ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
«ΠΡΟΗΓΜΕΝΑ ΥΛΙΚΑ»
ΤΜΗΜΑ ΜΗΧΑΝΙΚΩΝ ΕΠΙΣΤΗΜΗΣ ΥΛΙΚΩΝ
ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ

ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΑΤΡΙΒΗ

ΓΟΓΟΛΟΥ ΓΕΩΡΓΙΟΥ

ΤΙΤΛΟΣ ΜΕΤΑΠΤΥΧΙΑΚΗΣ ΔΙΑΤΡΙΒΗΣ

ΚΙΝΗΤΑ ΣΥΣΤΗΜΑΤΑ ΠΑΡΑΚΟΛΟΥΘΗΣΗΣ ΑΣΘΕΝΩΝ ΜΕ ΔΙΑΒΗΤΗ

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Η παρούσα Μεταπτυχιακή Διατριβή εκπονήθηκε στο πλαίσιο των σπουδών για την απόκτηση του Μεταπτυχιακού Διπλώματος Ειδίκευσης στην εξειδίκευση:

Βιοϋλικα και Βιοϊατρική Τεχνολογία

που απονέμει το Τμήμα Μηχανικών Επιστήμης Υλικών του Πανεπιστημίου Ιωαννίνων.

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ΥΠΕΥΘΥΝΗ ΔΗΛΩΣΗ

"Δηλώνω υπεύθυνα ότι η παρούσα διατριβή εκπονήθηκε κάτω από τους διεθνείς ηθικούς και ακαδημαϊκούς κανόνες δεοντολογίας και προστασίας της πνευματικής ιδιοκτησίας. Σύμφωνα με τους κανόνες αυτούς, δεν έχω προβεί σε ιδιοποίηση ξένου επιστημονικού έργου και έχω πλήρως αναφέρει τις πηγές που χρησιμοποίησα στην εργασία αυτή."

(Υπογραφή υποψηφίου)

ΠΡΟΛΟΓΟΣ

Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο του Προγράμματος Μεταπτυχιακών Σπουδών με τίτλο Προηγμένα Υλικά του τμήματος Μηχανικών Επιστήμης Υλικών του Πανεπιστημίου Ιωαννίνων. Η διπλωματική εργασία έλαβε εν μέρει χώρα στο εργαστήριο Ιατρικής Τεχνολογίας και Ευφυών Πληροφοριακών Συστημάτων του τμήματος Μηχανικών Επιστήμης Υλικών του Πανεπιστημίου Ιωαννίνων και εν μέρει στο ιατρείο Σακχαρώδη Διαβήτη της Β΄ Παθολογικής Κλινικής του Πανεπιστημιακού Νοσοκομείου Ιωαννίνων την χρονική περίοδο 2015 - 2016.

Με την ολοκλήρωση της διπλωματικής μου εργασίας θα ήθελα να ευχαριστήσω τον επιβλέποντα της εργασίας μου Καθηγητή κ. Δημήτριο Φωτιάδη που με έκανε δεκτό στην κατεύθυνση της Βιοϊατρικής Τεχνολογίας καθώς επίσης και για το ενδιαφέρον που έδειξε στη διάρκεια των σπουδών μου αλλά και για την καθοδήγησή του κατά τη διάρκεια εκπονήσεως της διπλωματικής μου εργασίας βοηθώντας με να ανοίξω νέους ερευνητικούς ορίζοντες σε ένα ενδιαφέρον μεν, αλλά άγνωστο αρχικώς κομμάτι της επιστήμης.

Επίσης θα ήθελα να ευχαριστήσω την υποψήφια διδάκτορα του τμήματος Μηχανικών Υπολογιστών και Πληροφορικής του Πανεπιστημίου Ιωαννίνων κα. Ελένη Γεώργα που με προμήθευσε με την απαραίτητη βιβλιογραφία αλλά και για τις συμβουλές της σχετικά με την πορεία της εργασίας, όπως επίσης θα ήθελα να ευχαριστήσω και τα υπόλοιπα μέλη του εργαστηρίου για την συμπαράστασή τους και για τις συμβουλές τους. Τέλος, θα ήθελα να ευχαριστήσω τον κ. Ευάγγελο Ρίζο, ιατρό στο Πανεπιστημιακό Νοσοκομείο Ιωαννίνων που μου επέτρεψε να συμμετάσχω στη διαδικασία των μετρήσεων που πραγματοποιήθηκε σε διαβητικούς ασθενείς και εν συνεχεία να συλλέξω τα απαραίτητα δεδομένα που απετέλεσαν τον κορμό της διατριβής.

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ΠΕΡΙΛΗΨΗ

Ο σακχαρώδης διαβήτης αποτελεί μια χρόνια μεταβολική νόσο που εμφανίζεται ξαφνικά δίχως να κάνει διακρίσεις σε ηλικία ή φύλο. Αποτελεί μια νόσο γνωστή στην ανθρωπότητα εδώ και χιλιάδες χρόνια, άλλωστε το όνομα της νόσου αυτής προέρχεται από τους αρχαίους Έλληνες. Τα συμπτώματα του διαβήτη έχουν επιπτώσεις στην καθημερινότητα του ασθενούς οδηγώντας σε κάποιες περιπτώσεις σε ανεπιθύμητες καταστάσεις και σοβαρές επιπλοκές.

Σύμφωνα με τον παγκόσμιο οργανισμό υγείας οι ασθενείς με σακχαρώδη διαβήτη αναμένεται να φτάσουν τους 340 εκατομμύρια ασθενείς μέχρι το 2030. Για το λόγο αυτό η παγκόσμια επιστημονική κοινότητα αλλά και ο παγκόσμιος οργανισμός υγείας κρούουν τον κώδωνα του κινδύνου. Διαρκώς έρευνες και μελέτες παρουσιάζουν νέα αποτελέσματα για τον έλεγχο και τη θεραπεία του διαβήτη προσφέροντας ελπίδα στους εκατομμύρια ασθενείς ανά τον κόσμο. Η παρούσα έρευνα εκπονήθηκε πρωτίστως στο χώρο του εργαστηρίου Ιατρικής Τεχνολογίας και Ευφυών Πληροφοριακών Συστημάτων του τμήματος Μηχανικών Επιστήμης Υλικών και δευτερευόντως στο χώρο του Πανεπιστημιακού Νοσοκομείου της πόλης μας. Ασθενείς με σακχαρώδη διαβήτη τύπου 1 συμμετείχαν σε μια διαδικασία μετρήσεων της συγκέντρωσης της γλυκόζης τους σε εικοσιτετράωρη βάση σε εβδομαδιαίο επίπεδο για δύο εβδομάδες σε συνδυασμό με εικοσιτετράωρη παρακολούθηση της σωματικής τους δραστηριότητας επίσης σε εβδομαδιαίο επίπεδο. Η διάρκεια της διαδικασίας των μετρήσεων ήταν δυο εβδομάδες για κάθε ασθενή. Την πρώτη εβδομάδα οι ασθενείς απέχουν από οποιαδήποτε μορφή σωματικής άσκησης ενώ την δεύτερη εβδομάδα τους υποδεικνύεται να ασκούνται καθημερινώς για τουλάχιστον τριάντα λεπτά με μία ώρα την ημέρα με ελαφρύ τρέξιμο ή γρήγορο βάδην. Η παρακολούθηση της γλυκόζης αλλά και της φυσιολογικής δραστηριότητας διασφαλίζεται με τη βοήθεια συσκευών συνεχούς καταγραφής. Συνεπώς ο απώτερος στόχος της παρούσας έρευνας είναι η μελέτη και εξαγωγή συμπερασμάτων σχετικά με την επίδραση της σωματικής άσκησης στη διακύμανση της γλυκόζης. Τα αποτελέσματα μπορούν να χρησιμοποιηθούν στη συνέχεια σε μοντέλα πρόβλεψης.

Στο πρώτο κεφάλαιο γίνεται αναφορά στη φυσιολογία του διαβήτη, τους τύπους διαβήτη αλλά και στα ιστορικά δεδομένα που διαθέτει η επιστημονική κοινότητα στα χέρια της όσον αφορά τη μεταβολική αυτή νόσο. Στη συνέχεια

αναφέρονται και περιγράφονται τα ανατομικά στοιχεία του παγκρέατος καθώς επίσης περιγράφεται και ο ρόλος της γλυκόζης στο ανθρώπινο σώμα.

