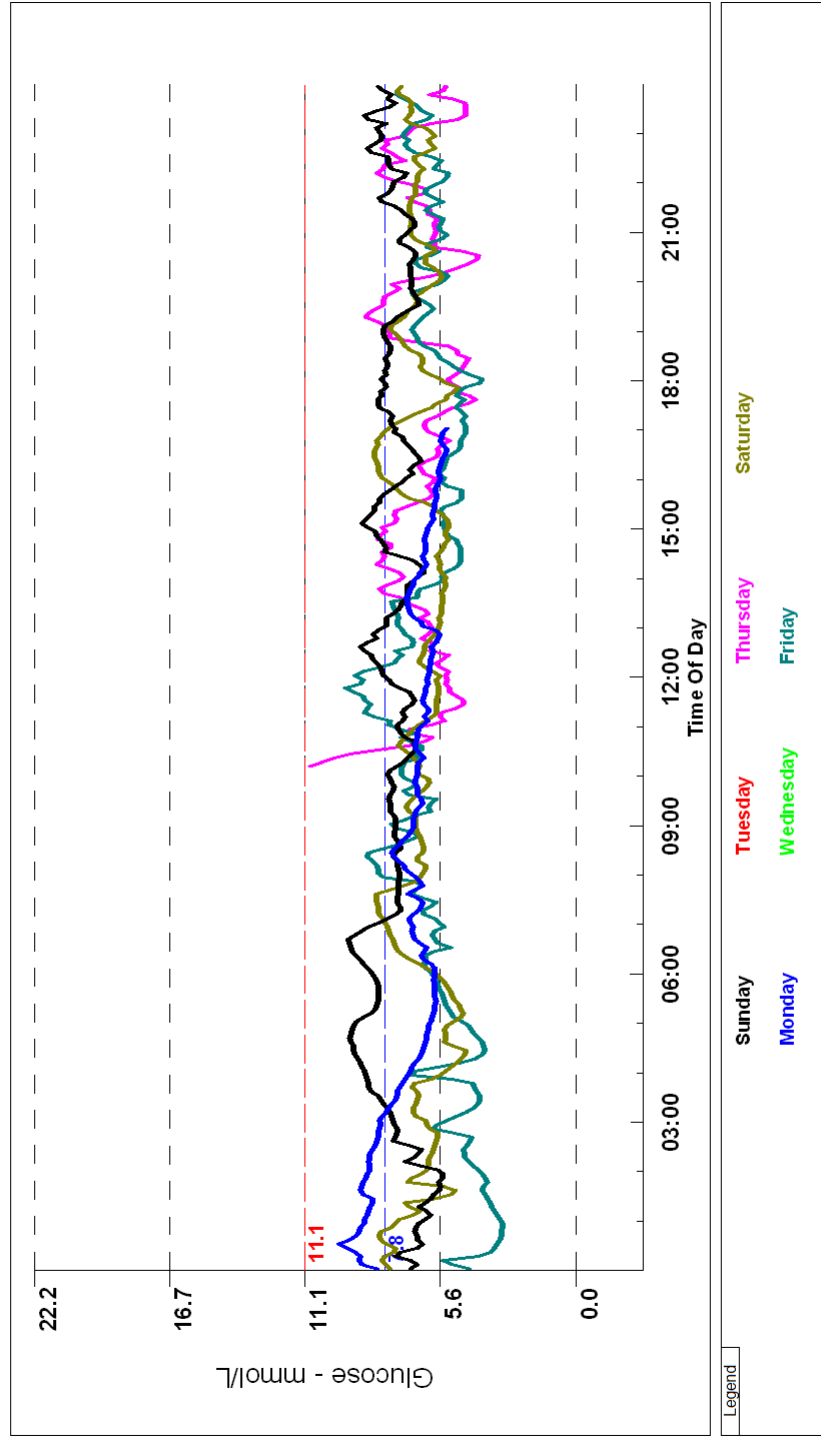
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-040b

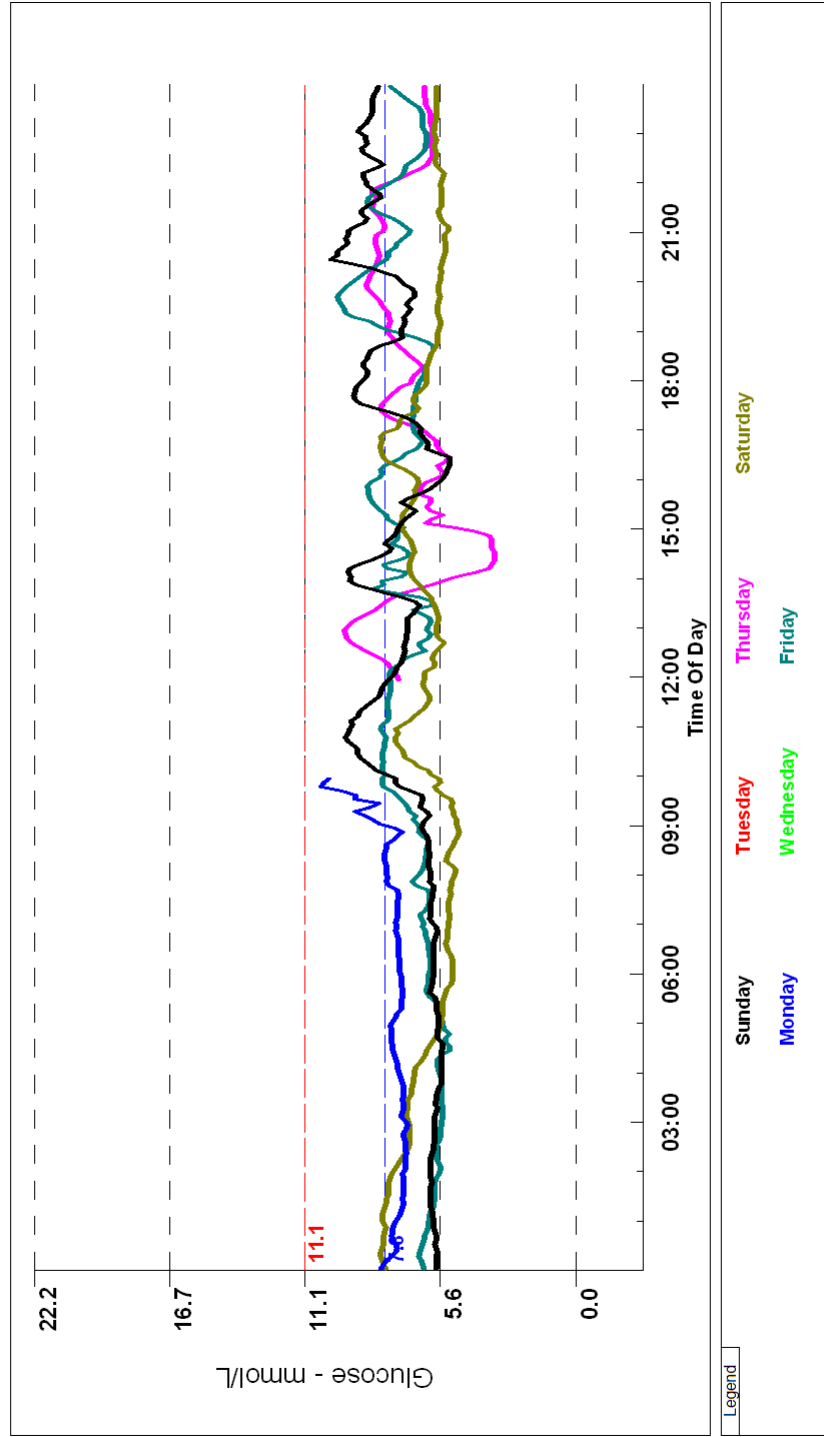
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-041a

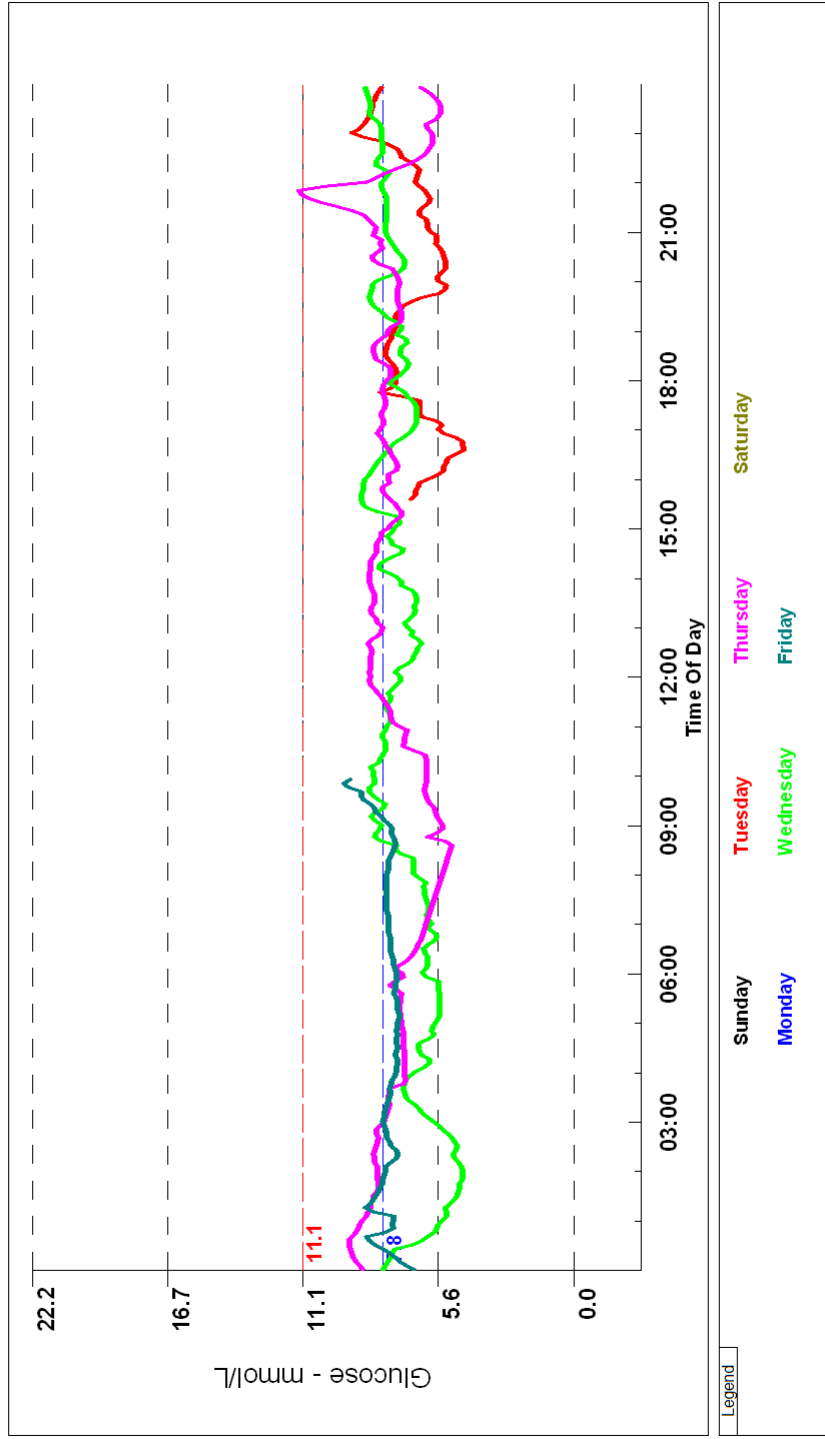
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-041aa

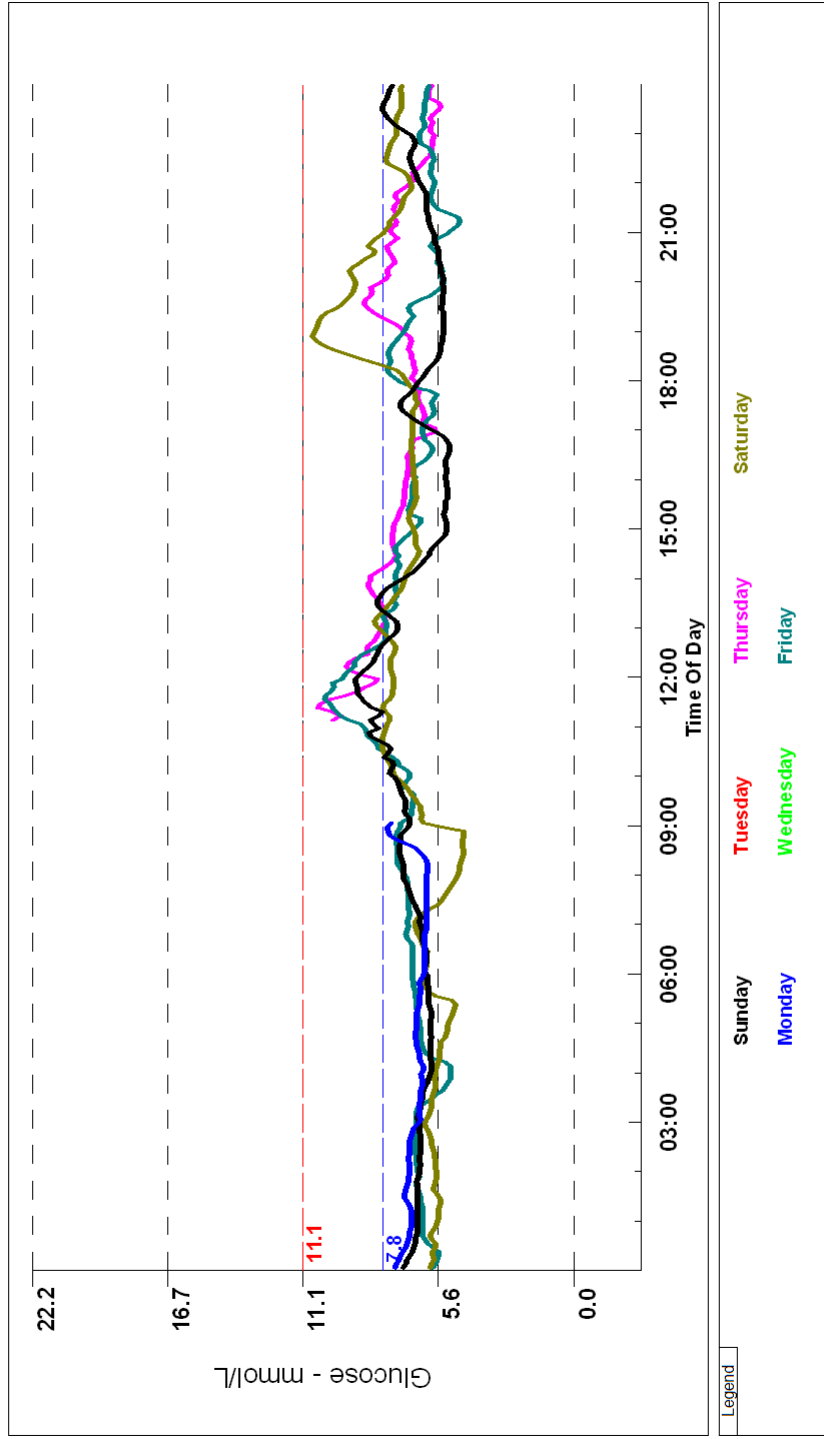
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-041b

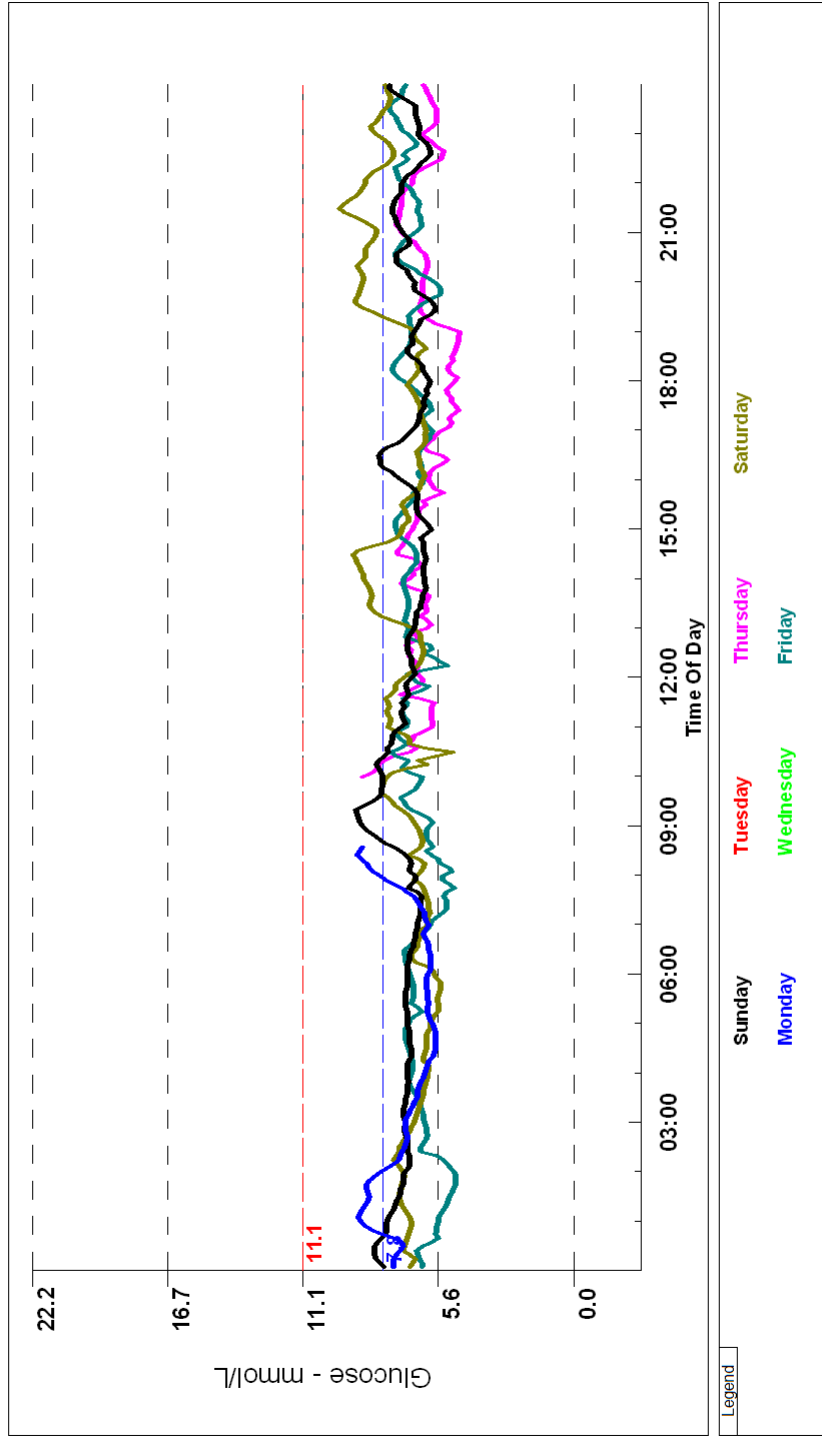
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-042b