Στο δεύτερο κεφάλαιο γίνεται λεπτομερής αναφορά στους αισθητήρες γλυκόζης όσον αφορά το είδος, τη λειτουργία και τα τεχνικά χαρακτηριστικά. Επίσης παρουσιάζεται η χρησιμότητά τους στη συνεχή παρακολούθηση της γλυκόζης αλλά και η εξέλιξή τους από τον πρώτο αισθητήρα του καθηγητή Leland C. Clark μέχρι το τι μέλλει γενέσθαι στην τεχνολογία και χρήση των αισθητήρων.

Στο τρίτο κεφάλαιο όπου και ξεκινά το κυρίως μέρος της εργασίας παρουσιάζονται τα χαρακτηριστικά των ασθενών που συμμετείχαν στη διαδικασία των μετρήσεων, οι συσκευές που χρησιμοποιήθηκαν στην ανωτέρω διαδικασία καθώς επίσης και το πρωτόκολλο που χρησιμοποιήθηκε και εγκρίθηκε από την επιστημονική επιτροπή του Πανεπιστημιακού Νοσοκομείου Ιωαννίνων για να πραγματοποιηθεί η παραπάνω διαδικασία.

Στο τέταρτο κεφάλαιο γίνεται αναφορά στα μέτρα που χρησιμοποιήθηκαν στα πλαίσια της ανάλυσης των δεδομένων ενώ στο πέμπτο κεφάλαιο παρουσιάζονται τα αποτελέσματα της επεξεργασίας και στατιστικής ανάλυσης των δεδομένων, αρχικώς με μορφή πινάκων και στη συνέχεια με μορφή διαγραμμάτων. Στη συνέχεια γίνεται αναφορά στα μέτρα που χρησιμοποιήθηκαν στη διαδικασία της ανάλυσης συσχέτισης των σημάτων φυσιολογικής δραστηριότητας και συγκέντρωσης της γλυκόζης και παρουσιάζονται τα αποτελέσματα αυτής οδηγώντας στα τελικά συμπεράσματα της έρευνας αυτής. Στο έκτο κεφάλαιο παρουσιάζονται τα συνολικά συμπεράσματα της παρούσας έρευνας.

Εν κατακλείδι, η παρούσα έρευνα εκπονήθηκε με στόχο να παρουσιάσει αποτελέσματα αναφορικά με τον συσχετισμό της φυσιολογικής δραστηριότητας με τη συγκέντρωση της γλυκόζης. Τα αποτελέσματα είναι ενθαρρυντικά, υπάρχουν ενδείξεις ότι η ήπια σωματική δραστηριότητα έχει θετική επίδραση στη διακύμανση της γλυκόζης καθώς δείχνει να περιορίζει τις τιμές της στο επιθυμητό εύρος μεταξύ [70 – 140] mg/dl. Μελλοντικά θα μπορούσε η παρούσα έρευνα σε μεγαλύτερη κλίμακα δοκιμών να επιφέρει ακόμη καλύτερα αποτελέσματα. Αναφορές και βιβλιογραφία παρουσιάζονται στο τέλος της εργασίας προκειμένου ο αναγνώστης να ανατρέξει και να αναζητήσει περαιτέρω στοιχεία σχετικά με την εργασία αυτή.

ABSTRACT

Diabetes mellitus is a chronic metabolic disease that appears suddenly without any discrimination on age or gender. It is a disease known to mankind since ancient times and its name comes from the ancient Greeks. The diabetes symptoms affect the patient's life, which in some cases leads to undesired situations and serious complications.

According to the World Health Organization (WHO), the diabetic population is expected to reach 340 million by 2030. The global scientific community and the world health organization sound alarm bells. Constant researches and studies show new outcomes regarding the control and treatment of diabetes, offering hope to millions of patients worldwide. This research was conducted, firstly, in the area of the Biomedical Technology and Intelligent Information Systems Laboratory of Materials Science and Engineering Department of Ioannina University and secondly in the area of our city's University Hospital. Patients with type 1 diabetes participated in a monitoring process of the concentration of glucose for 24 hours a day on a weekly basis for two weeks, in combination with monitoring their physical activity also on a weekly basis. The duration of the measurement procedure was two weeks for each patient. In the first week the patients must refrain from any form of physical exercise while in their second week, they are indicated to exercise daily for at least thirty minutes to an hour per day at a low tempo running or fast walking. The glucose monitoring and the physiological activity monitoring are ensured by means of continuous glucose monitoring devices and physiological activity monitoring devices, respectively. Thus, the ultimate goal of this thesis is to study and draw conclusions about the effect of exercise on glucose levels and variability. In turn, the results could be used in prediction models.

The first chapter refers to the physiology of diabetes, the types of diabetes, as well as to the historical data of the scientific community on this metabolic disorder. The anatomical details of the pancreas are described and in turn the role of glucose in the human body is described too.

In the second chapter, a detailed description of glucose sensor is given, regarding the type, operation and technical characteristics. Their utility is also shown in the continuous glucose monitoring and their evolution from the first sensor, of

Professor Leland C. Clark, to what will happen in the sensor technology in the close future is described.

In the third chapter, where the main part of the thesis begins, the characteristics of the patients who took part in the measurements procedure, the devices that are used in the above process, as well as the protocol which was used and in turn approved by the Scientific Committee of the University Hospital of Ioannina, are presented.

The fourth chapter refers to the measures used in the context of data analysis. The fifth chapter presents the results of data processing and statistical data analysis, initially in the form of tables and then in the forms of diagrams. Furthermore, there is reference to the measures used in the correlation process of analyzing the physiological activity signals and concentration of glucose and in turn the results are presented, leading to the final conclusions of this research in Chapter 6.

In conclusion, the present study has been prepared in order to present results regarding the correlation of physical activity with the glucose concentration. The results are encouraging; there is evidence that physical activity has an effect on the variation of glucose as it tends to limit its values in the desired range between [70 - 140] mg/dl. Future investigation on a wider scale trial could lead to more concrete results. References and bibliography are presented at the end of the thesis to the reader, to consult and seek further information on this work.

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1 DIABETES

1.1 ABSTRACT

Diabetes mellitus is a relatively common disease with chronic characteristics, accompanied by broad spectrum symptomatology. Diabetes mellitus is accompanied by serious complications, which affect the diabetic population's quality of life and their survival expectancy.

More specifically, diabetes mellitus constitutes a metabolic disorder characterized by an increase of the concentration of blood sugar, also known as hyperglycemia, and a disruption of glucose metabolism, either as a result of decreased insulin secretion or as a result of insulin's unsatisfactory activity. The disturbances in the metabolism of carbohydrates, fats and proteins, which are found in diabetes mellitus, are caused by the insufficient insulin action in target tissues (Nussey & Whitehead, 2001) (Diagnosis and Classification of Diabetes Mellitus, 2009).

There are number of different types of diabetes but the most common forms are, type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes (Gardner & Shoback, 2011). Diabetes mellitus is a chronic course and it has been shown that in some cases this mysterious metabolic disease could probably be responsible for the development of multiple complications including cardiovascular disease, renal failure, retinal damage, nerve damage, erectile dysfunction, etc. Insulin has been proven that plays a cardinal role in diabetes treatment and management. (Kitabchi A. E., Umpierrez, Miles, & Fischer, 2009).

Because of its nature, diabetes mellitus requires the active and continuous participation of diabetic individuals, their caregivers and physicians in order to be managed and well controlled (Georga, Protopappas, Bellos , & Fotiadis, 2014). The public's awareness regarding diabetes could help disease prevention, having a positive effect on health and the economy.

In Chapter 1 a wide scale reference is made concerning this metabolic chronic disease which plagues a large percentage of humanity. Firstly there is reference to the historical data of the disease and secondly to an analytical description about diabetes mellitus symptomatology and the types that diabetes mellitus appears. Subsequently, there is reference to the statistics regarding the spread of the disease in Greece,

Europe and its spread on a global scale too. Furthermore, Chapter 1 contains the essential characteristics of pancreas anatomy.

In conclusion, chapter 1 presents the ways of managing the disease and the medication that is required for the treatment of diabetes mellitus. Moreover, in chapter 1 the reader has the opportunity to visit the references and sources listed, which include further information for more details and examples.

1.2 PHYSIOLOGY OF DIABETES

Diabetes mellitus constitutes a chronic metabolic disorder affecting millions of people all over the world. These numbers increase day by day, leading to radical changes in the individual's daily routine, habits and lifestyle. Diabetes mellitus is considered by humanity a well-known and at the same time, mysterious disease since ancient years. For many years, physicians and scientists have been trying to achieve a better understanding of the origin of diabetes, as well as the diabetic genetic factors and consequently to find solutions regarding the better treatment and management of diabetes (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014).

Furthermore, if we want a better understanding regarding this metabolic disorder and give an answer to the question of what diabetes really is, it’s more prudent and wise to answer one more targeting question. What is the role of insulin in our body?