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

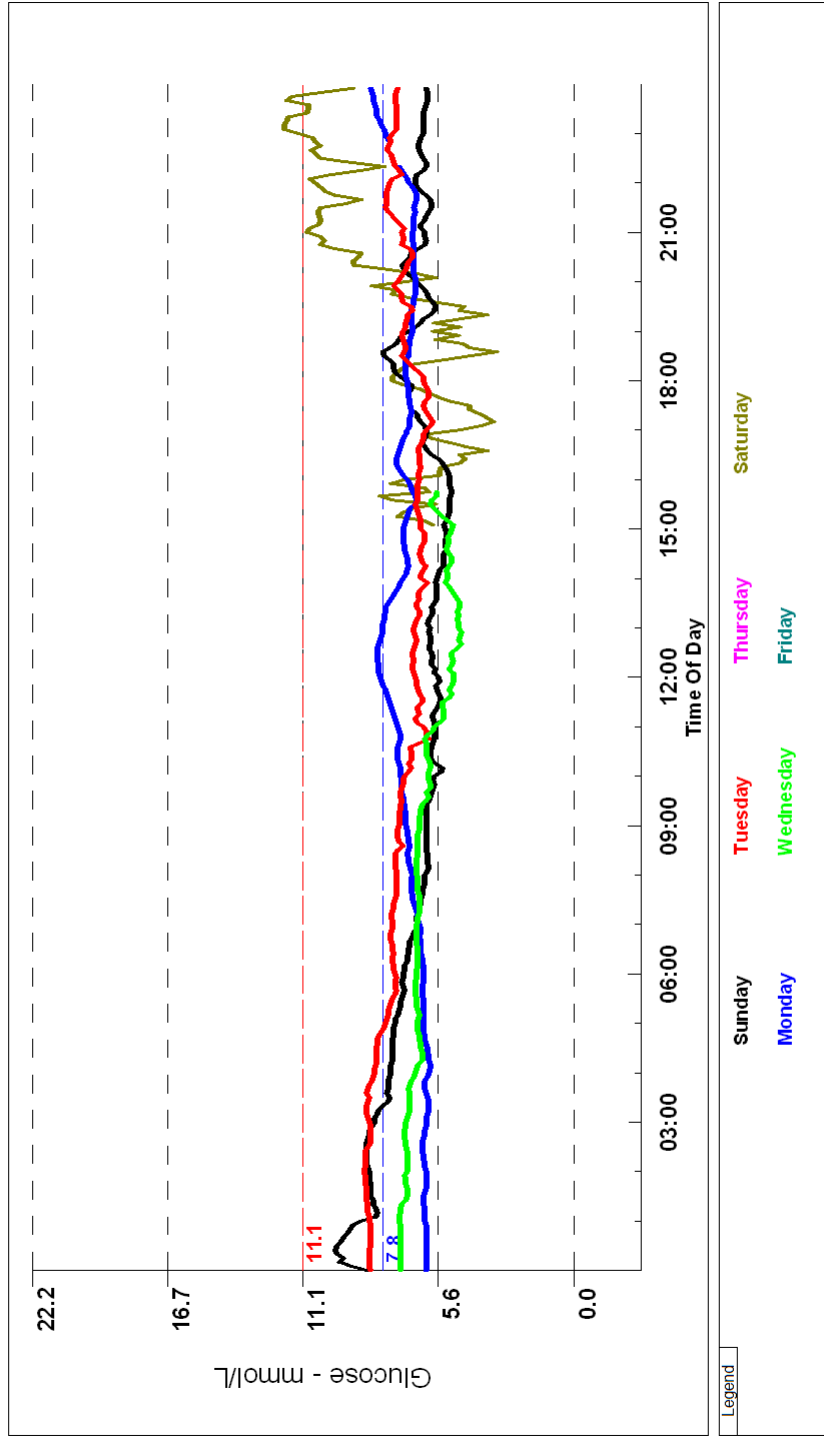




Sensor Modal Day

Patient:  
ID: ipro-043a

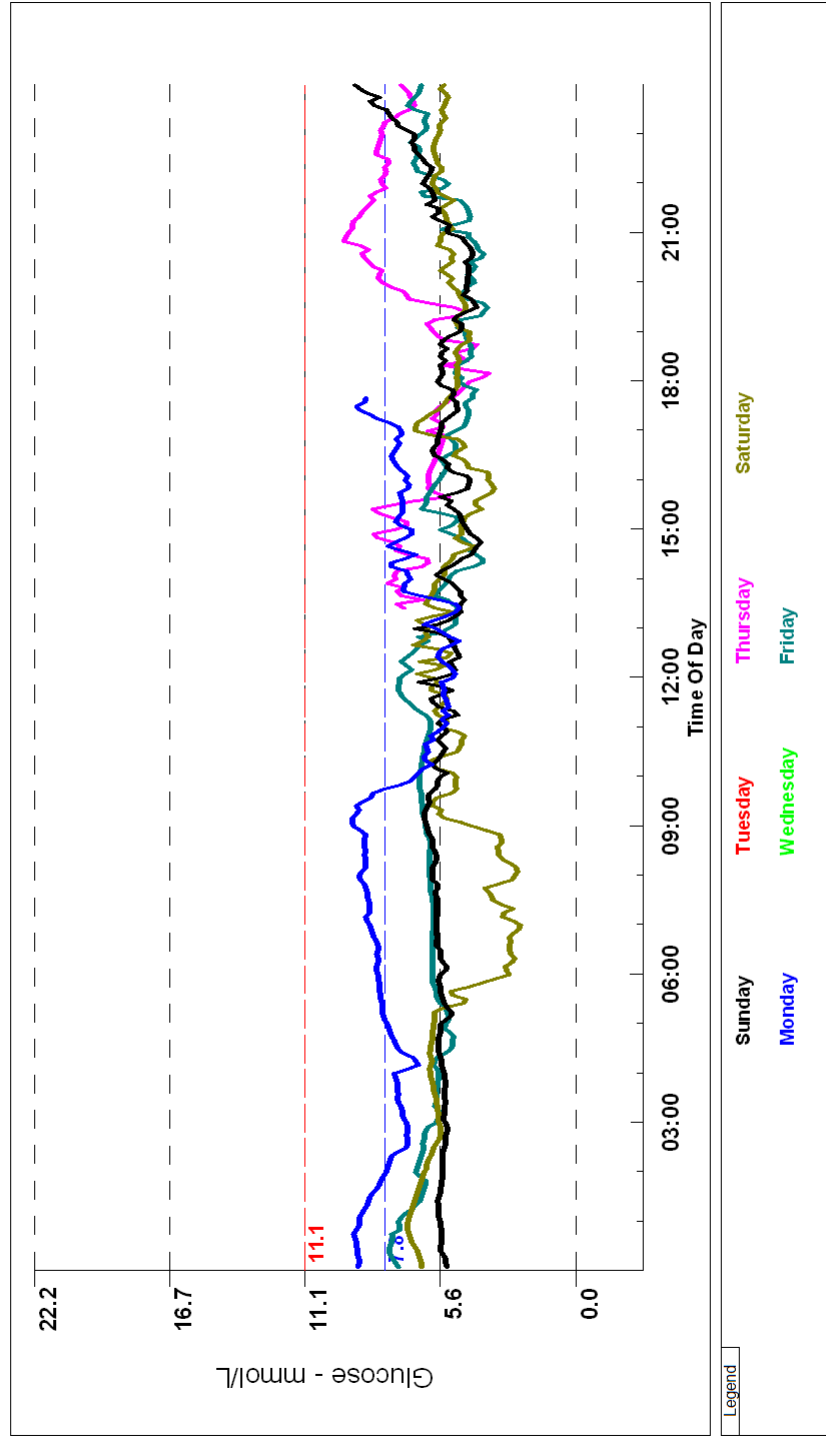
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: Ipro-044a

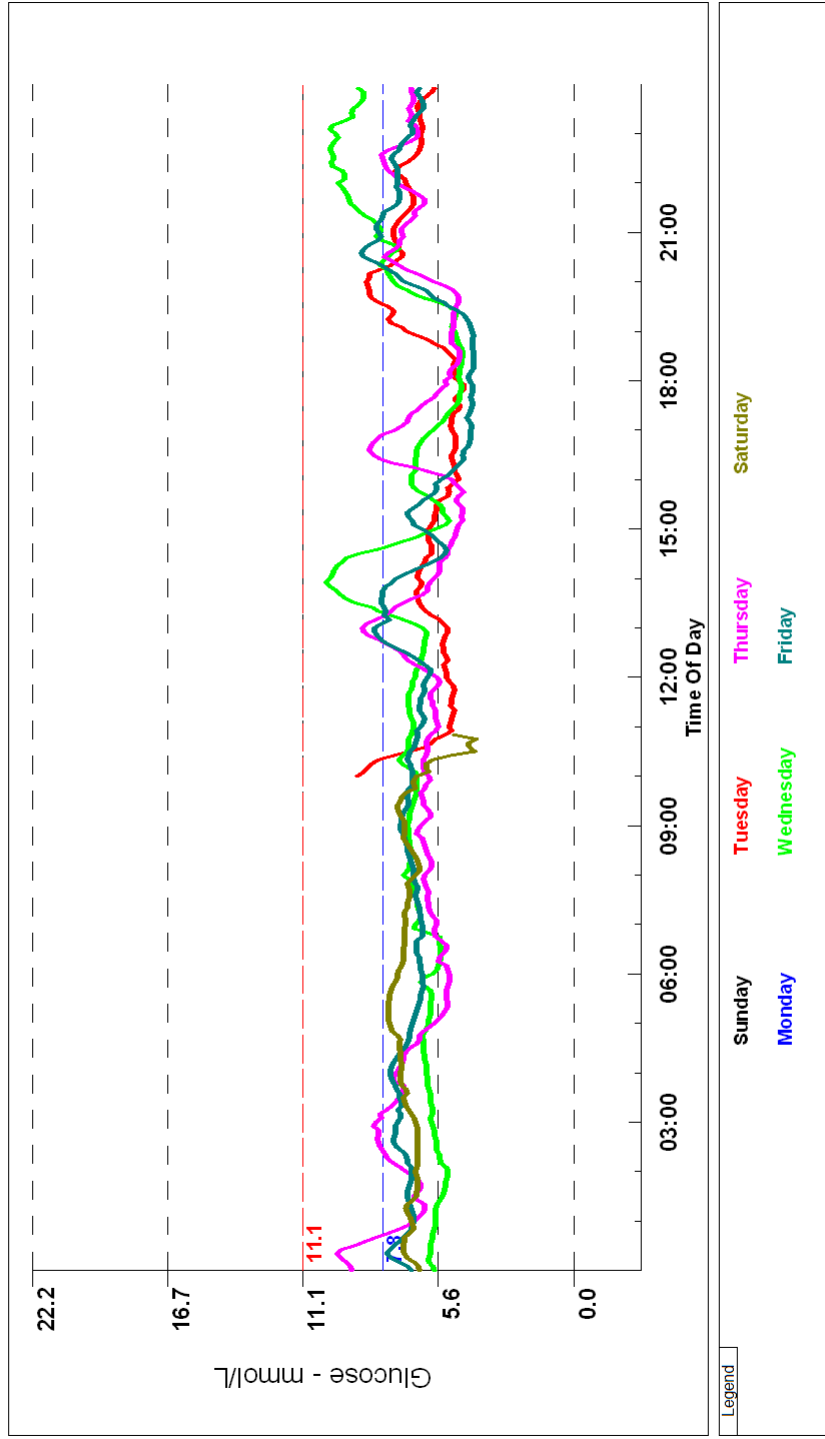
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-044b

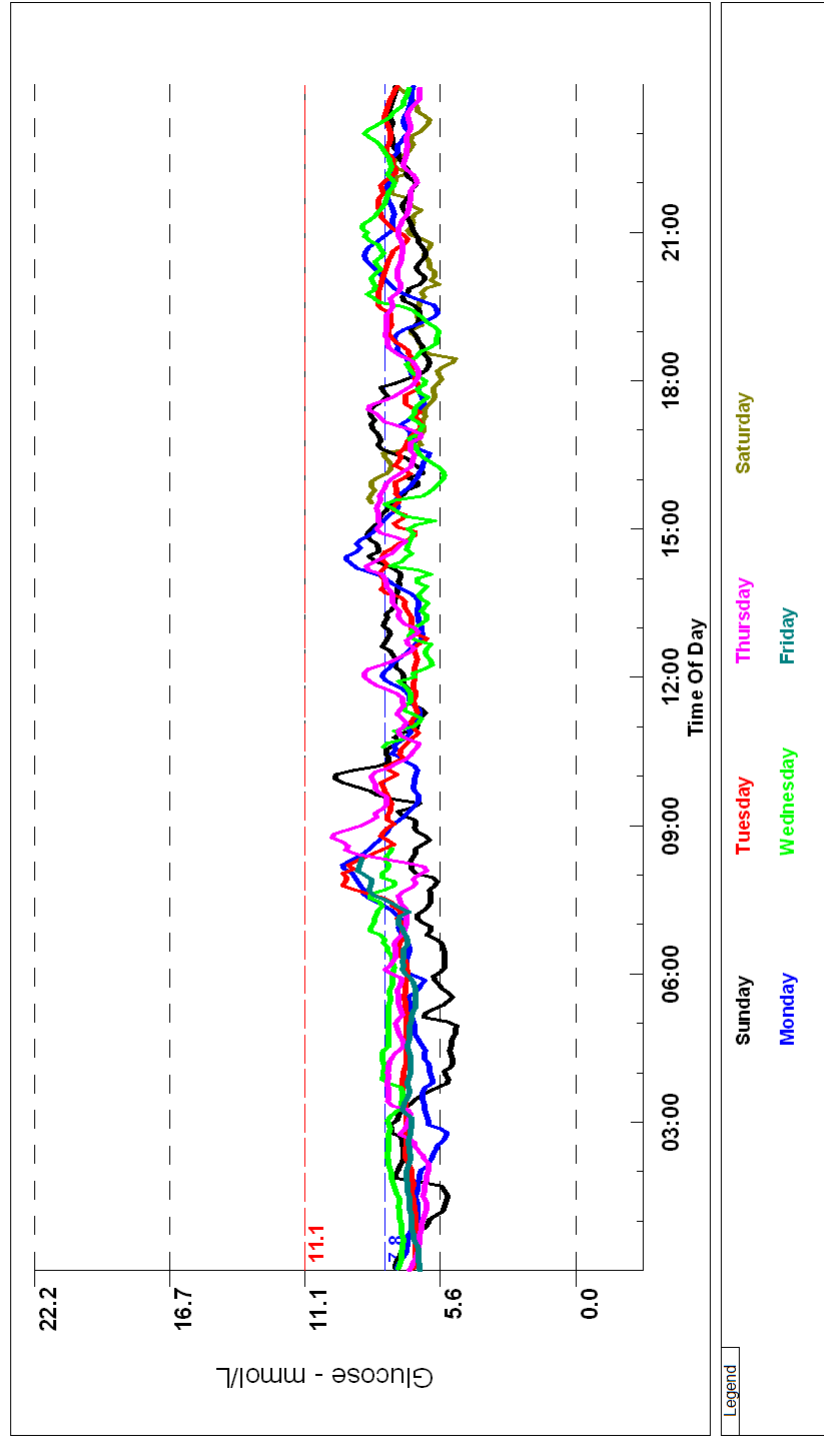
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-045a