When someone eats, our body turns food into sugars. In this stage, our pancreas is supposed to release insulin, a hormone that is produced in the pancreas by β-cells. Insulin plays a key role, helping our cells to open up and in turn allowing glucose to enter in them. Consequently, the glucose is used from our cells giving us the desired energy to continue our daily life. In the diabetic population, during the above procedure, problems arise and this system does not function as it should and therefore due to low or zero insulin secretion, the cells run out of glucose (energy). As a result, this low or zero secretion can possibly lead to several undesired complications such as hyperglycemia or hypoglycemia (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014).

In conclusion, to answer the question in the above text of what diabetes is, it’s worth noting that diabetes can strike anyone, at any point in their life, leading to

dramatic changes in the individual's daily life. Scientists and researchers are trying to understand diabetes better with a broader goal of finding ways to fight, manage and treat diabetes effectively.

1.3 HISTORY OF DIABETES

Diabetes mellitus has a long history throughout time and constitutes a well-known disorder by physicians since ancient times. Diabetes mellitus constitutes one of the first observed and described diseases (Ripoll, Brian, Leutholtz, & Ignacio, 2011). More specifically in 1962 in a noble tomb in Luxor of Egypt the first reference to diabetes was found, which was dated from 1500 B.C. The famous Greek doctor Aretaeus from Cappadocia (200 – 120) B.C gave the name diabetes which derives from the verb 'διαβαίνω', meaning traverse in Greek, because when an ill person drank water, the water passed, without changing, through the urine. During the same period in China, the physician Li Hsuan observed that diabetics were prone to lung infections. Avoiding sex and alcohol were famous treatments by Li Hsuan. In India two physicians, famous in their time, Sushruta and Charaka were the first who observed and described the two major types of diabetes mellitus. The Arab doctor Avicenna (980 – 1037) A.D was the first doctor who described diabetic gangrene in the leg (Poretsky, 2009).

In the 17th century the term 'mellitus' was added by Thomas Willis, when it was discovered that urine had a sweet taste, something that was noticed by the ancient Greeks too (Mandal, 2012). In the following century (18th) the presence of glucose in urine constitutes an admission. A medical student in Berlin, Paul Langerhans (1847 – 1888 A.D) notes, with the use of a microscope, that piles of cells exist in a rabbit's pancreas which in turn, he named islets, a name that in Latin is known as insulae, a term that is used by the scientific community even nowadays (Brian, February 2004). In 1889, two doctors from Strasbourg, Minkowski and Mering proceeded to the removal of the pancreas from dogs and saw that the characteristic displayed symptoms of diabetes like polyuria and polydipsia appeared (Von Mehring & Minkowski, 1890). Subsequently, the Romanian doctor Paulescu published a pancreatic extract that could cure diabetes (Paulescu).

In 1921, two doctors from Toronto, Banting and Best discovered that lack of insulin causes diabetes. They isolated the islets of Langerhans from several types of

pancreas and with chemical treatment they made insulin which was given to their dying dog. In 1923, the team of Toronto won the Nobel Prize for this significant discovery. In January 1922, this insulin was granted for the first time in humans (Poretsky, 2009). The winner was the young Leonard Thompson, a fourteen years old child whose weight was 29 pounds when he saw the end approaching (Leonard Thompson Biography).

1.4 TYPES OF DIABETES MELLITUS

There are number of types of diabetes mellitus, striking anyone and at any point of life. According to the type of diabetes, treatment and management of this metabolic disorder can be adjusted appropriately, due to the fact that every type has its own characteristics and behavior. This is something that is mirrored in a diabetic's daily life and routine. The most known types of diabetes mellitus are described in detail, as it is presented in the next sections.

1.4.1 Diabetes Mellitus Type 1

Type 1 diabetes mellitus, is one of the major types of this metabolic disorder. Diabetes type 1 is usually an insulin dependent type and is characterized by the destruction of β-cells in pancreas, which in turn is responsible for the production of insulin resulting in lack of insulin secretion which can vary from total to minimal one. The sensitivity of cells to insulin is usually normal, especially in the early stages (Cihakova, MD, & PhD). Diabetes type 1 is the main cause of childhood diabetes, that's the reason that it has been given the nomenclature juvenile, but it also affects adults too (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014) (Aanstoot, et al., 2007). The destruction of pancreatic β-cells, in most cases, has an autoimmune etiology, meaning more specifically that our body's immune system attacks our own pancreas due to the fact that it mistakenly perceives the insulin producing cells as foreign and in turn proceeds to their destruction (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014). The largest percentage of patients with diabetes type 1 is detected in the circulation of one or more types of autoantibodies. However, in a small percentage of patients with diabetes type 1, these autoantibodies are not detectable and this type of diabetes is called idiopathic diabetes (Knip, et al., 2005).

Type 1 diabetes usually strikes quickly and often leads to the development of ketosis, if the deficit of insulin isn't treated. The patient totally depends on an exogenous form of insulin, so that the blood sugar is maintained at normal levels. The disease frequency varies from 8 to 17 per 100.000 persons in Northern Europe and in the U.S.A; the highest is about 35 per 100.000 persons in Scandinavia and the lowest is 1 per 100.000 persons in Japan and China (Kasper, et al., 2004). Type 1 diabetes mellitus is characterized as high risk diabetes by its insulin dependent nature and can be fatal unless it's treated with insulin. Insulin injection is the most common method of insulin administration, although in the last decades other methods, including insulin pumps and inhaled insulin, have successfully made their appearance (Perry, 2008).

Type 1 diabetes can be distinguished from diabetes type 2 through a measurement of C-peptide (Jones & Hattersley, 2013). Treatment should never be stopped since there is no indication of having any significant side effects in everyday activities, but it can be adequate if patient education, awareness, appropriate care and discipline in testing and dosing of insulin are followed according the guidelines (Roglic, et al., 2005).

However, the treatment still remains too complicated for many people. Complications probably can be associated with both low and high blood sugar levels, which are largely due to the abnormal way in which insulin is replaced. If the patient takes too much insulin then the body will burn too much glucose and as a result, the blood sugar falls to very low levels closely related to hypoglycemic events. These low blood sugar levels (Hypoglycemia) can lead to undesired conditions including seizures and unconsciousness and requires emergency treatment (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014). On the other side of the coin, if the patient takes too little insulin, the body will starve from the needed energy and in turn blood sugar levels will have an acute growth rate, leading to hyperglycemic events. High blood sugar (Hyperglycemia) can result in increased fatigue and long-term damage to organs (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014) (Roglic, et al., 2005). The amount of the replaced insulin is linked to several daily life factors including food, stress, exercise, the emotional state and general health of the individuals. So, it is very important to precisely calculate the amount of insulin that needs to be taken (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014).

1.4.1.1 Signs and symptoms of type 1 diabetes mellitus

Diabetes mellitus shows a wide scale of symptomatology, but the classic symptoms of diabetes type 1 can come on quickly and may include one or more of the following signs and symptoms:

- Polyuria, which constitutes a condition that, is characterized by frequent urination, usually greater than 2,5L -3L over 24 hours in adults (Shah, 2013).
- Polydipsia, which constitutes a condition that, is characterized by an increase feeling of thirst (Disease and Conditions: Type 1 Diabetes, 2014).
- Hyperphagia, which constitutes a condition that, is characterized by an increase feeling of appetite (Disease and Conditions: Type 1 Diabetes, 2014).
- Xerostomia (dry mouth syndrome), which constitutes a condition that is characterized by intense dryness in the mouth (Disease and Conditions: Type 1 Diabetes, 2014).
- Fatigue and weakness (Disease and Conditions: Type 1 Diabetes, 2014).
- Weight loss, usually with unintended character (Disease and Conditions: Type 1 Diabetes, 2014).
- Bedwetting, usually in children who previously didn't wet the bed during their nocturnal sleep (Disease and Conditions: Type 1 Diabetes, 2014).
- Blurred vision ability (Disease and Conditions: Type 1 Diabetes, 2014).
- Vaginal yeast infection in females (Disease and Conditions: Type 1 Diabetes, 2014).

Before a person realizes that he suffers from diabetes, many symptoms may have already appeared. At the same time, blood sugar levels are very high for long periods of time, something that could potentially lead to diabetic ketoacidosis, resulting in a variety of symptoms such as dry skin, fast and deep breathing, drowsiness, abdominal pain and vomiting (Cooke & Plotnick, 2008).

1.4.1.2 Causes of type 1 diabetes mellitus

The occurrence of type 1 diabetes has kept science pre-occupied for many years. The exact cause of type 1 diabetes remains a mystery. As it has previously been mentioned, diabetes type 1 has an autoimmune origin and our own immune system, which normally faces undesired harmful microorganisms and viruses, proceed to face

its own cells in its own pancreas (Diabetes Research Institute Foundation: The Best Hope for Our Cure, 2014). The factors that generate type 1 diabetes mellitus still remain unknown, but studies and researches have shown that several of the following factors may be responsible for type 1 diabetes mellitus:

- Genetic factors (Knip, et al., 2005) (Disease and Conditions: Type 1 Diabetes, 2014).
- Diabetogenic factors, like drugs or other chemical substances (Knip, et al., 2005).
- Exposure to a virus or an antigen, like the Epstein Barr virus, Coxsackie virus, mumps and cytomegalovirus (Knip, et al., 2005) (Disease and Conditions: Type 1 Diabetes, 2014).
- Family history, if someone in the family is type 1 diabetic, then their children present an increased possibility to develop diabetes type 1 too (Disease and Conditions: Type 1 Diabetes, 2014).
- If someone has a mother who showed preeclampsia during her pregnancy (Disease and Conditions: Type 1 Diabetes, 2014).
- Being born with jaundice (Disease and Conditions: Type 1 Diabetes, 2014)

The risk of a child developing diabetes mellitus type 1 is approximately 10%, if the father is diabetic, about 10% if one of its siblings is diabetic and about 4% if the mother is diabetic and was, at the age of 25 or younger, or around 1% if the mother was older than 25 years old by the time the child was born (Knip, et al., 2005) (Bluestone, Herold, & Eisenbarth, 2010) (Dr.Warram).

1.4.2 Diabetes Mellitus Type 2

Diabetes type 2 is characterized by a phenomenon known as insulin resistance. More specifically, patients with type 2 diabetes in contrast to type 1 diabetic patients, produce insulin but their cells do not use it as well as they should. In the early stages of the disease, the decrease in sensitivity towards insulin is the cardinal disorder and thus the blood insulin levels are elevated (Kumar, et al., 2005).

Studies and researches have shown that, type 2 diabetes is the most common form of diabetes in adults. Obesity seems to play an important role; people who are obese present a much higher risk in developing diabetes type 2 than people who follow a healthy lifestyle. It has been proven that obesity constitutes a very

significant parameter which increases the possibilities of the development of insulin resistance. Other predisposing factors include age and family history. In diabetes type 2 the symptoms are mild and the occurrence of diabetic ketoacidosis is small (Melmed, Polonsky, Larsen, & Kronenberg, 2011).

Nevertheless, long term and serious complications can afflict the diabetic population without the right treatment. The first step in treating type 2 diabetes is changing the patient's lifestyle, targeting weight loss, increasing physical activity and healthy eating. If it is necessary, the patients might use antidiabetic medications. If the treatment fails, then the necessary insulin administration should be applied (Athyros, et al., 2009). During the last fifty years, the rates of diabetes have presented a significant increase, along with obesity. Since 2010 approximately 285 million people have been diagnosed with diabetes, whereas in 1985 patients amounted to 30 million (American Diabetes Association).

1.4.2.1 Signs and symptoms of diabetes type 2

The classic symptoms of diabetes are similar to diabetes type 1 symptoms. In contrast to diabetes type 1, diabetes type 2 strikes slowly and not sharply like type 1 diabetes. Other common symptoms that have been found, usually by diagnosis, include blurred vision, itchiness in several parts of the body, peripheral neuropathy, and fatigue (Vijan, 2010). Type 2 diabetic symptoms are presented in detail, as follows:

- Polyuria, which constitutes a condition that, is characterized by frequent urination, usually greater than 2,5L -3L over 24 hours in adults (Shah, 2013).
- Polydipsia, which constitutes a condition that is characterized by an increased feeling of thirst (Disease and Conditions: Type 1 Diabetes, 2014).
- Hyperphagia, which constitutes a condition that is characterized by an increased feeling of appetite (Disease and Conditions: Type 1 Diabetes, 2014).
- Xerostomia (dry mouth syndrome), which constitutes a condition that is characterized by intense dryness in the mouth (Disease and Conditions: Type 1 Diabetes, 2014).
- Fatigue and weakness (Disease and Conditions: Type 1 Diabetes, 2014).
- Weight loss, usually unintentionally (Disease and Conditions: Type 1 Diabetes, 2014).

- Blurred vision (Disease and Conditions: Type 1 Diabetes, 2014).
- Slow healing, sores or frequent infections, which constitutes a condition that affects body's ability to heal and resist infections (Diseases and Conditions: Type 2 Diabetes, 2014)
- Dark skin areas, usually near the neck (Diseases and Conditions: Type 2 Diabetes, 2014).

However, it has been proven that many individuals do not show any symptoms in the early years and the diagnosis can be made after a routine blood test. In fact it is possible that someone has diabetes type 2 for years and has not realized it (American Diabetes Association, 2013).

1.4.2.2 Complications of diabetes mellitus type 2

Diabetes mellitus type 2 constitutes a long term metabolic disorder, which in some cases, leads patients to undesired situations. Studies and researches have shown that diabetes mellitus can reduce life expectancy up to ten years. This is partly due to the fact that it is connected with various complications which include two to four times greater risk of developing cardiovascular disease, foot damages, skin conditions, ischemic heart disease, stroke, increased lower extremity amputations by 20 times and increased hospitalization rates (Melmed, Polonsky, Larsen, & Kronenberg, 2011) (Diseases and Conditions: Type 2 Diabetes, 2014) (Mavridis, 2014).

In developed countries and increasingly in other parts of the world, diabetes type 2 is the most important cause of renal failure as well as visual disturbances leading to non-traumatic blindness (Ripsin, Kang, & Urban, 2009). Moreover, studies and researches have shown that diabetes type 2 can increase the risk of developing cognitive dysfunction and dementia (Pasquier, 2010) (Mavridis, 2014).

1.4.2.3 Causes of diabetes mellitus type 2

The development of diabetes type 2 occurs due to the combination of genetic factors and lifestyle (Mavridis, 2014) (Ripsin, Kang, & Urban, 2009) (Riserus, Willet, & Hu, 2009). While some factors, such as diet and obesity is in the hands of the individual themselves , there are others, such as passage of age, the female gender and

genetic factors that can't be controlled (Melmed, Polonsky, Larsen, & Kronenberg, 2011) (Mavridis, 2014). Lack of sleep has also been proven that can be an indicator of diabetes mellitus type 2 because of its effect on metabolism (Touma & Pannain, 2011). It has been suggested that the dietary habits of a mother, during fetal development may also play a significant role (Mavridis, 2014) (Christian & Stewart, 2010). The diabetic risk factors are presented in detail below:

- Obesity; it has been proven that being overweight increases the possibility of developing diabetes type 2. More fatty tissue, more resistance by the cells becoming to insulin (Diseases and Conditions: Type 2 Diabetes, 2014).
- Inactivity, because when someone exercises this can help the body to remain at a normal weight (Diseases and Conditions: Type 2 Diabetes, 2014).
- Family history, just like diabetes type 1, if one parent or sibling has diabetes type 2 then the possibility to develop diabetes mellitus type 2 remains high (Diseases and Conditions: Type 2 Diabetes, 2014).
- Race, there are several studies and researches which have shown that some races, develop diabetes type 2 more easily, including Africans, Asians, Indians in contrast with the Caucasians (Diseases and Conditions: Type 2 Diabetes, 2014).
- Gestational Diabetes; it has been proven that if a mother develop diabetes during her pregnancy then the possibility to develop type 2 diabetes in her life remains high (Diseases and Conditions: Type 2 Diabetes, 2014).

The mechanism that helps to amend the DNA is methylation. Although diabetes is a disease that usually appears among adults, children and teenagers are highly possible to develop the disease. Although the etiology remains a mystery, scientists maintain that it seems to be not an autoimmune destruction of cells. The risk of developing type 2 diabetes increases with age, obesity, sedentary life or in some cases with lack of sleep at night (Melmed, Polonsky, Larsen, & Kronenberg, 2011) (Mavridis, 2014) (Diabetes.co.uk:The global diabetes community, 2015).

1.4.3 Gestational Diabetes

Gestational diabetes is defined as glucose intolerance which appears for the first time at women during their pregnancy. The effect of this type of diabetes increases due to various factors, like obesity and advanced maternal age. This type of diabetes

is similar to diabetes type 2, and characterized from concomitant reduced insulin secretion and reduced sensitivity of cells to insulin. Women with obesity have a greater probability of developing gestational diabetes (Mavridis, 2014) (National Institute of Diabetes and Digestive and Kidney Diseases , 2014).