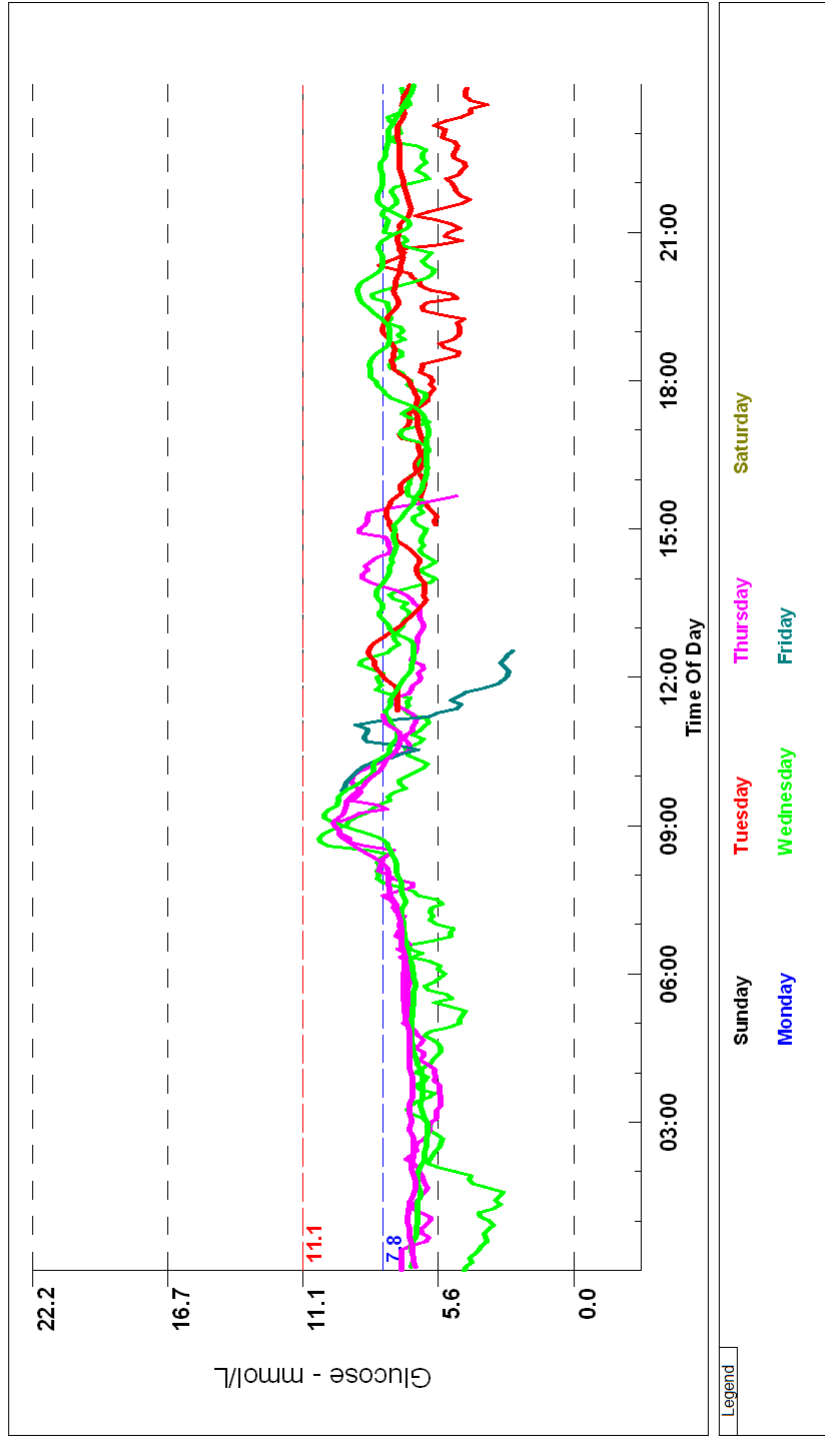
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-046a

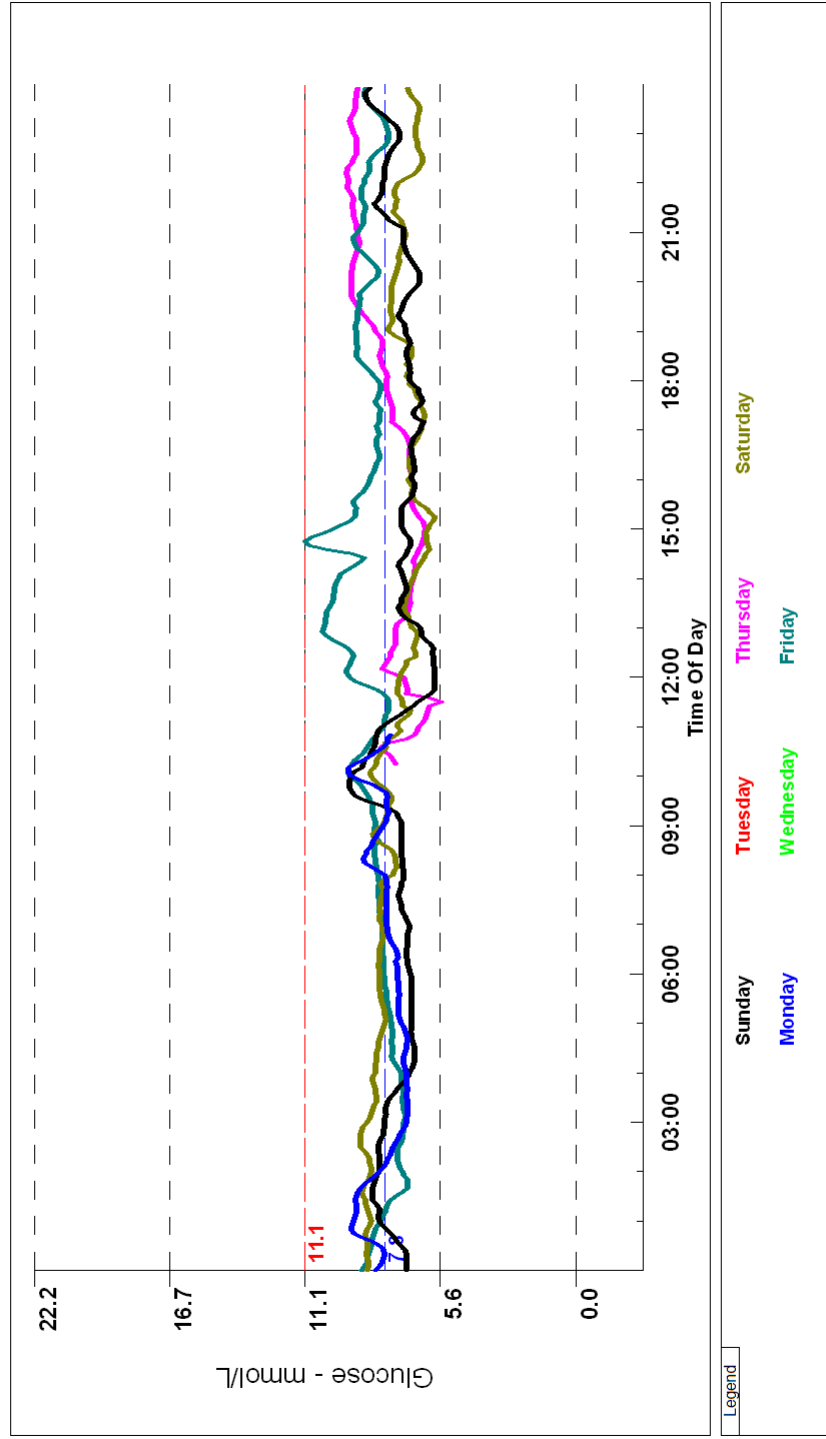
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-046b

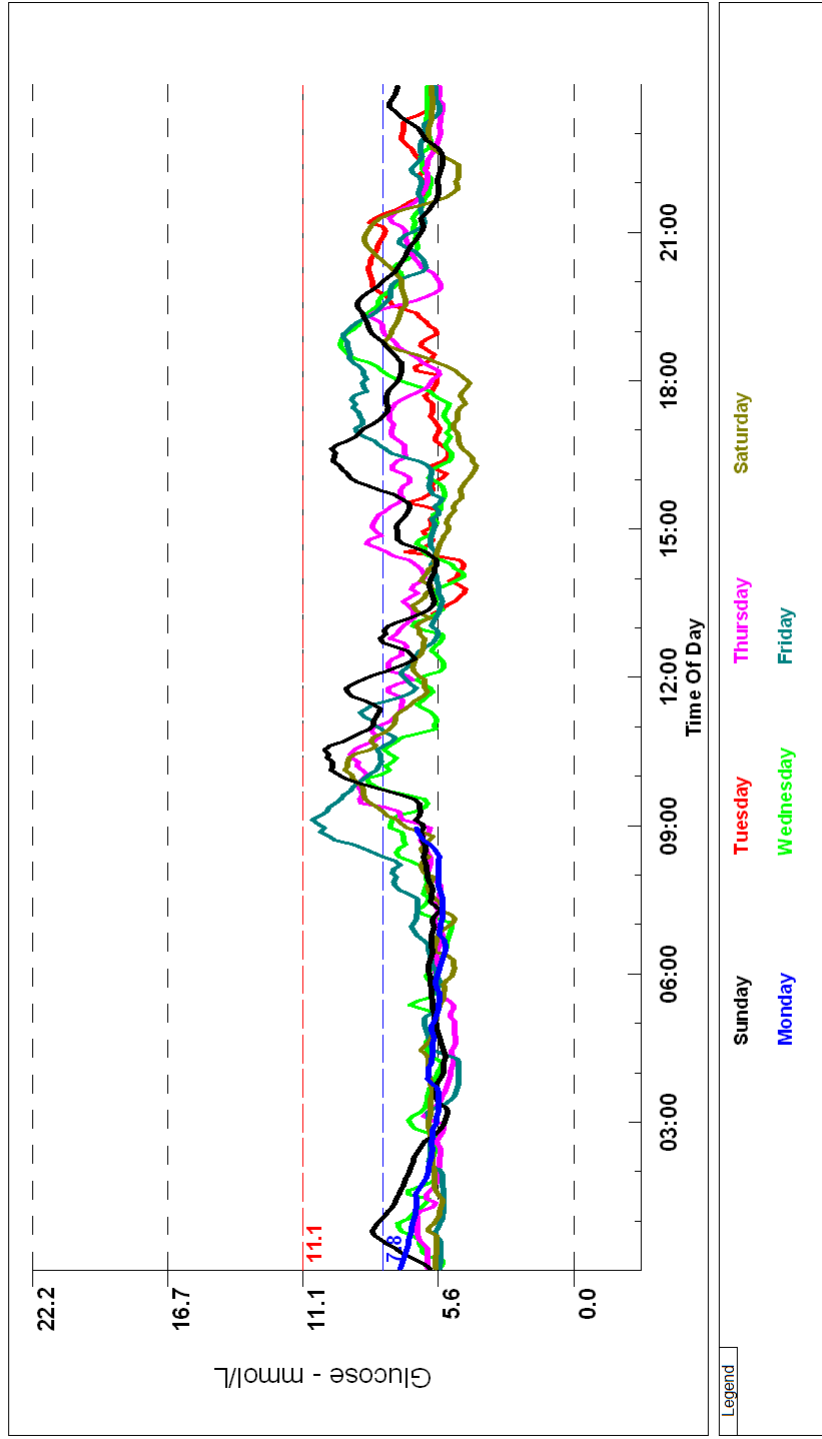
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-047a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



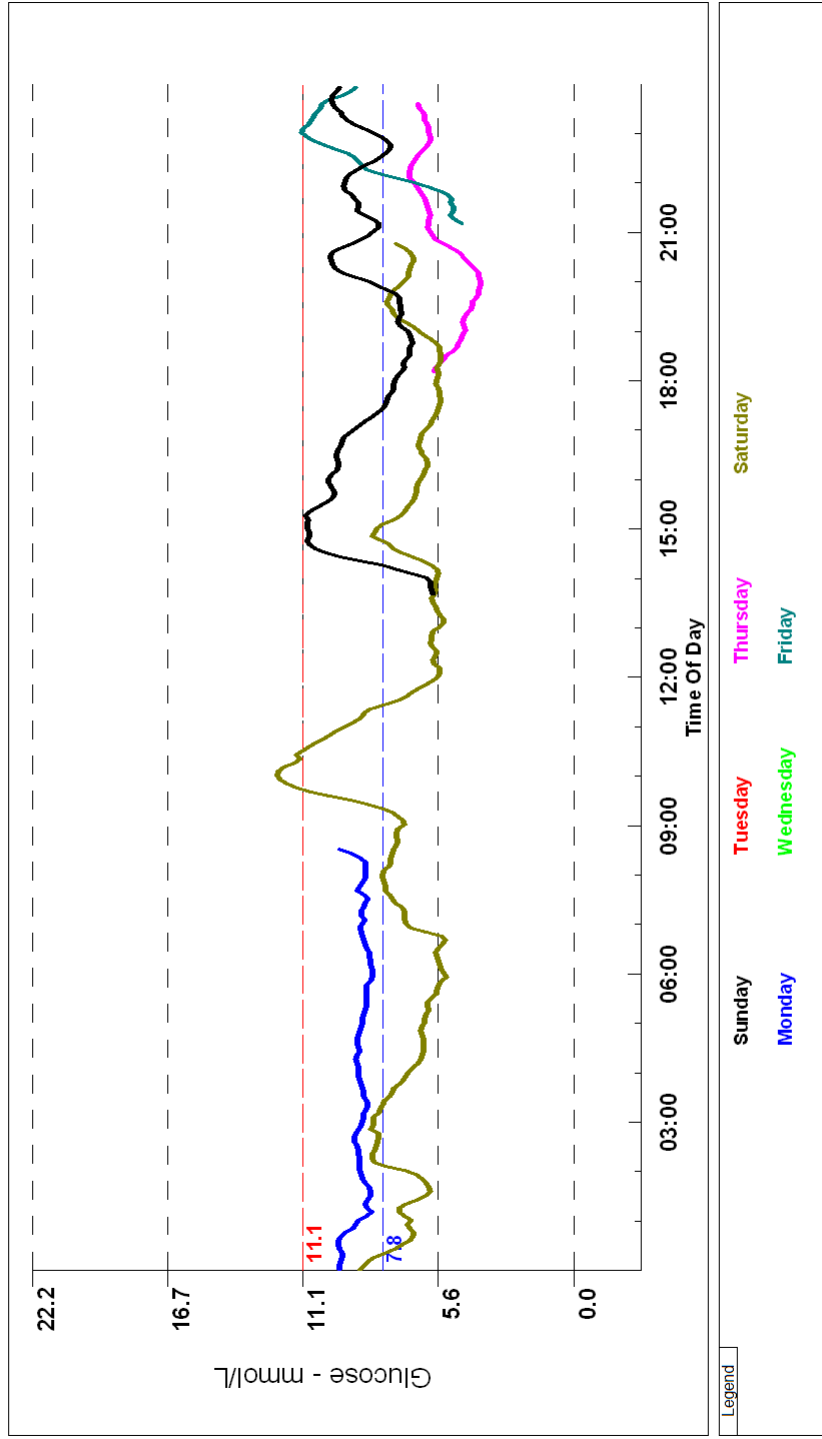
## **APPENDIX 4 – CGM PROFILES: MOST VARIABILITY**



Sensor Modal Day

Patient:  
ID: ipro-001a

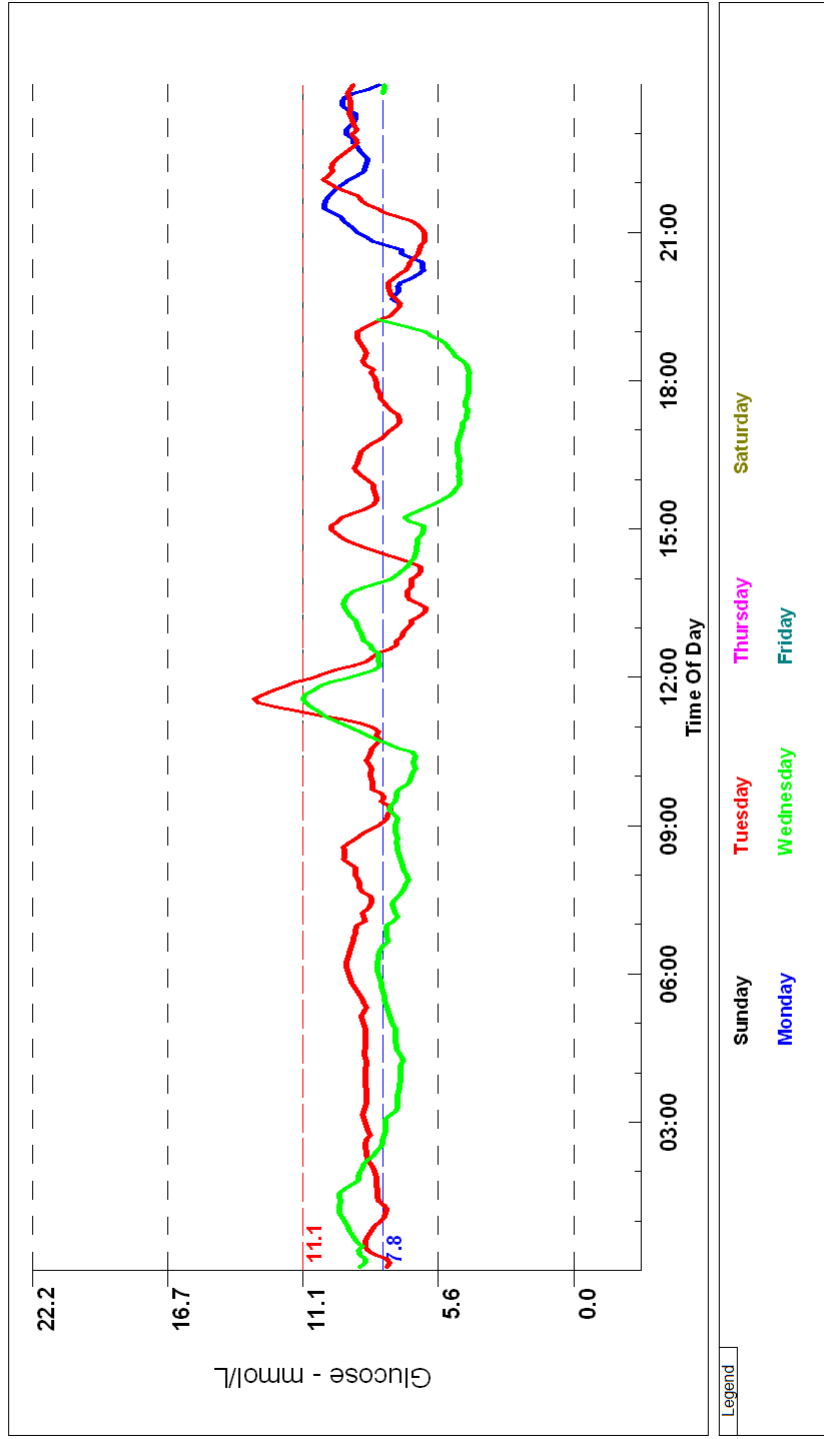
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-001aa