In gestational diabetes, the symptoms are similar to type 2 diabetes symptoms, but after the baby is born, the symptoms disappear and in turn blood sugar returns to normal levels. Of course that doesn't mean that it shouldn't be given the appropriate attention, because gestational diabetes might be harmful for the baby's health and the possibility of the mother developing diabetes type 2 later in her life, is high (Diseases and Conditions: Type 2 Diabetes, 2014).

In conclusion, it is worth noting that just as in other types of diabetes, science still hasn't an answer why some women develop gestational diabetes during their pregnancy (Disease and Conditions: Type 1 Diabetes, 2014) (Diseases and Conditions: Type 2 Diabetes, 2014). Gestational diabetes is responsible for developing diabetes mellitus in 5% of pregnant women. A percentage of about 30-40% of the people affected by gestational diabetes will develop diabetes type 2 later in their life. The gestational diabetes is reversible and disappears after the baby's delivery but can cause the mothers health problems as well as the newborns (Mavridis, 2014) (National Institute of Diabetes and Digestive and Kidney Diseases , 2014).

1.4.4 Neonatal Diabetes

This type of diabetes mellitus usually appears in the first six months of life. Neonatal diabetes mellitus is rarely noticed and is linked with several etiologies, which need special assessment and special treatments. More specifically, its rare condition appears in one in 100,000 to 500,000 live births (Monogenic Forms of Diabetes: Neonatal Diabetes Mellitus and Maturity-onset Diabetes of the Young, 2007).

Furthermore, Neonatal diabetes presents similar symptoms and complications to type 2 diabetes mellitus. For example, some children diagnosed with neonatal diabetes are rarely associated with severe autoimmunity, but they have specific mutations and are treated better with Sulphonylureas after initial therapy with insulin (Mavridis, 2014) (Monogenic Forms of Diabetes: Neonatal Diabetes Mellitus and

Maturity-onset Diabetes of the Young, 2007) (National Institute of Diabetes and Digestive and Kidney Diseases, 2007).

1.4.5 Other Types of Diabetes

Diabetes mellitus major types are type 1, type 2 and gestational diabetes but this chronic metabolic disease can appear in the following cases too:

- Genetic defects of the β-cells of the pancreas (Mavridis, 2014) (World Health Organization, 2014).
- Genetic defects of insulin action (Mavridis, 2014) (World Health Organiztion, 1999).
- Pancreatic diseases (Mavridis, 2014) (World Health Organization, 2014).
- Hormonal disorders and endocrine diseases (Mavridis, 2014) (World Health Organization, 2014).
- Diabetes due to the use of drugs (Mavridis, 2014) (World Health Organiztion, 1999).

However, these types of diabetes mellitus are very rare but awareness should be raised, as in the cases of the most popular types of diabetes mellitus.

1.5 EPIDEMIOLOGICAL DATA OF DIABETES MELLITUS

1.5.1 Worldwide Epidemiological Data of Diabetes Mellitus

According to the World Health Organization, diabetics were more than 170 million in 2006, in 2030 this number will be expected to increase to 340 million, because this metabolic disorder increases its appearance among the population rapidly. According to the international diabetes federation, the global percentage of the disease among adults (20-79 years) is expected to increase from 8,3% in 2011 to 9,9% in 2030. It is estimated that in Greece, 5,9% of the general population suffers from diabetes. According to the American Diabetes Association (A.D.A), in 2014 among diabetic individuals, 75% of deaths were due to cardiovascular disease. In comparison to the general population, the diabetic population presents higher mortality numbers after a myocardial infarction. Cerebrovascular accidents (strokes) are responsible for the percentage of 15% of deaths in patients with diabetes (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Melmed, Polonsky, Larsen, & Kronenberg,

2011) (American Diabetes Association) (Mavridis, 2014) (World Health Organization, 2014) (International Diabetes Federation, 2014).

Diabetes mellitus commonly appears in the developed world, particularly type 2 diabetes, which emerges more and more every day. Diabetes has been proven that is linked to the so-called western lifestyle that includes living in large urban centers, unhealthy diets and minimum physical exercise. Diabetes mellitus is considered responsible for a large percentage of deaths worldwide (Mavridis, 2014) (World Health Organization, 2014) (International Diabetes Federation, 2014) (Wang J. , 2008).

The largest percentage of diabetics is within the range of 40 and 59 years old. In 2013 the number of people with undiagnosed diabetes is estimated to have caused more than 5 million deaths, more specifically, every six seconds a person dies from diabetes. According to the world health organization, diabetes was recently ranked in 7th place in the leading causes of mortality (Mavridis, 2014) (World Health Organization, 2014) (International Diabetes Federation, 2014).

1.5.2 Epidemiological Data in Europe

In Europe, has been a significant increase in type 2 diabetes mellitus among people under the age of 20 to 30 years old. Surveys indicate that more than half of the Europeans suffer from hypoglycemia or diabetes throughout their lifetime, while, according to an epidemiological study, approximately 5% of Europe's population is affected by type 2 diabetes. The number of diabetic population in Europe is estimated at 56.300.000 people. The countries which present the highest percentage of diabetics are mostly in Western Europe, including Germany, Spain, Italy, France and United Kingdom (Mavridis, 2014) (World Health Organization, 2014) (International Diabetes Federation, 2014).

1.6 CLINICAL SIGNS OF DIABETES MELLITUS

The increase of blood glucose causes fluid loss by osmotic diuresis. Signs of dehydration include cold and dry skin, dried mucous, rough and dry tongue are observed when the fluid loss isn't adequately compensated (Khardori) (Mavridis, 2014).

Diabetic ketoacidosis is a severe condition, whose main characteristic is the Kussmaul breath. This consists of deep and prolonged breathing, accompanied by sighs and the exhalation of acetone. Blurred vision, decreased responsiveness to stimuli, drowsiness and coma follow if the ketoacidosis isn't treated (Mavridis, 2014) (Khardori).

1.7 DIAGNOSIS

The diagnosis of diabetes mellitus is easy when the usual symptoms make their presence felt and sufficient to bear out the measurement of blood sugar. The diagnosis is also easy when the individual shows symptoms and signs of diabetic ketoacidosis. The diagnostic criteria established for the diagnosis of diabetes are presented as follows (Vijan, 2010) (Mavridis, 2014) (Diabetes Programme):

- Presence of the common symptoms of diabetes and glucose value at any time >200mg/dl (Vijan, 2010) (Mavridis, 2014) (Diabetes Programme).
- Fasting blood sugar (obtained at least 8 hours after digestion/last meal) >126mg/dl (Vijan, 2010) (Mavridis, 2014) (Diabetes Programme).
- Sugar value two hours after loading with 75g oral glucose >200mg/dl (Vijan, 2010) (Mavridis, 2014) (Diabetes Programme).
- Another useful indication is the value of glycosylated hemoglobin (HbA1c), which monitors the regulation of blood sugar, as it represents the average blood sugar levels of the last three months before the checkup. Percentages of HbA1c that are lower than 6% are considered indicative of good glycemic control (Vijan, 2010) (Mavridis, 2014) (Diabetes Programme).

1.8 COMPLICATIONS OF DIABETES MELLITUS

It is observed that when the blood sugar levels are controlled better, diabetic complications have a reducing rate. A wide scale number of factors, such as smoking, increased cholesterol levels, obesity, hypertension and sedentary lifestyle, accelerate the development of diabetes. Complications of diabetes are divided in two major categories, acute and chronic and are presented in detail as follows (Melmed, Polonsky, Larsen, & Kronenberg, 2011) (Vijan, 2010) (Mavridis, 2014).

1.8.1 Acute Complications

1.8.1.1 Diabetic ketoacidosis and diabetic coma

Diabetic ketoacidosis constitutes a very dangerous condition, which is considered a medical emergency and requires immediate hospitalization. The patient shows signs of dehydration, Kussmaul breathing (deep, prolonged and accompanied by sighs) and exhalation of acetone. These signs are often accompanied by diffuse abdominal pain (Mavridis, 2014) (Khardori) (Ramlo-Halsted & Edelman, 2000) (Polumeris, 2009).

Diabetic Ketoacidosis is observed more commonly in patients with type 1 diabetes, but under certain circumstances, it can also affect type 2 diabetic patients. Due to low or zero insulin secretion, our body responds to this condition by burning fatty acids and producing acidic ketone bodies that are responsible for most symptoms. The level of consciousness isn't affected initially, but can progressively result to loss of consciousness, somnolence and eventually coma. Excluding the infections that allow the frequent appearance of diabetic ketoacidosis, this complication appears in adolescents, who skip their insulin injections or in those individuals who present the so-called labile diabetes. In serious cases hypotension and circulatory collapse (shock) is observed. With proper and immediate treatment, diabetic ketoacidosis is completely reversible. It's presented more in type 1 diabetics (Mavridis, 2014) (Khardori) (Ramlo-Halsted & Edelman, 2000) (Polumeris, 2009) (Kitabchi A. , Umpierrez, Miles, & Fisher, 2009).