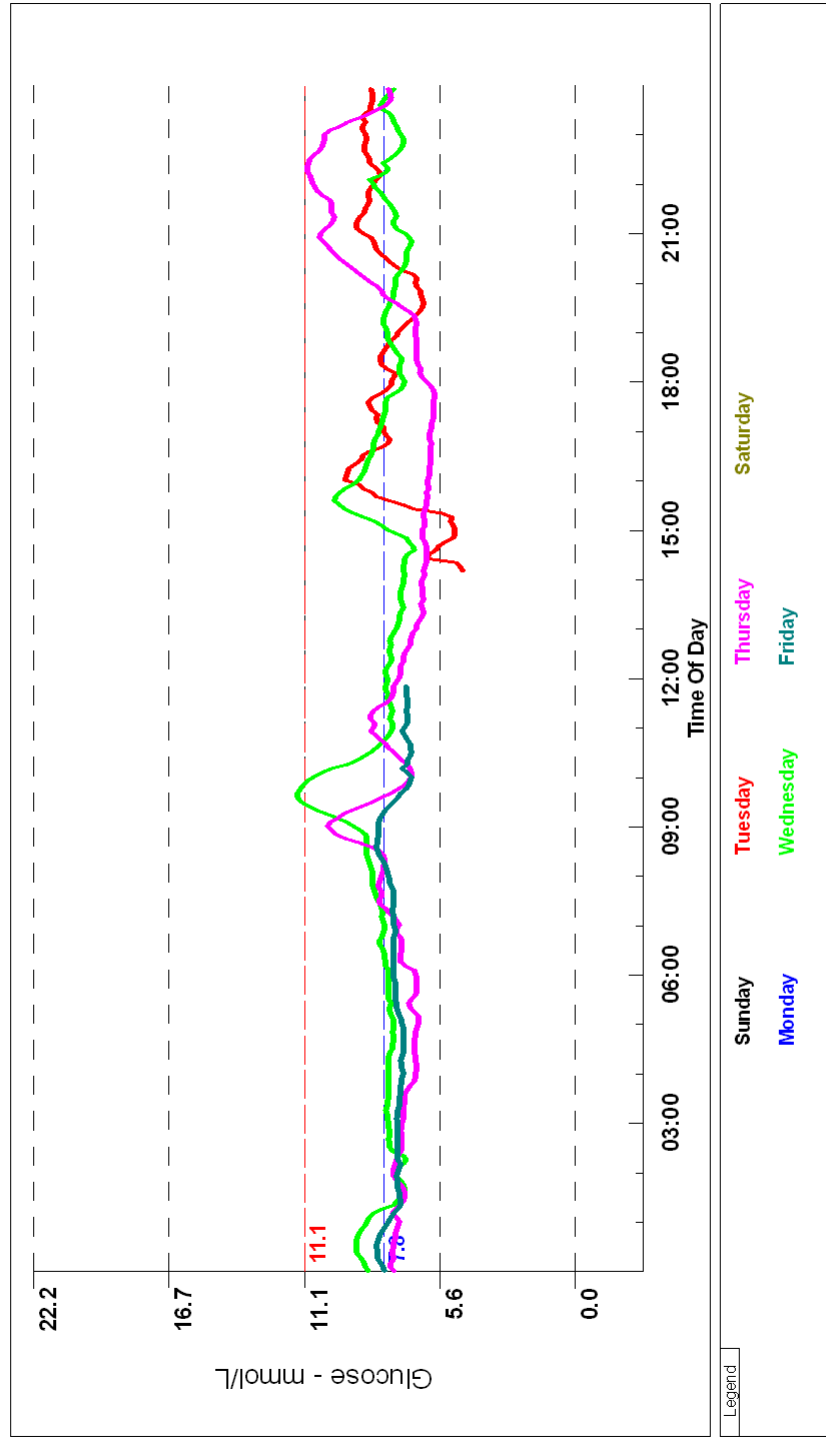
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-001B

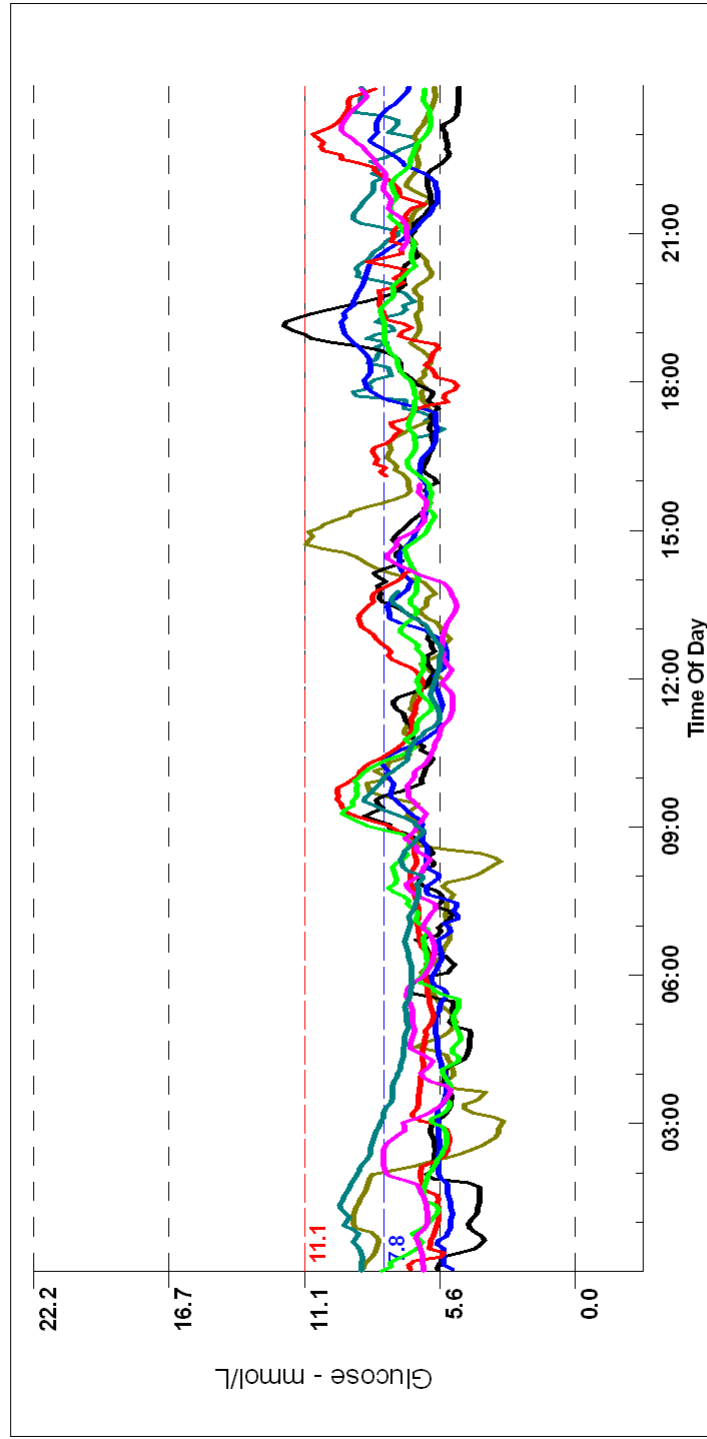
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-002a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Legend

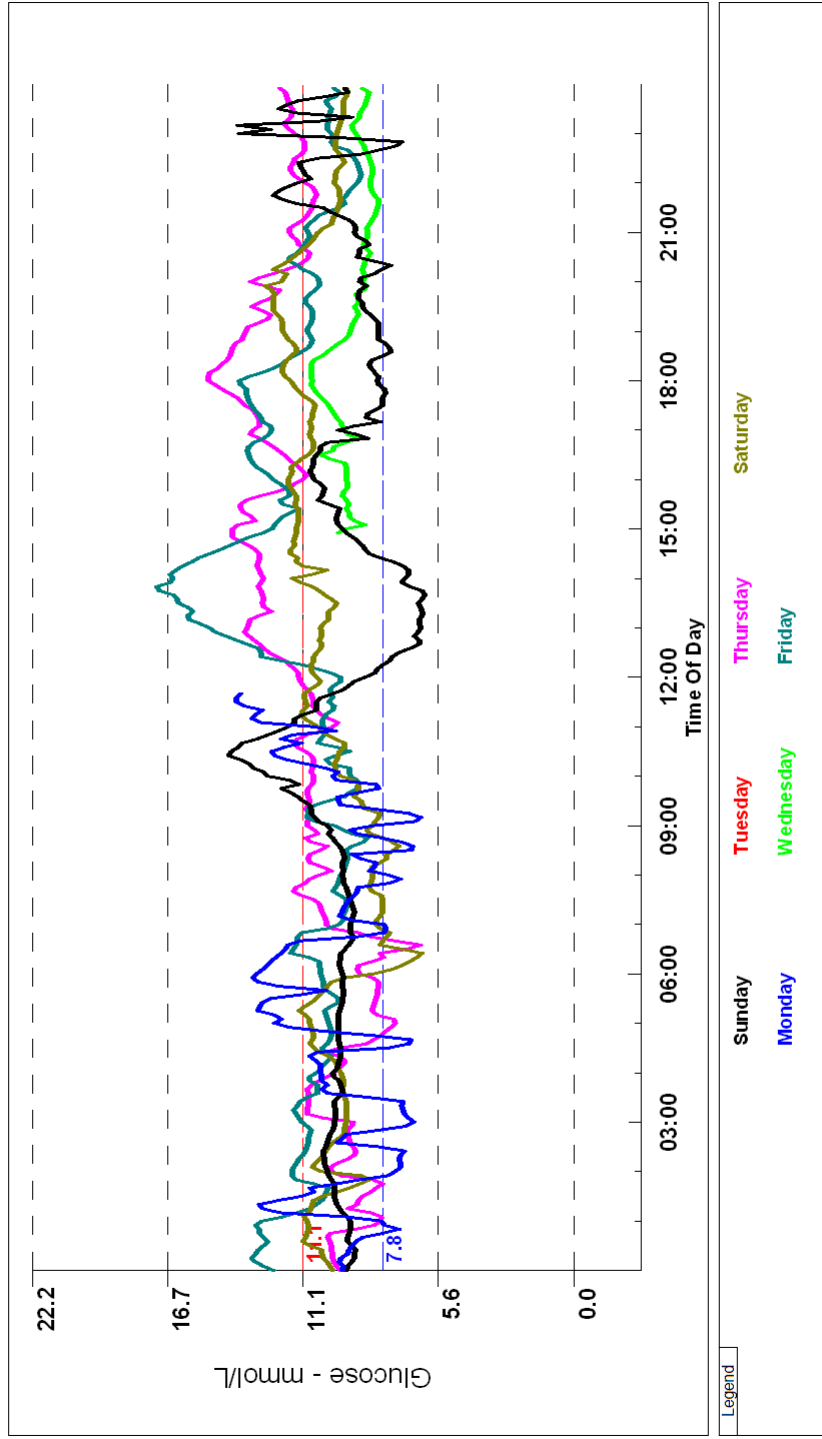
Sunday  
Monday  
Tuesday  
Wednesday  
Thursday  
Friday  
Saturday

Report Printed: 27-Feb-08 05:54

Sensor Modal Day

Patient:  
ID: ipro-003b

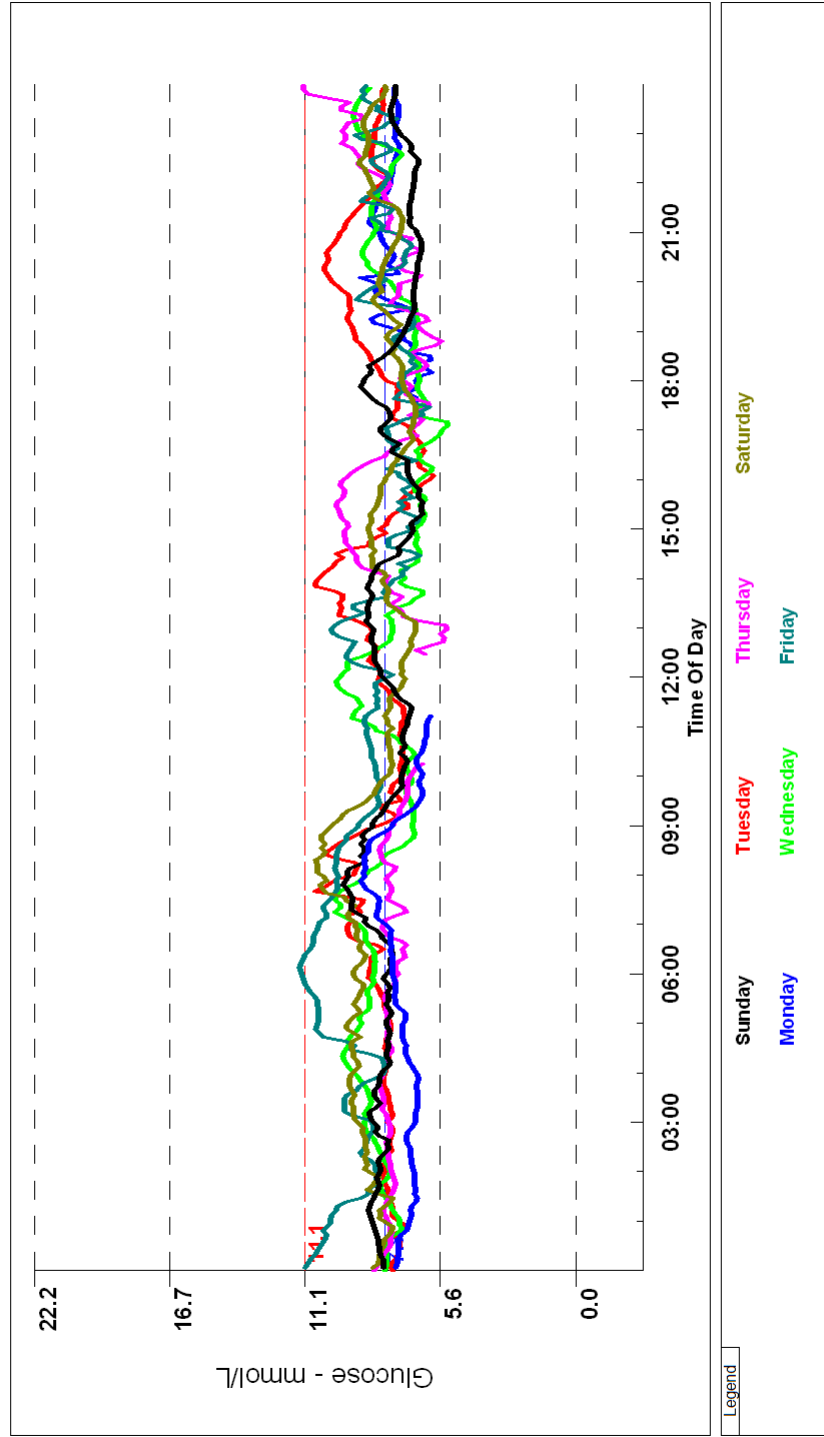
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-006a

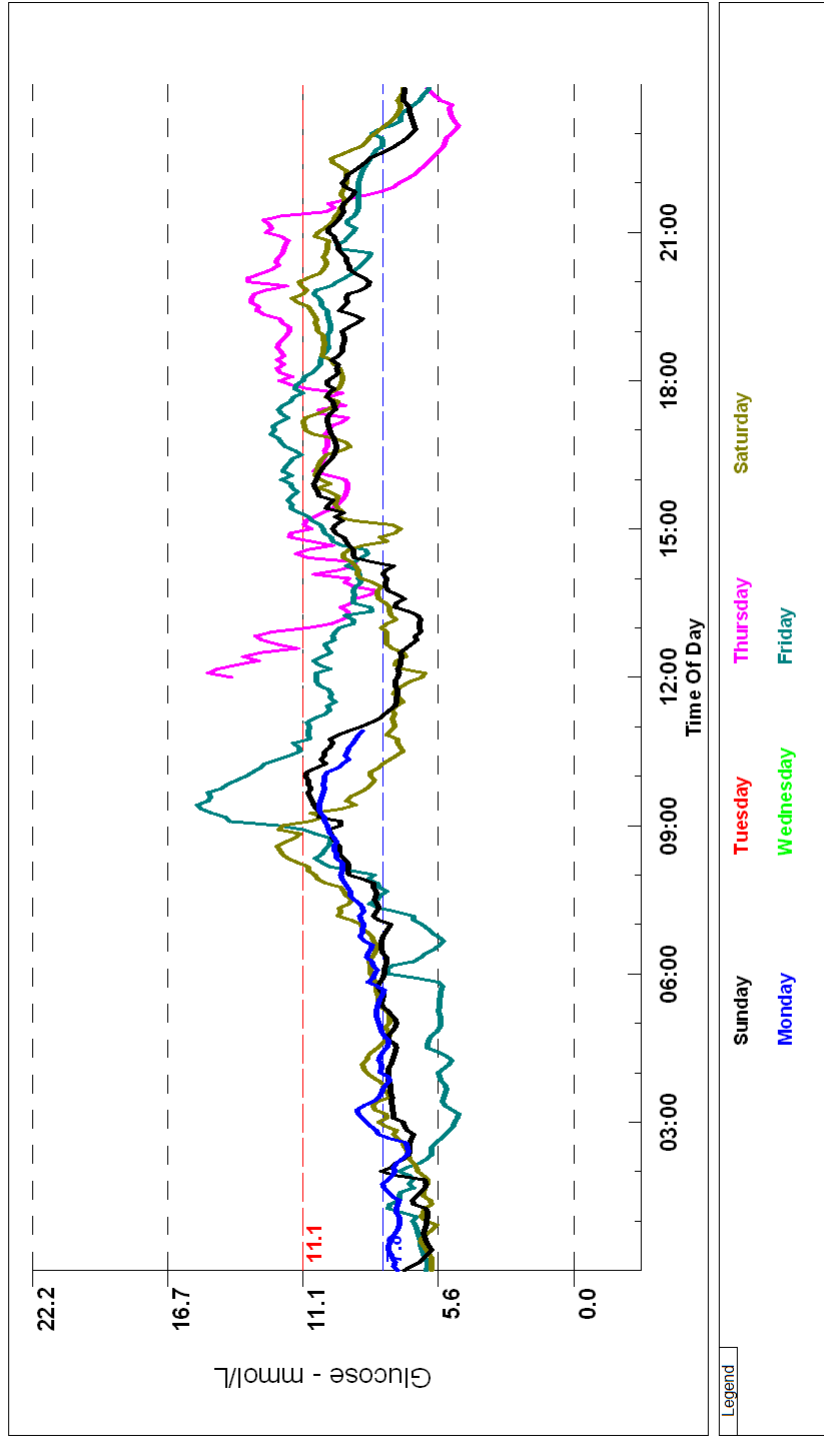
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-006b