1.8.1.2 Hyperosmolar coma non ketotic

Hyperosmolar (Non Ketotic) Coma is characterized by an increase in plasma osmolality, blood sugar levels higher than 600mg/dl and no ketoacidosis. In addition to these, impaired consciousness, as well as electrolyte imbalance may appear. This occurs more frequently in older diabetic individuals and constitutes a dangerous situation. More specifically, blood sugar levels present a continuous growth rate and in turn our body tries to get rid of the excess sugar by passing it through our urine. In this stage the symptoms of diabetes can become more intense (Mavridis, 2014) (Polumeris, 2009).

Several causes can trigger the onset of hyperosmolar non ketotic coma, such as sepsis, myocardial infraction, pancreatitis, gastrointestinal bleeding and medication (Mavridis, 2014) (Polumeris, 2009).

1.8.1.3 Hypoglycaemia

Hypoglycemia is referred to the complication that can often appear during the treatment of diabetes. It can be caused by incorrect dosage around administration of insulin (increased dose), intense physical activity or decreased sweating and can cause disorders of consciousness, loss of consciousness or coma (Mavridis, 2014) (Filandra, 2009).

Immediate glucose uptake by mouth such as sweets and sugary soft drinks is required if it is possible, or in undesired conditions of unconscious patients, an intravenous sugar solution might be the best choice (Mavridis, 2014) (Filandra, 2009).

Hypoglycemia results in various symptoms and signs, such as confusion, clumsiness, trouble talking, loss of consciousness, seizures and in some extreme situations death. These symptoms usually come quickly accompanied by sweating and weakness (Mavridis, 2014) (Filandra, 2009).

1.8.1.4 Hyperglycaemia

High blood sugar or Hyperglycemia, as it is often called, constitutes a complication in which a very high percentage of glucose remains in constant circulation in the blood plasma. This blood sugar level is, in general higher than 200mg/dl. Symptoms of Hyperglycemia are possible to remain unnoticeable, until even higher values such as 250 to 300 mg/dl appear (Mavridis, 2014) (Diabetes.co.uk/Diabetes and Hyperglycemia).

The origin of the term Hyperglycemia is Greek in which hyper (υπέρ) meaning excessive, glyc (γλύκ) meaning sweet and emia (αιμία) meaning blood (Wikipedia the free encyclopedia). As mentioned previously, while blood glucose levels can have a growth rate well above normal levels for large periods, the individual continues his daily life without the perception of any permanent effects or symptoms. Furthermore, it is worth noting that, as a result, chronic hyperglycemic conditions, at levels slightly above normal, have the ability to generate a diversity of serious complications or undesired conditions over a significant period of time, including renal failure,

neurological damage, cardiovascular damage, retinal damage or damages to feet and legs. A result of long-term hyperglycemia may be diabetic neuropathy (Mavridis, 2014) (Diabetes.co.uk/Diabetes and Hyperglycemia) (Pais, Hallschmid, & Jauch-Chara, 2007).

Nevertheless, chronic hyperglycemia constitutes a very dangerous condition and as mentioned before, it is important to pay attention when hyperglycemic symptoms appear. Hyperglycemia has the following symptoms:

- Polyphagia
- Polydipsia
- Polyuria
- Blurred Vision
- Fatigue
- Weight Loss
- Dry Mouth
- Dry Skin
- Erectile Dysfunction
- Stupor
- Coma
- Seizures
- Cardiac Arrhythmia

It is worth noting that chronic hyperglycemia that persist even in fasting periods, is usually caused by the disease of diabetes. It is a fact that, chronic hyperglycemia is one of the major characteristics of diabetes metabolic disorder. Intermittent hyperglycemia is possible to appear in pre-diabetic stages. The appearance of acute hyperglycemic events without an obvious cause may indicate the development of diabetes or they might operate as a harbinger of this metabolic disorder (Mavridis, 2014) (Diabetes.co.uk/Diabetes and Hyperglycemia) (Pais, Hallschmid, & Jauch-Chara, 2007).

Diabetic patients present hyperglycemic symptoms and signs which usually have a resistance in insulin origin. More specifically, hyperglycemia is usually caused by zero insulin secretion or low insulin levels, something that depends on the type and state of diabetes. Nevertheless, chronic hyperglycemia may be a result of other several factors too. Some of these factors are several drugs for other diseases

which can also increase the risk of hyperglycemia. Some of these drugs are corticosteroids, beta blockers and epinephrine (Mavridis, 2014) (Diabetes.co.uk/Diabetes and Hyperglycemia) (Pais, Hallschmid, & Jauch-Chara, 2007).

Critical illness may be another factor causing chronic hyperglycemia. In addition, stress is another factor causing hyperglycemia which is accompanied by dysfunction of the thyroid, several pancreas diseases and also several diseases in blood glucose may be seen in certain infections and sepsis (Mavridis, 2014) (Diabetes.co.uk/Diabetes and Hyperglycemia) (Pais, Hallschmid, & Jauch-Chara, 2007).

1.8.2 Chronic Complications

1.8.2.1 Diabetic microangiopathy

The term microangiopathy refers to the angiopathy which has the ability to affect small blood vessels in the body (Dorland's Medical Dictionary for Helathcare Consumers, 2007). Diabetic microangiopathy constitutes one of the major chronic diabetic complications (Vojtkova, Ciljakova, & Banovcin, 2012). Although the etiopathogenesis of diabetic microangiopathy remains a mystery, there is great suspicion that long-lasting hyperglycemia lies behind this dangerous condition (Vojtkova, Ciljakova, & Banovcin, 2012). Massive microangiopathy could cause:

- Diabetic retinopathy, which constitutes a condition where we observe hyperplasia of the capillaries in the retina surface and attack to the macula, which results in loss of vision. It is worth noting that diabetes is very often responsible for the cause of total vision loss in the western world (Mavridis, 2014) (Vojtkova, Ciljakova, & Banovcin, 2012) (Kumar, Abbas, Fausto, & Aster, 2009).
- Diabetic nephropathy, which constitutes a condition of kidney damage with glomerular lesions, vascular and interstitial tissue that can lead to kidney failure (Mavridis, 2014) (Vojtkova, Ciljakova, & Banovcin, 2012) (Kumar, Abbas, Fausto, & Aster, 2009).
- Diabetic neuropathy, which constitutes a condition of neuropathy of the autonomic nervous system. Sensory loss (initially in the leg), and erectile dysfunction are often described as the major characteristics of this dangerous

condition (Mavridis, 2014) (Vojtkova, Ciljakova, & Banovcin, 2012) (Kumar, Abbas, Fausto, & Aster, 2009).

1.8.2.2 Diabetic macroangiopathy

It is a condition that is characterized by the obstruction of blood flow within the large blood vessel walls. Diabetic macroangiopathy has also been linked with the appearance of arteriosclerosis among the diabetic population. Arteriosclerosis appears earlier and is more severe in diabetics than in the non-diabetic population. Although the etiopathology of diabetic macroangiopathy is unknown, it has been observed that serious problems in medium and large arteries appear during this condition. These problems are presented as follows (Mavridis, 2014) (International Diabetes Federation, 2014) (The Free Dictionary, 2002).

- Coronary artery disease, which can lead to angina or acute myocardial infraction (Mavridis, 2014) (International Diabetes Federation, 2014).
- Stroke, especially ischemic type (Mavridis, 2014) (International Diabetes Federation, 2014).
- Peripheral vascular disease (Mavridis, 2014) (International Diabetes Federation, 2014).

1.8.2.3 Other Complications

- Susceptibility to infections. In this condition the body is vulnerable and the possibility of infection becomes higher (Mavridis, 2014) (International Diabetes Federation, 2014).
- Fatty Liver Disease (Mavridis, 2014) (International Diabetes Federation, 2014).
- Periodontitis (Mavridis, 2014) (International Diabetes Federation, 2014).

1.9 TROUBLESHOOTING

Diabetes mellitus constitutes a very demanding disorder and the patient plays a very significant role in the management and treatment of diabetes. The diabetic population should pay attention to the diet needed to be followed because it constitutes a key player in order to keep the glucose concentration between the normal

levels. Another key factor is the administration of insulin, which the patient needs practicing with. Since both medical treatment and lifestyle choices are directly related to glucose metabolism, it is important for the diabetic individual to actively work towards the self-management and health maintenance (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Hellenic Diabetes Association) (Georga et al, 2014).