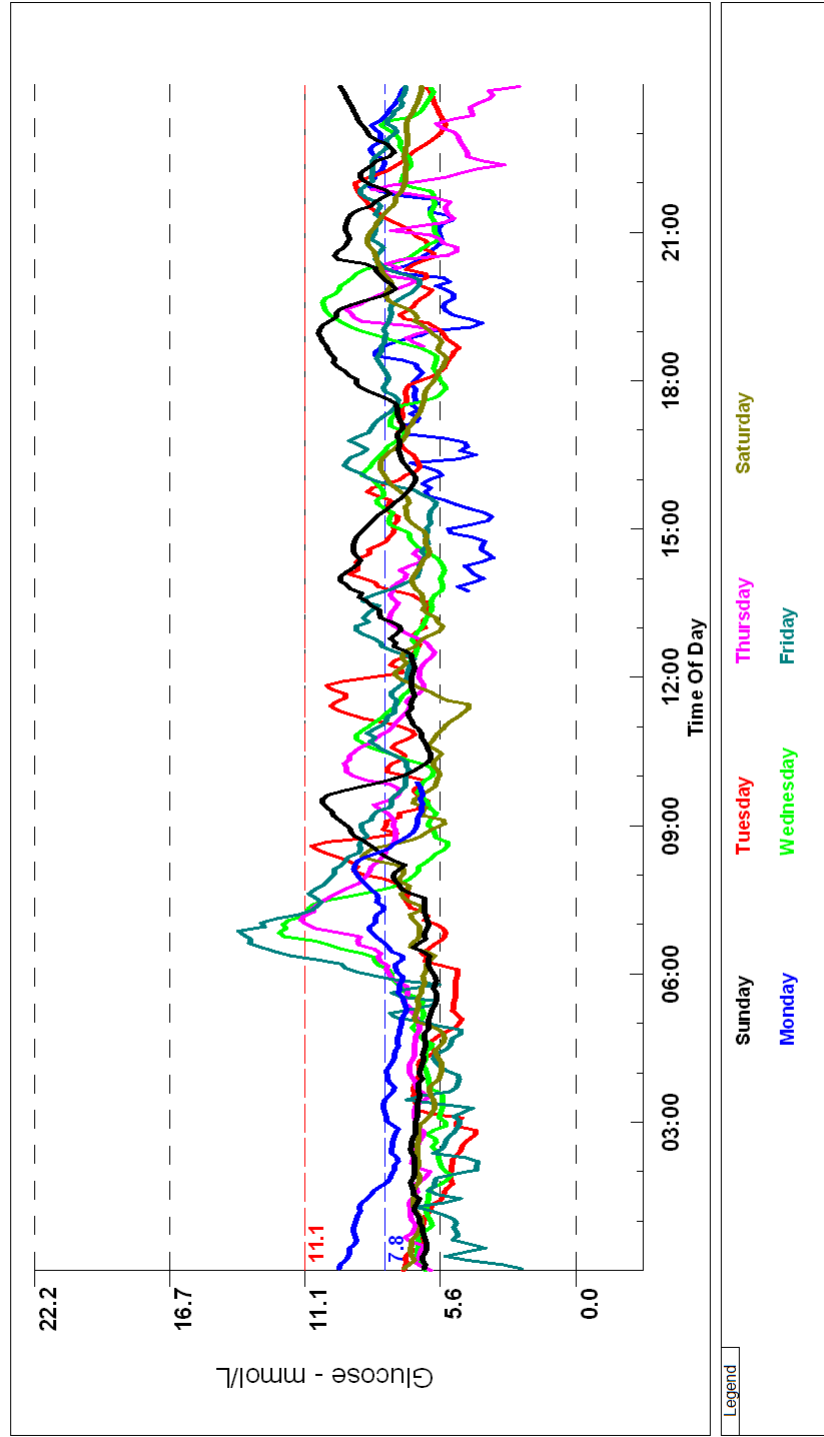
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-010a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

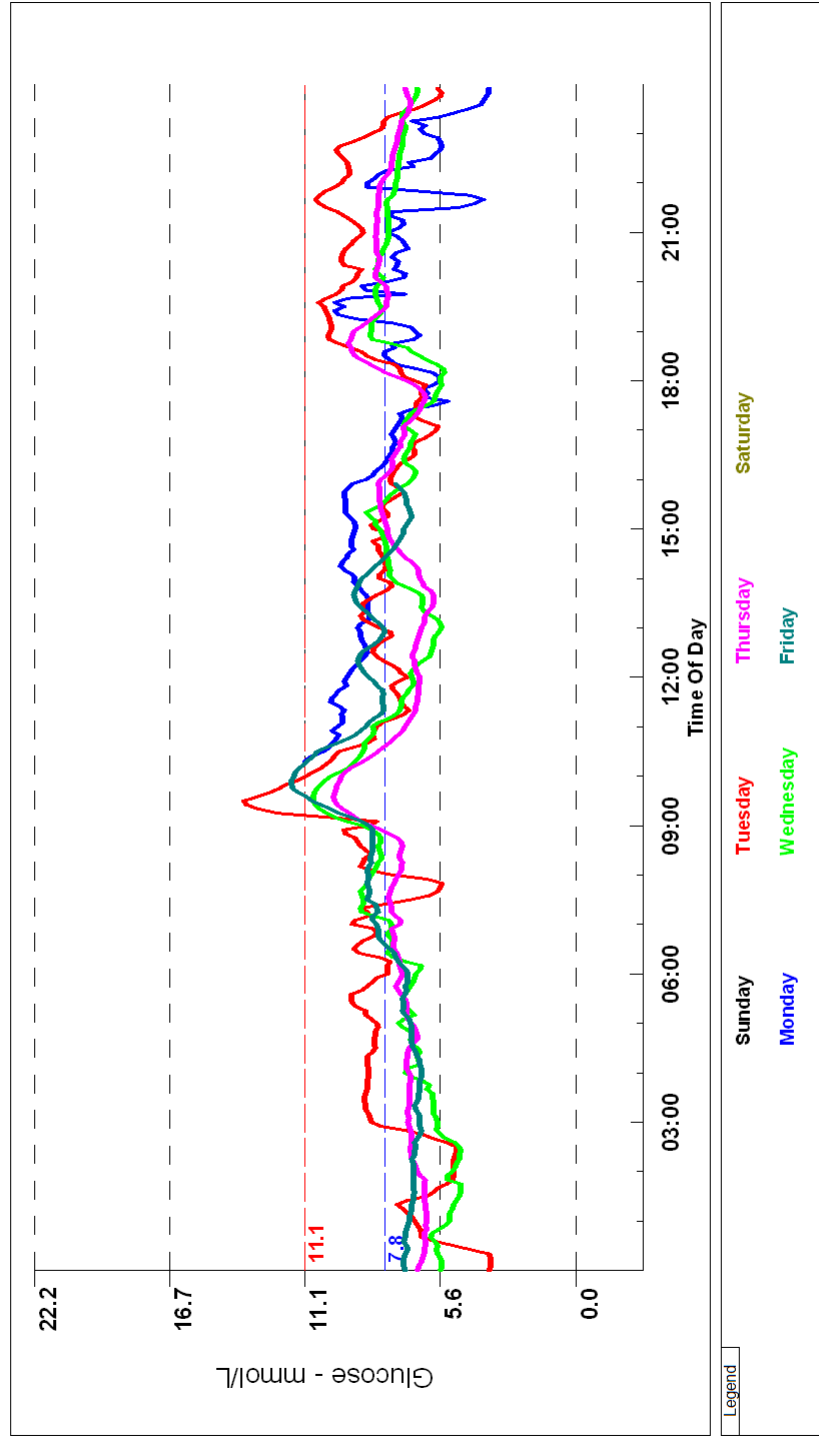




Sensor Modal Day

Patient:  
ID: ipro-011b

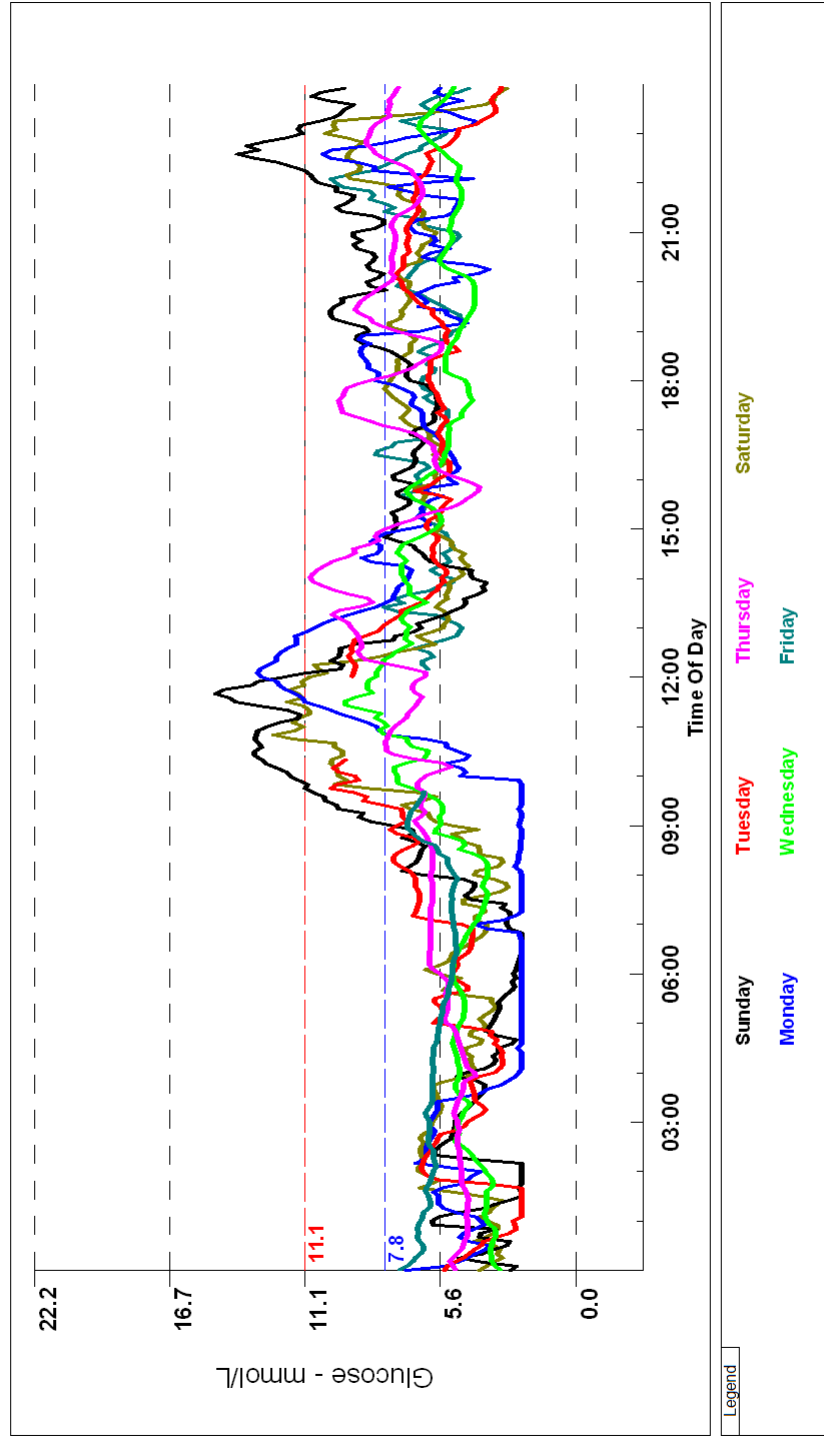
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-014a

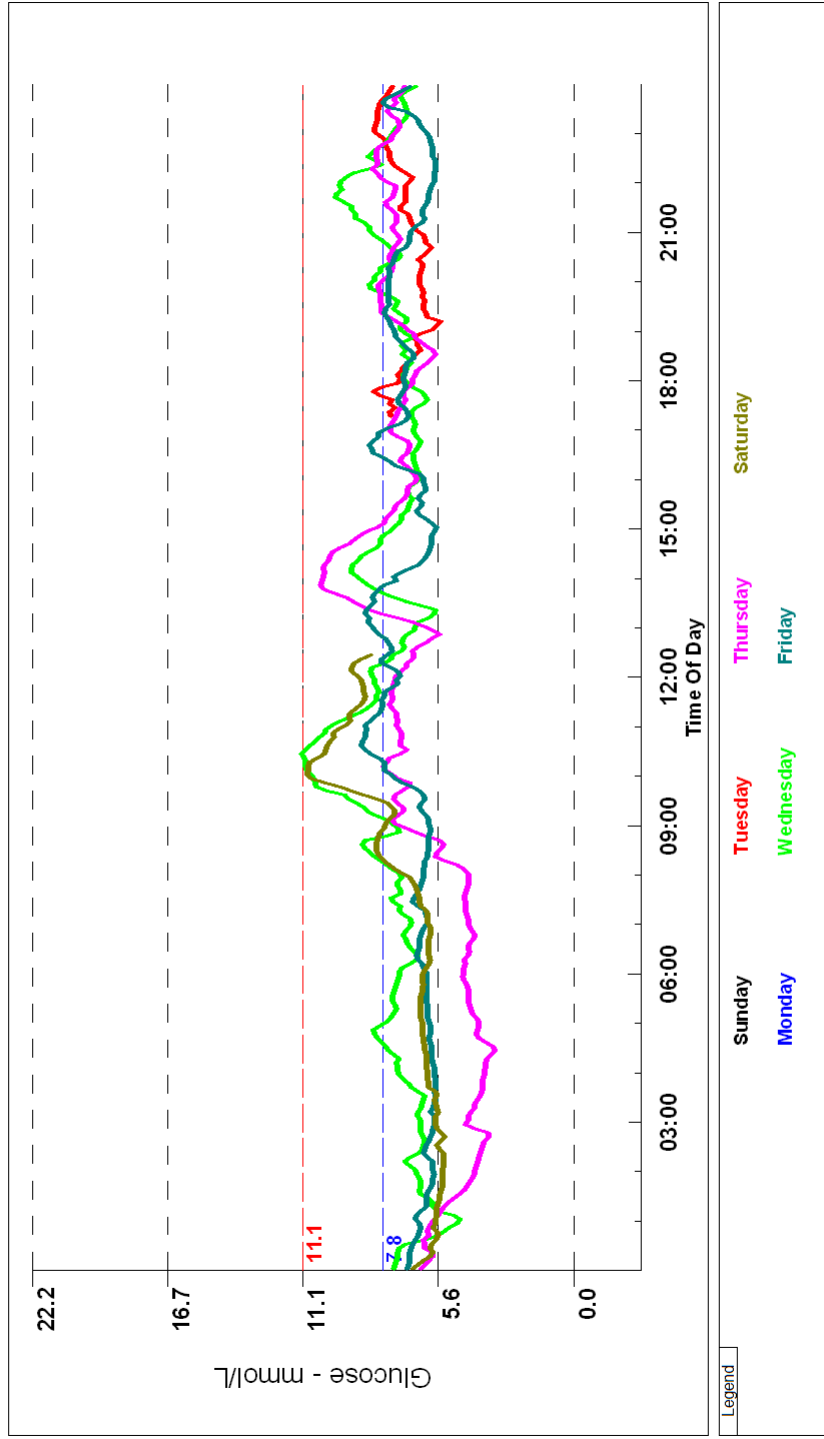
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-014b

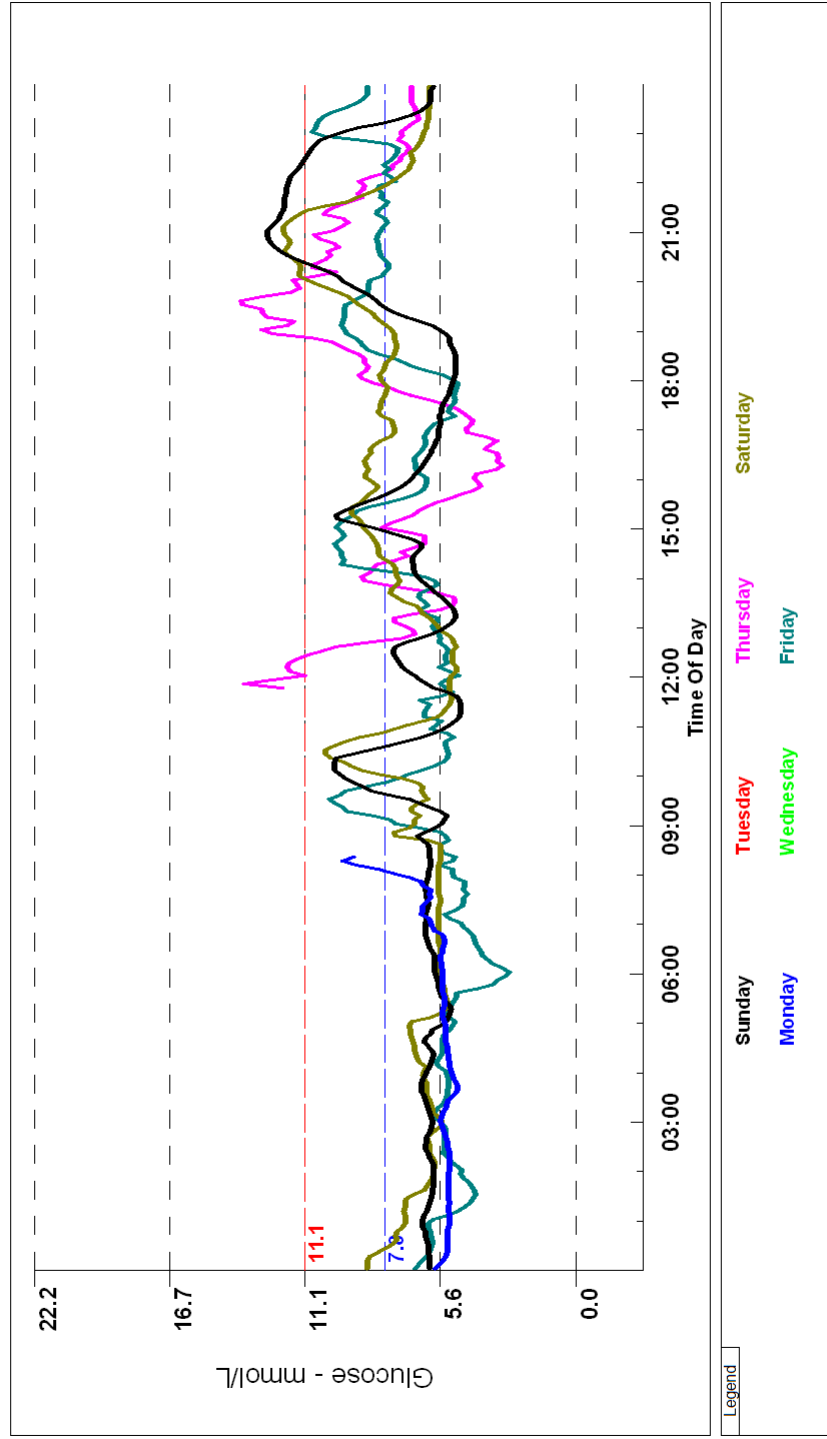
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-018b