The primary target focuses on the long term glycemic control of the patient. At the same time the objective to eliminate the factors that can increase the risk of chronic complications of the disorder is still on the map. Quitting smoking, controlling blood pressure and cholesterol levels, increasing physical activity and adopting healthy lifestyles are top priorities for the patient. For individuals with type 1 diabetes, insulin administration is necessary to achieve the desired blood sugar levels. On the contrary, for patients with type 2 diabetes, glycemic control may be initially achieved with weight control and diet or administration of antidiabetic drugs orally (Hellenic Diabetes Association).

Insulin should be supplied to patients, if the glycemic control fails, with the measures that were described before. Subcutaneous administration constitutes the most known way of insulin administration to diabetics. The type of insulin, the dose and frequency of administration depends exclusively on the needs of each patient. Therefore, the type of insulin is completely individualized (Hellenic Diabetes Association).

1.10 ECONOMIC COST OF DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder with chronic characteristics. These chronic characteristics are related to the cost of diabetic treatment. In many countries, the public health institutions and pension funds cover a high percentage of the cost of treatment. According to the American Diabetes Association (ADA) the cost of diabetes treatment presents an increasing rate, something that is mirrored in the fact that in 2012 the economic cost was about 245$ billion, while in 2007 the same cost was about 174$ billion, when the economic cost was last examined (www.diabetes.org, 2012).

Furthermore, the economic cost of medical expenditures is high and can be distributed in percentage regarding the total medical cost:

- Hospital inpatient care (43%)

- Prescription drugs to manage the complications of diabetes mellitus (18%)
- Antidiabetic agents and supplies for diabetes mellitus (12%)
- Visits in doctor offices (9%)
- Needs for nursing (8%)

The largest percentage of the economic cost for diabetes care in U.S.A is covered by government insurance (62,4%) while the rest is covered by private insurance (34,4%) while the uninsured are estimated at 3,2%. The diabetic population who is not covered by private or public health insurance have 79% fewer visits to any physician's office and are also prescribed about 68% fewer pharmaceutical medications, than people with insurance health coverage but at the same time they present 55% more emergency department visits than individuals who were covered by an insurance health (American Diabetes Association, 2013) (Mavridis, 2014).

In the United States of America in 2008, approximately one million people were diagnosed with diabetes type 1. The economic cost of this metabolic disease is estimated at 10.5 billion dollars in annual medical costs and an additional 4.4 billion in indirect costs (American Diabetes Association) (American Diabetes Association, 2013) (Mavridis, 2014).

In conclusion this research highlights the heavy burden that diabetes mellitus places on our society. During the last decades diabetes mellitus presents an increasing index, consequently the cost presents an increasing rate, too (American Diabetes Association, 2013) (Mavridis, 2014).

1.11 GLUCOSE

Glucose constitutes a simple monosaccharide which can be found in plants and is commonly known as sugar, too. Glucose ($C_6H_{12}O_6$) along with fructose and galactose is one of the human body nutrition monosaccharides which can absorbed by the body directly into the bloodstream during digestion. It’s the most important carbohydrate in biology, since it can be used by the cells as their primary source of energy. Glucose is one of the main products of photosynthesis and is used as energy source for cellular respiration (Bunn & Higgins, 1981).

Our glucose levels usually range between [70 - 110 mg/dl]. More specifically our blood sugar levels range close to 100 milligrams per deciliter in the morning before breakfast, but after breakfast, 1 or 2 hours later, the blood glucose levels

should be less than 140 milligrams per deciliter (Bunn & Higgins, 1981). In diabetic patients these glucose levels exceed these limits and in turn hyperglycemic or hypoglycemic events appear.

1.11.1 Glycosylation of Hemoglobin

The glycosylated hemoglobin is a form of abnormal hemoglobin which derives from the chemical union with glucose. Increased blood glucose, increases the rate of binding between glucose and hemoglobin via a reaction which is called non-enzymatic glycosylation, because it occurs without the mediation of any enzyme and it occurs through the life of the red cells, which is about 120 days. The higher the blood glucose is, the more increased the percentage of glycated hemoglobin is. The concentration of glycosylated hemoglobin in the blood is an indicator of the average glucose concentration in the blood (Salway, 2006).

1.11.2 Blood Glucose Level

The term blood glucose level or blood sugar concentration, as it is usually called, describes the amount of glucose that occurs in blood of a human or an animal. The natural regulation of glucose in blood constitutes a part of the metabolic homeostasis (Walkers & Rodgers, 2006).

The transportation of glucose is carried from the intestines or the liver to body cells through the bloodstream and in turn becomes available for cell absorption via insulin which constitutes a hormone that is produced by the body, mainly in the pancreas as described before (Walkers & Rodgers, 2006).

Researches and studies have shown that glucose levels are usually cut-rate in the morning, before the patient's first meal. On the other hand, glucose levels present an increasing rate after any meal for a time space between one to two hours by a few millimolars. Outside the normal range, blood sugar levels are possible to be an indicator (harbinger) of an undesired medical condition. The persistently high blood sugar levels are referred to as Hyperglycemia and the persistently low blood sugar levels are referred to as Hypoglycemia (Walkers & Rodgers, 2006).

Diabetes Mellitus has a persistent hyperglycemic character from any of several causes and constitutes the most prominent disease which is related to failure of blood sugar regulation. It is worth noting that alcohol intake tends to increase the blood

sugar levels for a small period of time and later tends to cause blood sugar levels to fall. Another factor that affects the change of blood glucose levels is the body's response to intensive insulin therapy because in many cases it increases the risk of severe Hypoglycemia, with all its consequences. In addition, certain drugs have the ability to increase or decrease glucose levels (Walkers & Rodgers, 2006).

The international standard method for measuring the glucose concentration is in terms of a molar concentration, which is measured in mmol/L (millimoles per litre). In the United States of America and in many other countries too, mass concentration measurements are recorded in mg/dL (milligrams per deciliter) (Abbot Diabetes Care, 2015).

1.12 INSULIN

Insulin constitutes a hormone which is produced in the pancreas from β-cells. These cells produce a first precursor proinsulin, which is converted to insulin after removing the middle portion. Insulin plays a major role in the metabolism of carbohydrates in the body and it stimulates the liver to store glucose. In addition to this function of regulating the glucose, insulin is involved in maintaining adequate energy supplies (Sonksen & Sonksen, 2000).

More specifically, insulin operates as a key and unlocks our cells, in turn, glucose receives the green light to enter in our cells and give us energy to continue our daily life. The fact that the central nervous system has a key role in both afore mentioned functions, and also that both the body weight and the blood glucose are regulated primarily by the same hormone, is the subject of an ongoing research (Dr.Warram) (Sonksen & Sonksen, 2000).

Nevertheless, insulin is capable of activating regulatory peptides and neurotransmitters which can activate processes related to food behavior, learning and memory. Additionally, it is potentially involved in the intercom brain structures, and more particularly in the hypothalamus and in limbic system (Dr.Warram) (Sonksen & Sonksen, 2000).

It is generally accepted that the effective action of insulin in the brain is essential for maintaining the energy level of glucose and lipid homeostasis. The deficiency of insulin is responsible for diabetes mellitus. This hormone has been synthesized since 1921 and is used in patients with diabetes. Also it can be acquired

by animals too, especially pigs (Paulescu). Nowadays, another source of insulin can be secured through genetic engineering as well as from bacteria.

In conclusion, it is worth noting that, if insulin is secreted in large amounts, the glucose is spread into the cells rapidly, thus reducing the concentration of the blood. This condition is named hypoglycemia which is very dangerous and can lead to coma. Insulin may be prescribed by a physician, an endocrinologist and a diabetologist specialist (Dr.Warram) (Sonksen & Sonksen, 2000).

1.13 PANCREAS

Pancreas constitutes an organ of our body with a very important function and is a mixed gland of the digestive tract. More specifically, in humans it is located in the abdominal cavity behind the stomach (Khan, 2014) (Cancer of Pancreas, 2014).

As an endocrine gland, pancreas is able to produce several very useful hormones. One of them is insulin, the hormone which has a cardinal role in diabetes. The part of the pancreas with the endocrine function is made up of cell clusters which are known as the islets of Langerhans. Four major types of cells exist in these islets. The α-cells secrete glucagon, which increases glucose in the blood. The β-cells secrete insulin which decreases glucose in the blood. The δ-cells secrete somatostatin which regulates α-cells and β-cells. The fourth major type is the γ-cells which secrete the pancreatic polypeptide. Pancreas as a digestive organ also has the ability to secrete the pancreatic juice which contains digestive enzymes. These digestive enzymes give a helping hand at the stage of digestion and also participate in the absorption of nutrients inside the small intestine. Their role is to further break down the carbohydrates, proteins and lipids (Khan, 2014) (Cancer of Pancreas, 2014).