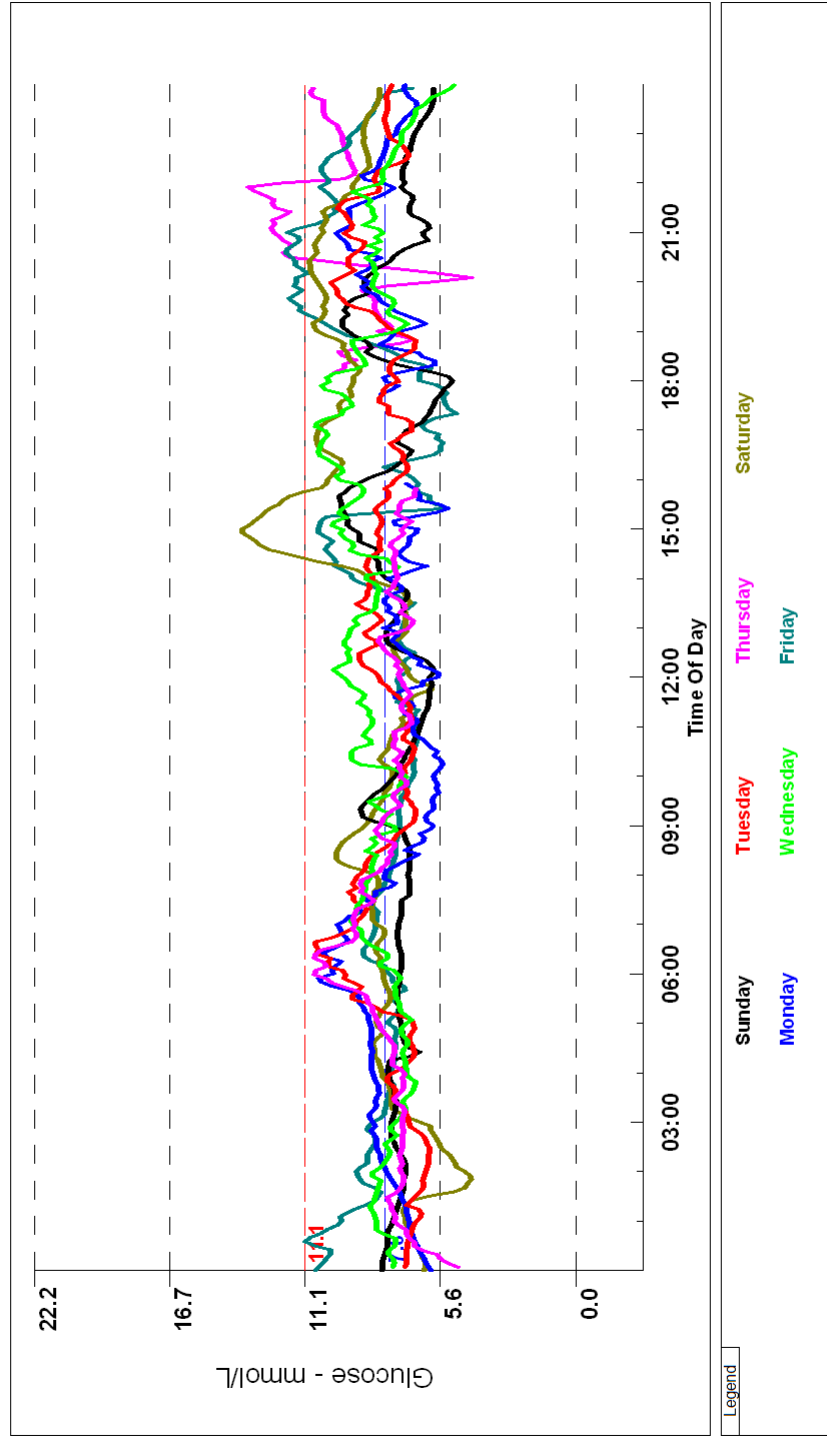
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-019a

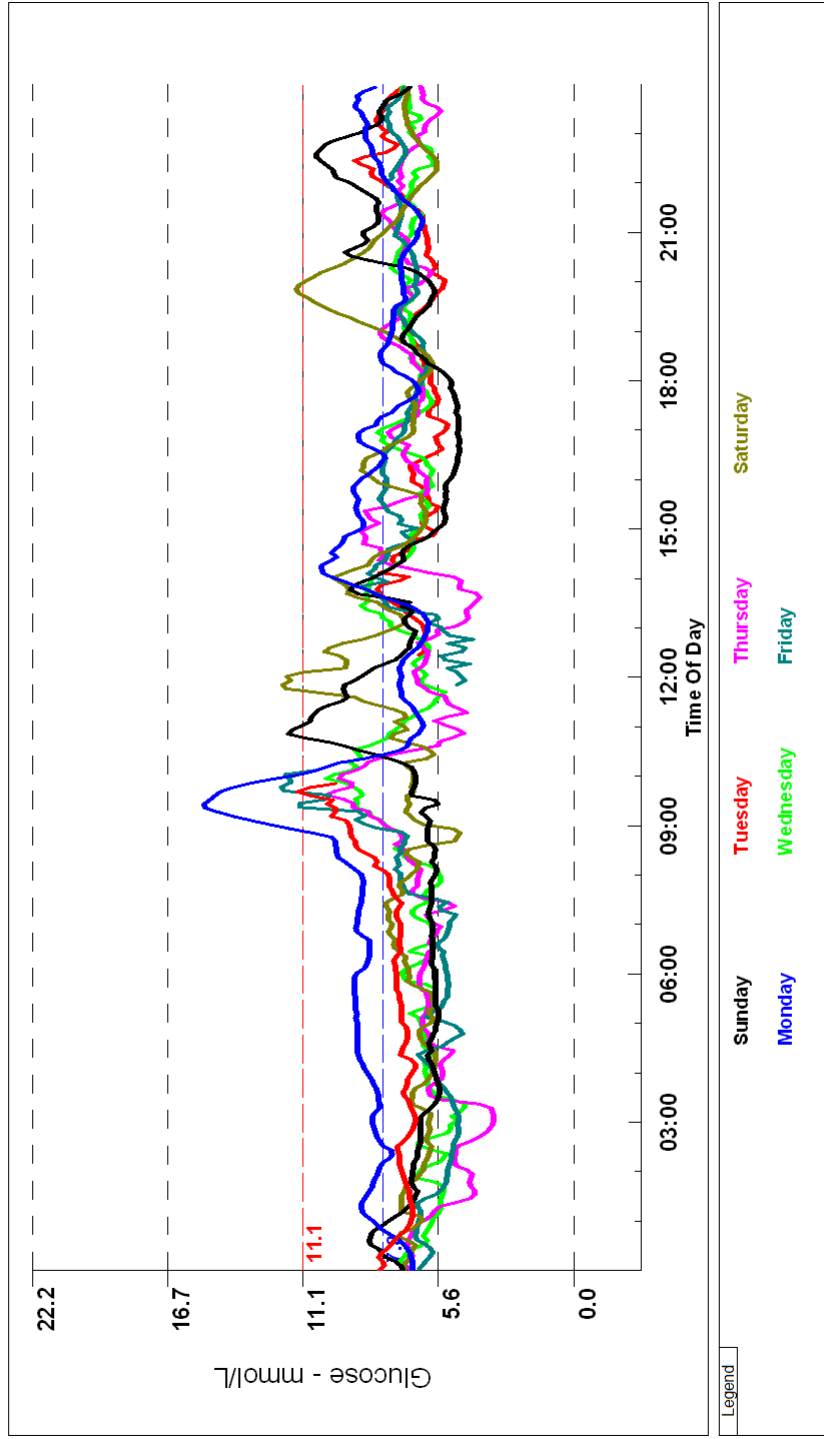
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-021a

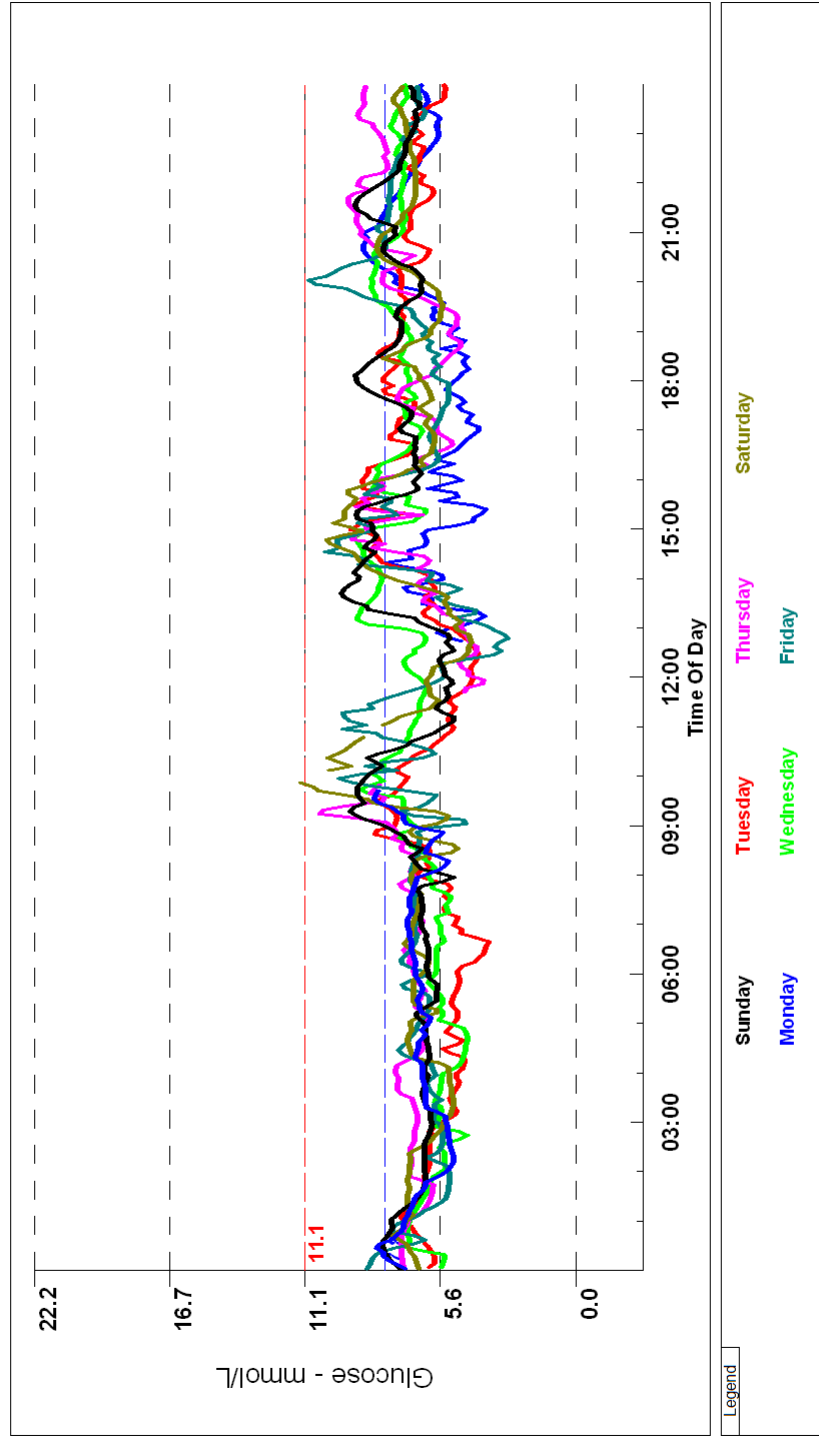
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-022a

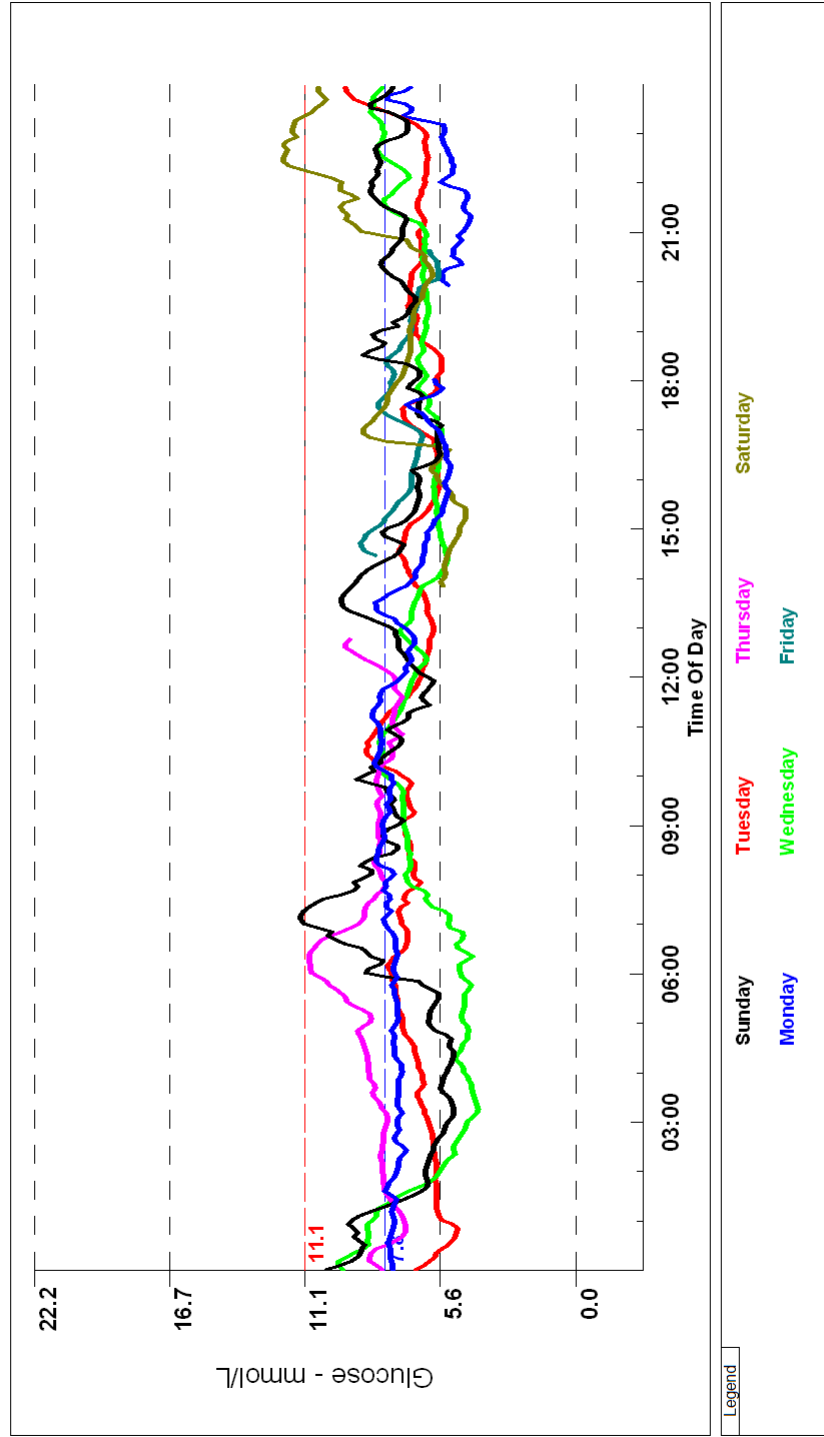
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-24a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

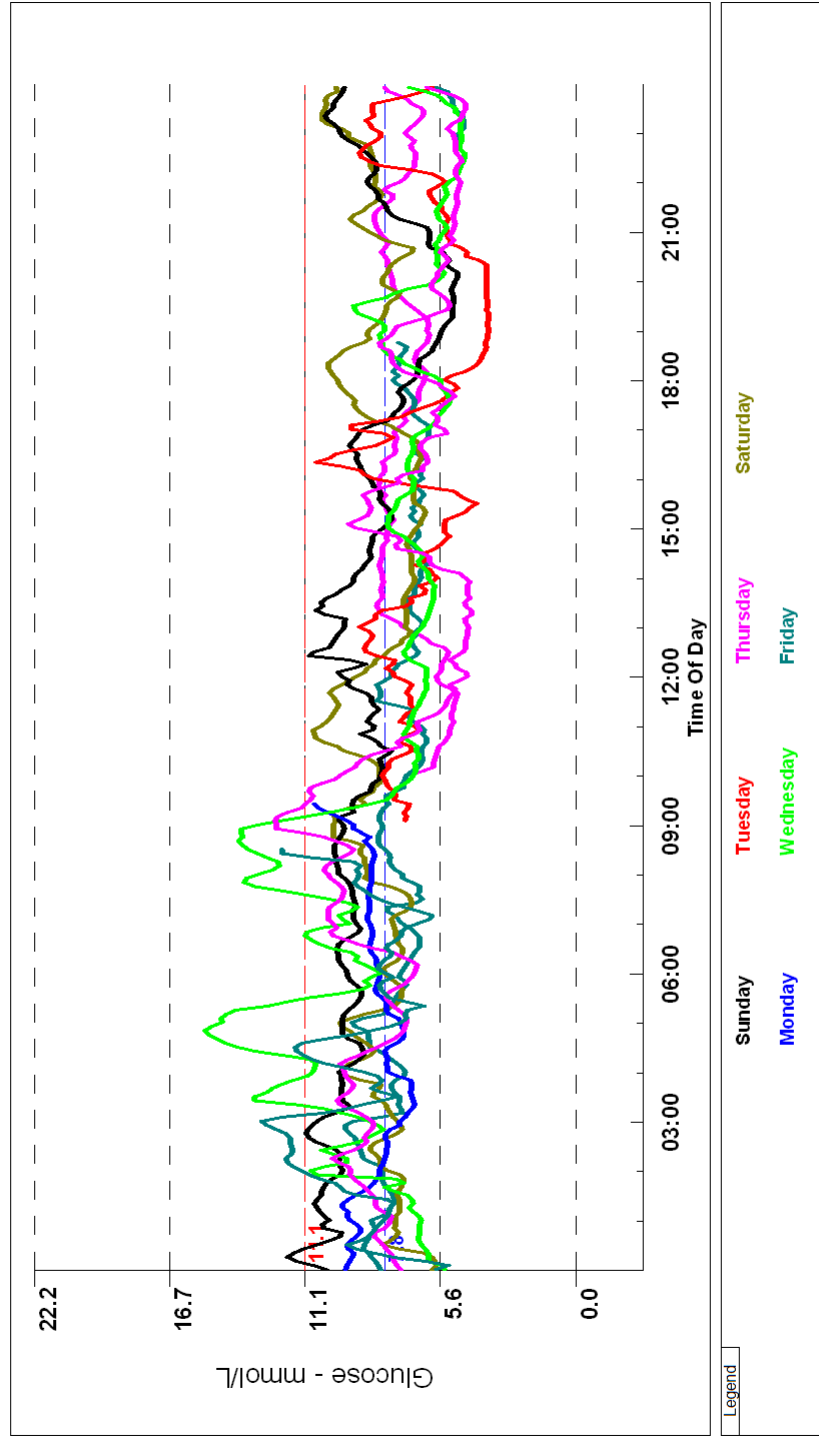




Sensor Modal Day

Patient:  
ID: ipro-028a

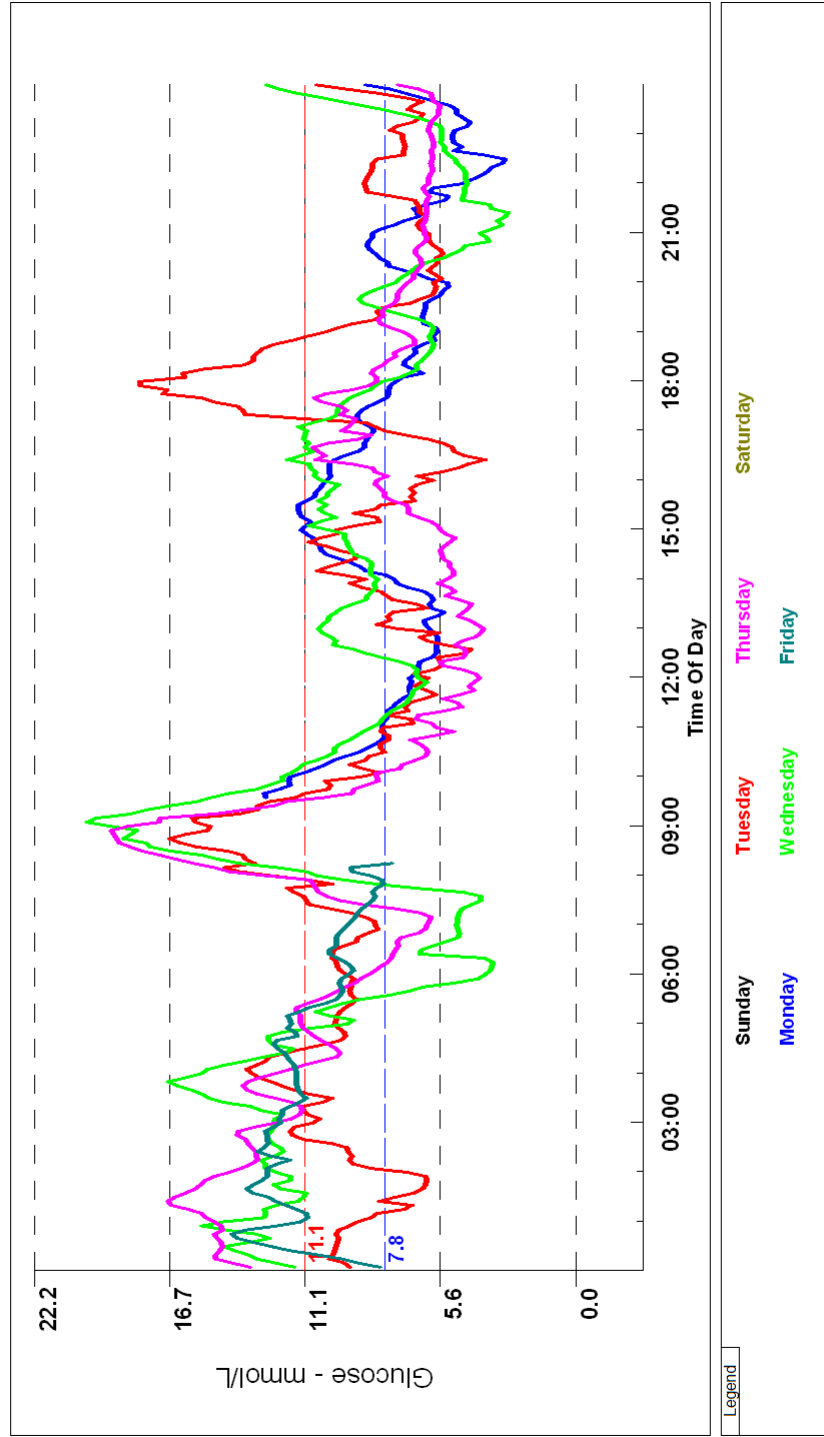
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-028b

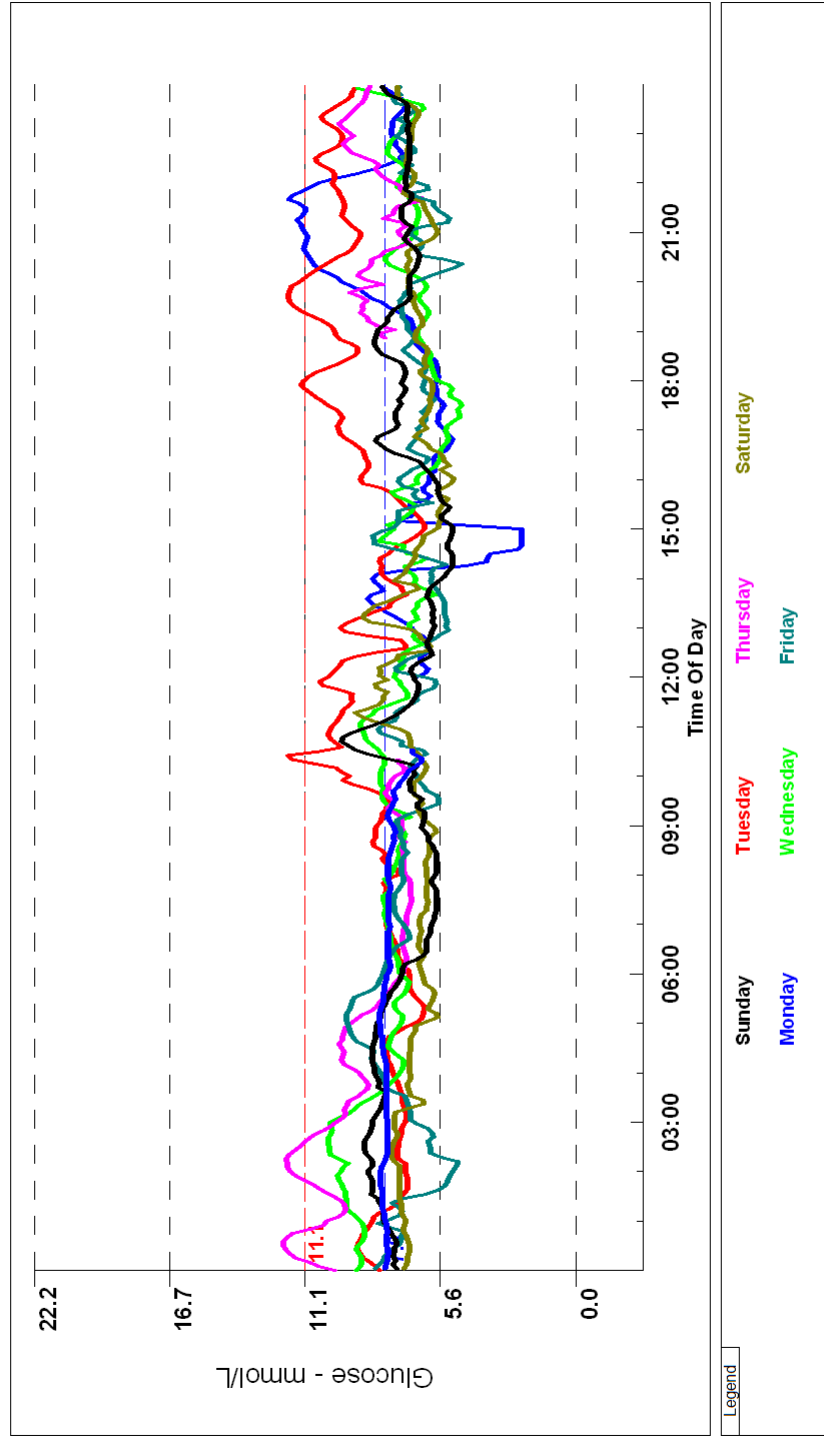
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-031a

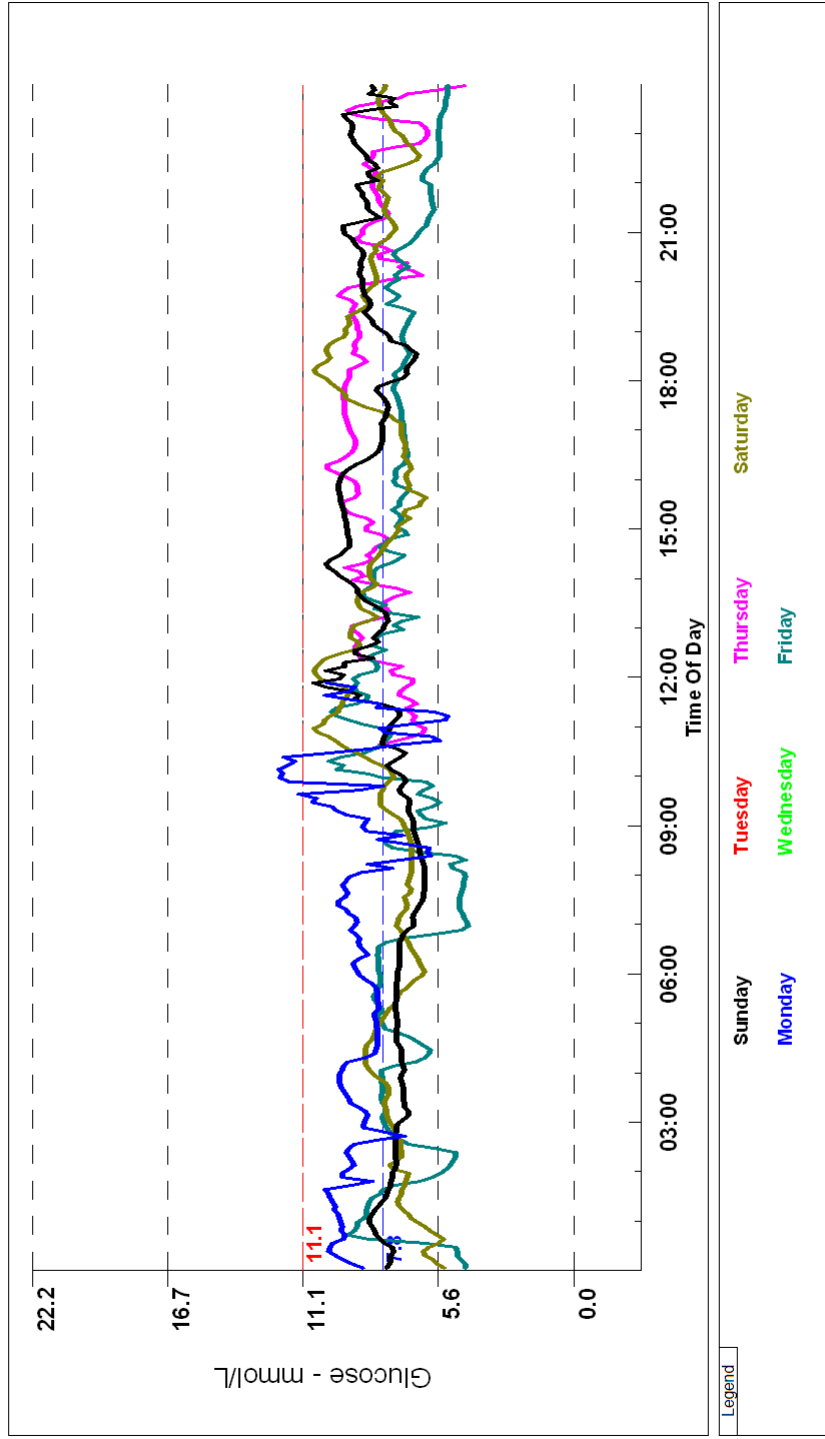
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-031b

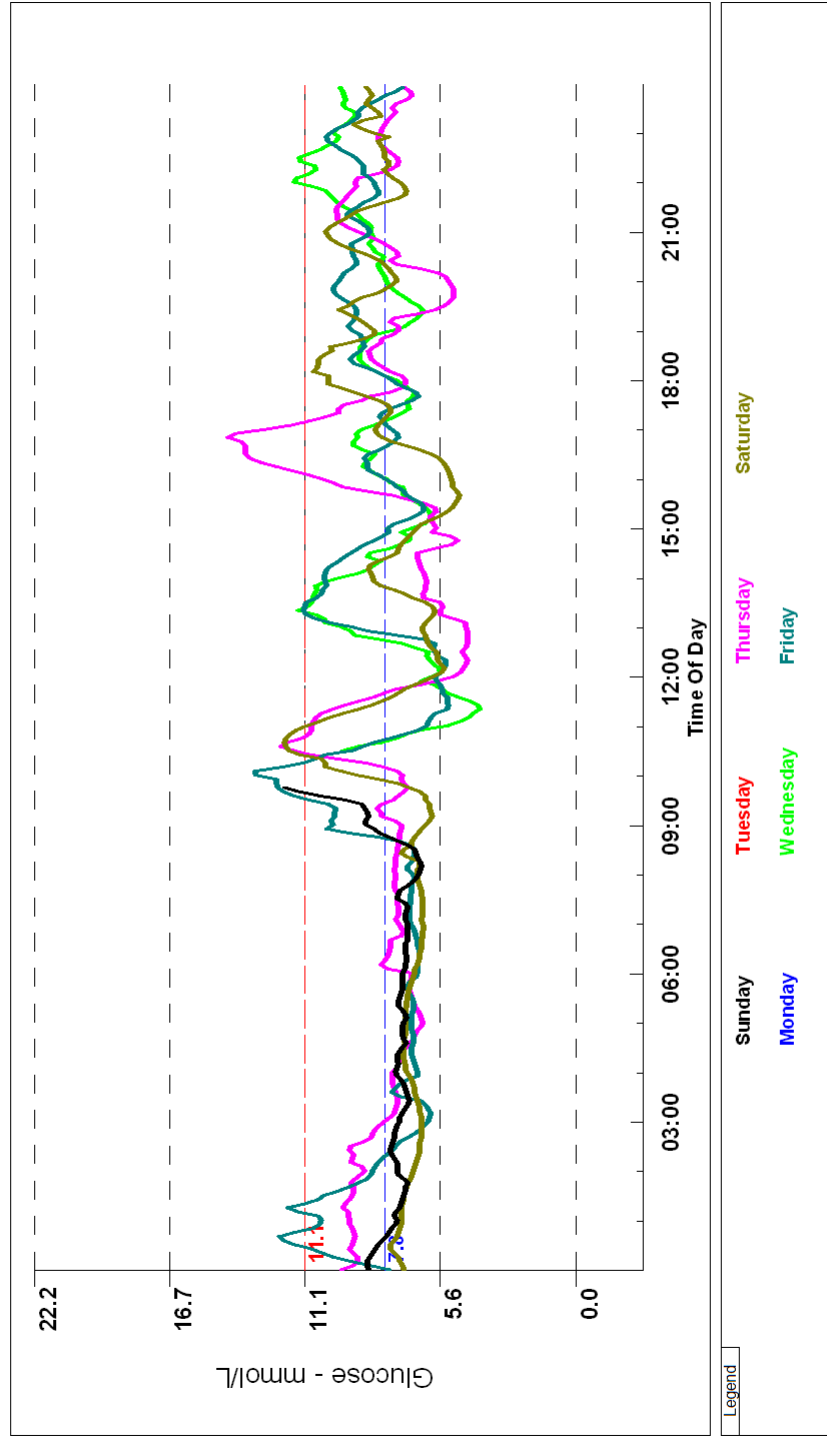
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-035b

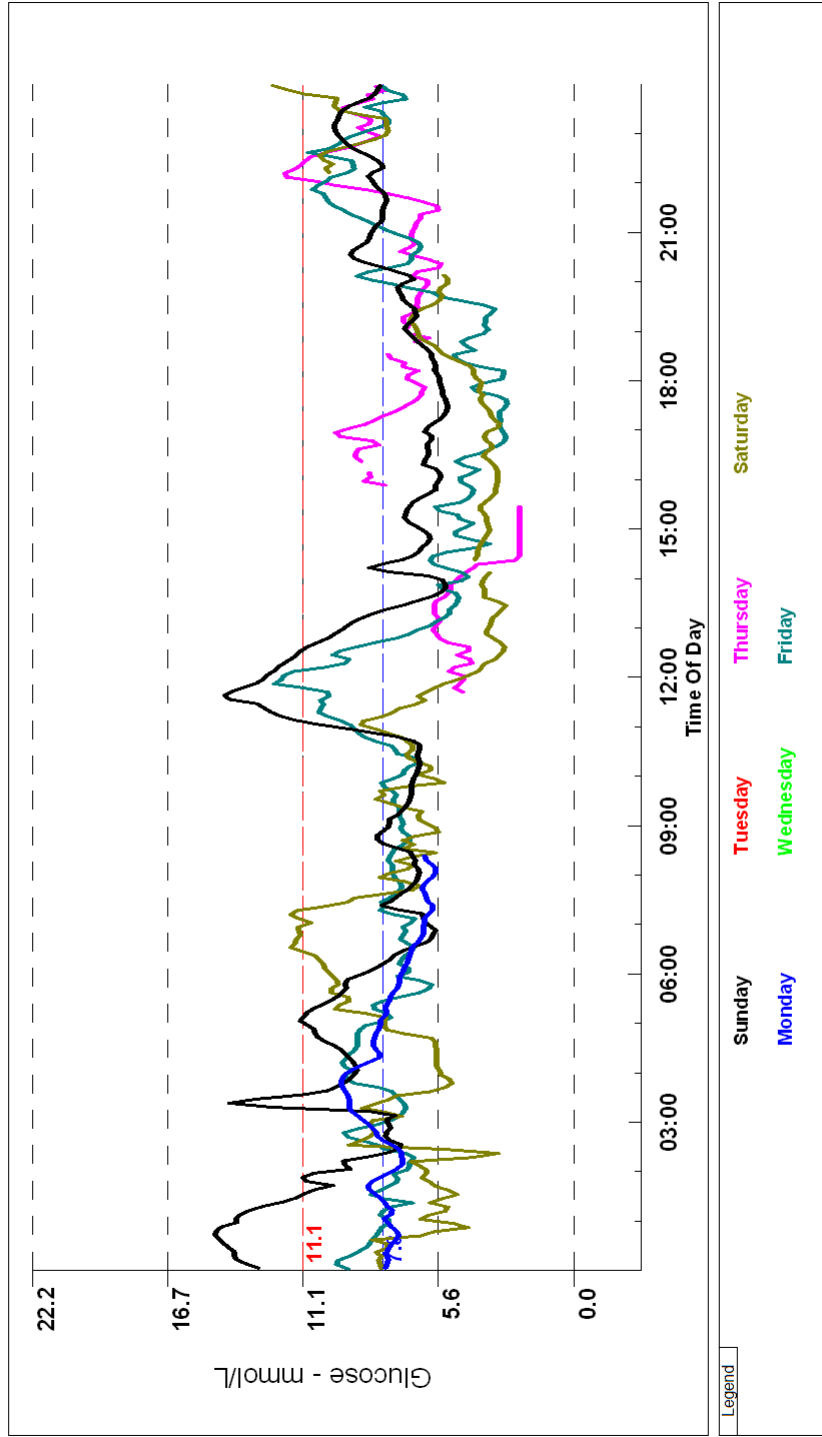
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-036b

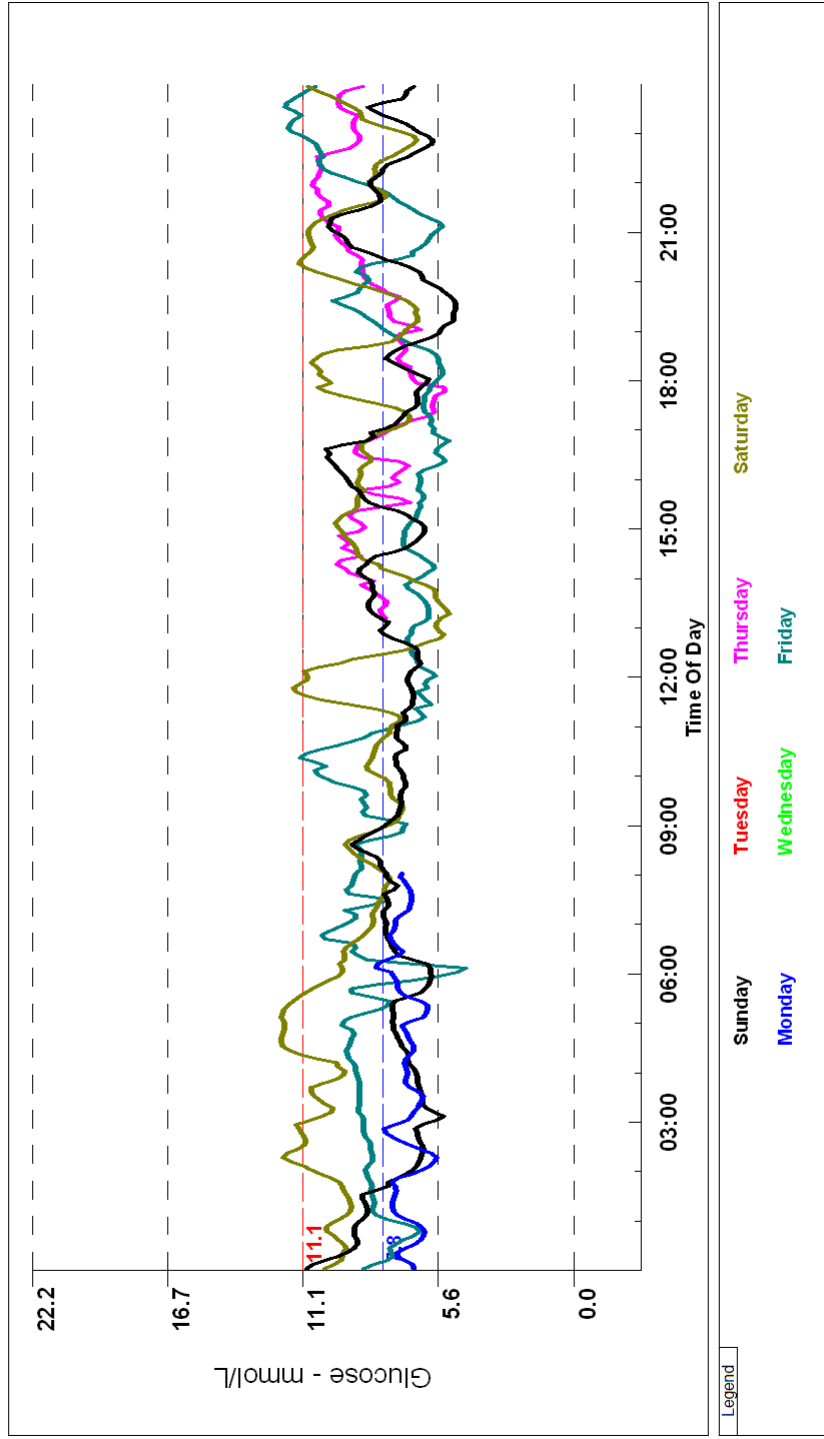
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-038b

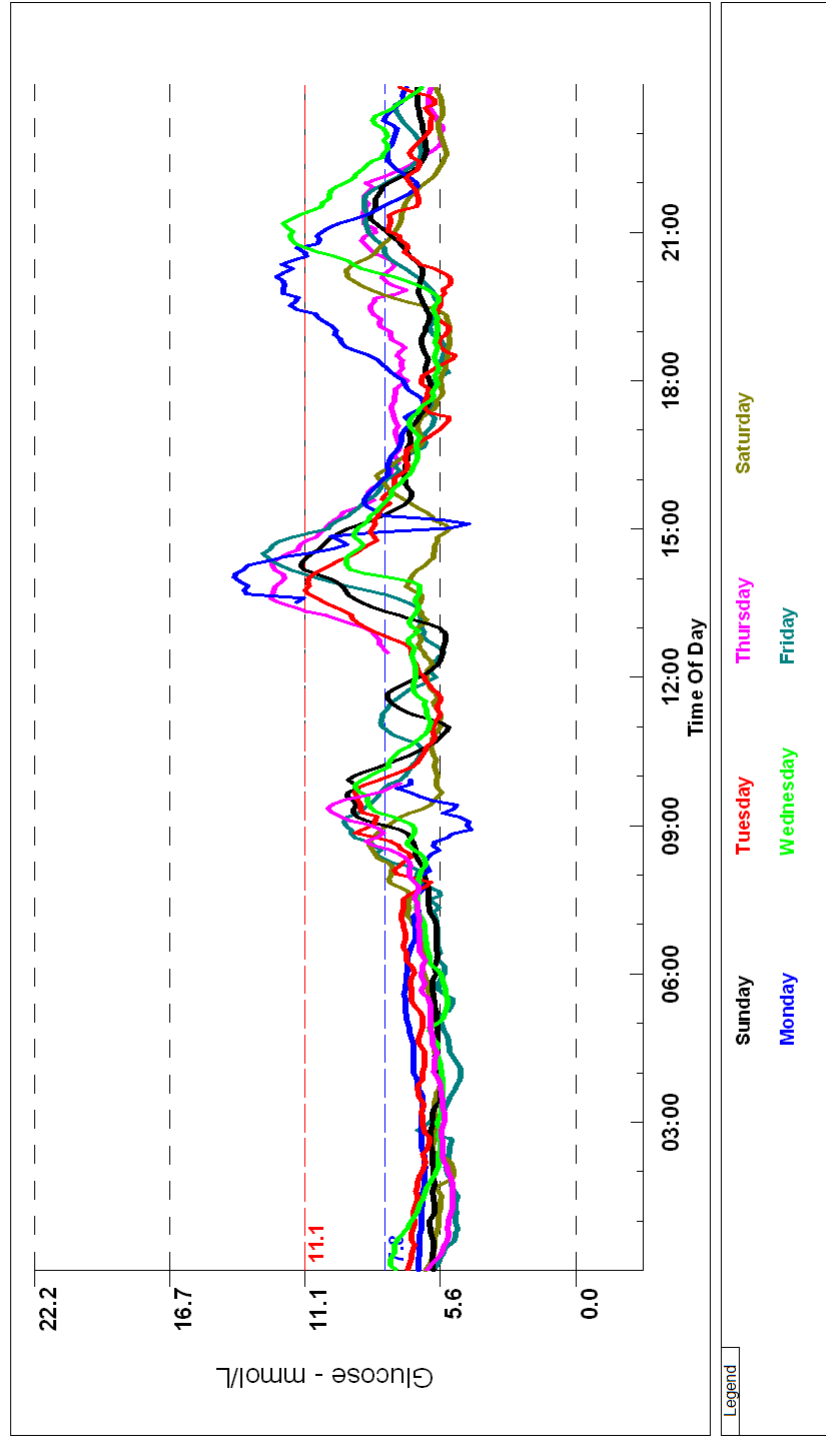
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-039a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

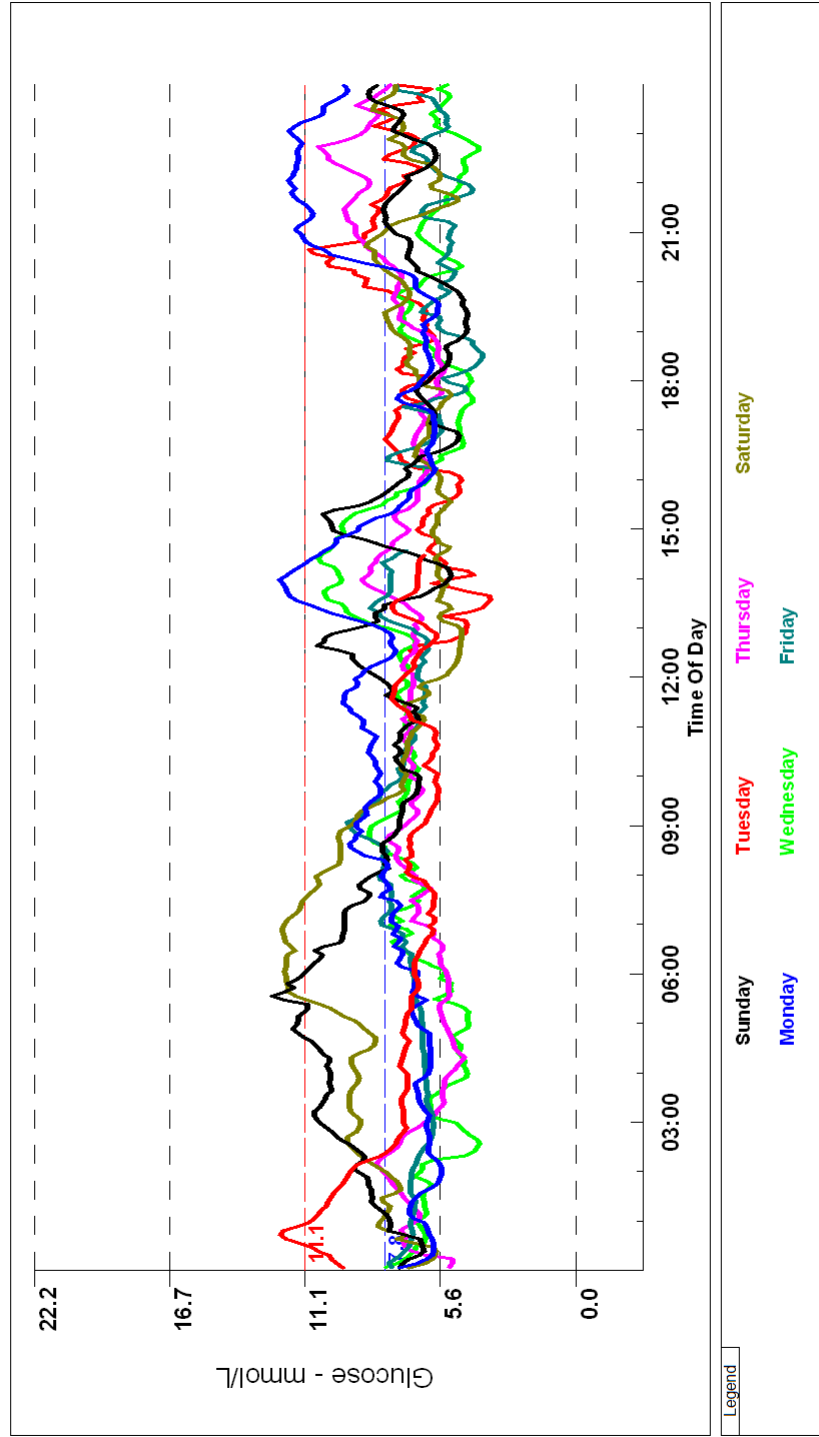




Sensor Modal Day

Patient:  
ID: ipro-040a

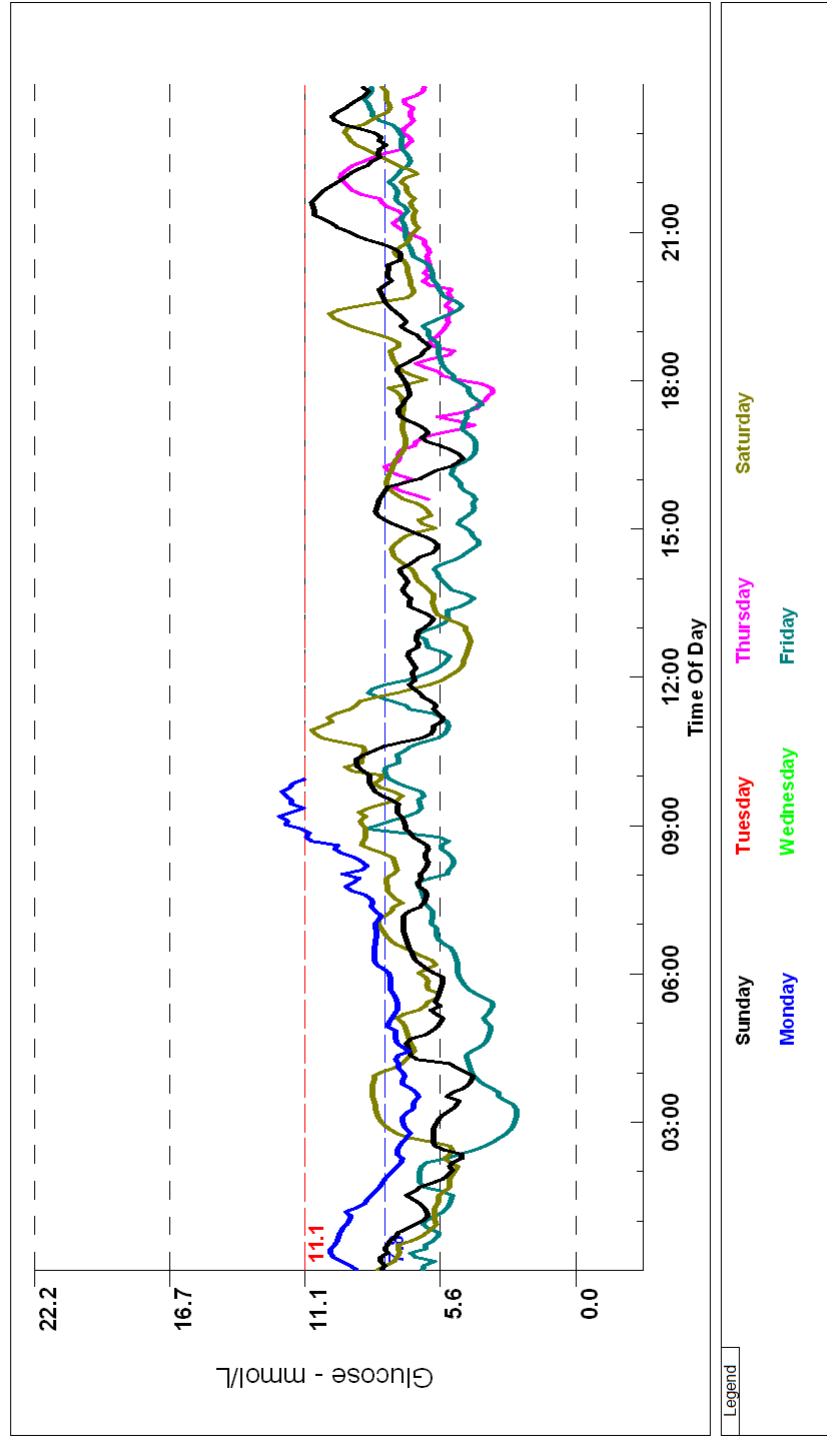
Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-042a

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A



Sensor Modal Day

Patient:  
ID: ipro-045b

Medtronic Solutions: CGMS iPro  
CGMS iPro 2.2A