According to the above, pancreas has exocrine functions too. As an exocrine gland, the pancreas is very important for the digestive system. In the human body the secretory activity of the pancreas is adjusted through the hormone effect in the blood on the Langerhans islets and directly through the autonomic nervous system effect on the blood flow (Khan, 2014) (Cancer of Pancreas, 2014).

The pancreas varies in shape and is located in the anterior para renal space. The head of the pancreas lies between the curve of the duodenal loop and the inferior vena cava. The head of the pancreas is the most bulbous part of the gland, which is thinner as it goes to the pancreatic neck. The tail of the pancreas is related to the spleen, left

adrenal gland, and upper pole of the left kidney (Khan, 2014) (Cancer of Pancreas, 2014).

In conclusion, the head of the pancreas is about 2,5 – 3,5cm, the body is about 1,75 – 2,5cm and finally the tail about 1,5 – 3,5cm. The pancreas is about 15cm long. As mentioned before, the pancreas size varies considerably, so the size of this gland alters with age. Pancreas size, especially, shows a decrease of its size with the passing of time (Khan, 2014) (Cancer of Pancreas, 2014).

The picture below depicts all that was referred to in the above text regarding pancreas anatomy and physiology:

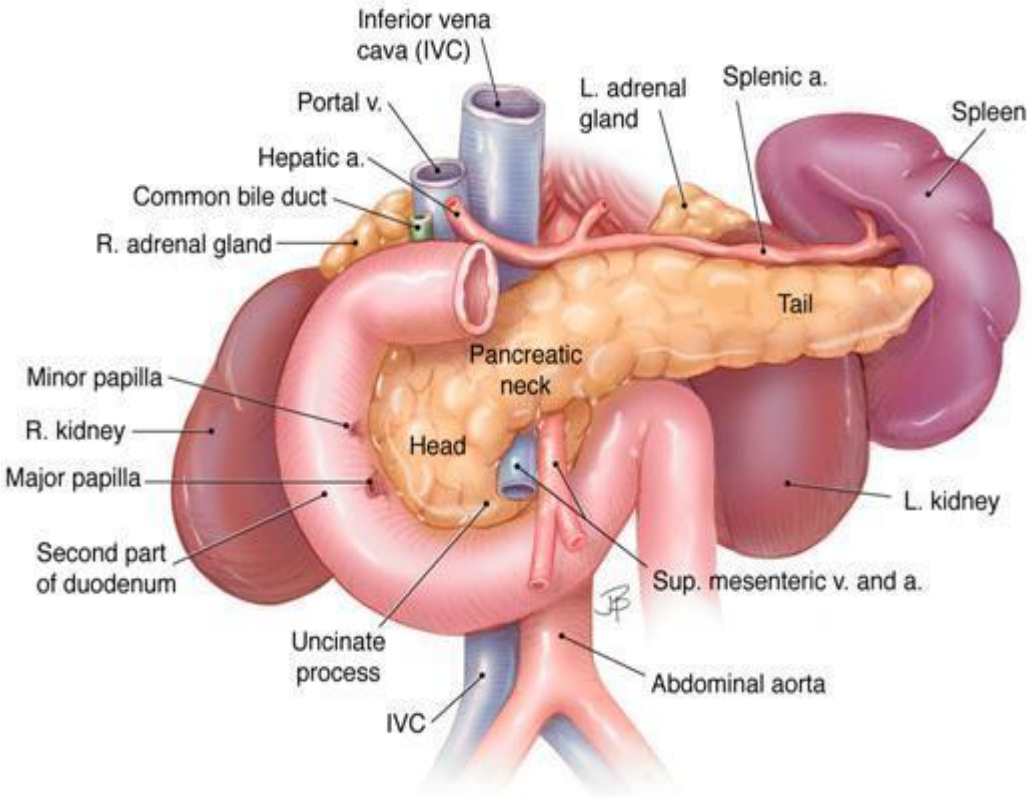


Figure 1-1 Pancreas Physiology (Longnecker, 2014)

2 SENSORS

2.1 ABSTRACT

Sensors are all around us and literally invading our everyday life, even though they are not noticed very often. If a definition had to be given of what meant by the word sensor, then the following definition could be a suggestion:

'By sensors, we mean all the devices which are useful for the detection of a physical quantity and from which a measurable output can be produced."

Furthermore, sensors are useful everyday devices, in everyday objects with so many applications, such as motion detectors or presence detectors, lift buttons which have touch sensitive ability and light bulbs that have the ability to emit brighter or softer light when something touches their base. The mercury thermometer constitutes a glaring example of using sensors in everyday life. The change in height of the mercury column in this example constitutes the desired output signal. The uses of sensors are countless in so many ways that most people do not notice them, as mentioned before. Sensors have many and several applications finding use in material science, automobile manufacturing, machine manufacturing, electronic and electrical devices, aeronautical engineering, industry and factory production, mechanical engineering and biomedical engineering. It is clearly understood that sensors are critical components to all measurement and control systems (Rang et al, 2003) (Dorf, 2006) (Elgar, 2003).

Furthermore, even the human body has thousands of sensors that we are not aware of most of the time. For example, the human tongue has 9,000 taste sensors some of which are lost with the passing of time. However, just like taste sensors we have sensors all over our body including the muscle system, eyes, ears, nose, etc (Barretto, et al., 2015).

In this chapter, a wide scale reference was made to the most important parts of sensors and biosensors science and technology and in which way they can be clearly and easily understood. In the first part of chapter 2 the characteristics of sensors are presented in detail. In the second part of chapter 2, a wide scale reference to biosensor's system technology is also made, as well as how this technology can be a useful weapon in the hands of the diabetic population. Furthermore, the operating procedure of biosensors as a part of glucose meters is presented. The third part of this

chapter presents the future of glucose biosensors and how they can be part of the artificial pancreas, giving hope to millions of diabetic patients.

2.2 CHARACTERISTICS OF SENSORS

As mentioned before, sensors are useful everyday devices with several applications. Sensors have some characteristics which allow us to understand their utility and importance. Based on these characteristics, each sensor is intended for a different use. The characteristics that really matter are sensitivity, range, accuracy, selectivity, stability and error.

More specifically, selectivity constitutes one of the major characteristics of sensors and especially regarding electrochemical biosensors. It expresses the sensor's ability to measure a single parameter like detecting a certain analyte. The term range mirrors the ability of the device to operate reliably within certain specified limits. The term accuracy reflects the solvency of the output value which is related to the input value or more simply it expresses the degree of rightness of measurements in relation to the actual value of the measured size. Error refers to the difference between the measured value and the actual value. Sensitivity is a term that refers to the relation between the output and the input change, which reflects the difference between the output values to the corresponding input values. The term stability refers to the change in output over a long period, without changing the input (Elgar, 2003).

In conclusion, there are many attributes of an ideal sensor which indicate the use of each one. Nowadays, the level is high but not ideal, as a consequence, science continues to work on the improvement of sensors by making another step further in sensor technology.

2.3 PHYSIOLOGICAL SENSORS

The primary goal of physiological monitoring is the measuring of vital signs of the human body, in order to improve physical and mental performance, to enhance learning and entertainment but most importantly, physiological monitoring leads to a better and deeper understanding of health itself. In the last decade physiological sensors have shown great public acceptance and are often found in use of intermittent and continuous monitoring of a variety of human vital signs including blood pressure, heart rate, respiration rate, oxygen saturation, body temperature and physiological

activity. The conclusions can provide indicators of health condition and present significant diagnostic value (O'Connel, 2013) (Pantelopoulos et al, 2010) (Patel, Park, Bonato, Chan, & Rodgers, 2012).

Physiological sensors find use in every day devices, although physiological monitoring bounded in biomedical engineering sphere, nowadays finds use in several aspects of everyday life from personal sports to advance military training (O'Connel, 2013). Another area that physiological sensors can be applied in is wearable health monitoring, which in the last few years is at a peak. A common wearable health monitoring system consists of several parts such as sensors, wireless communication modules, control and processing units, graphical user interface and advanced algorithms. The obtained measurements are communicated to a central node like a smartphone which displays, processes or transmits the aggregated vital signs (Pantelopoulos et al, 2010) (Fotiadis, 2015) (Cheng Hii & Wan Young, 2011).

In conclusion, physiological sensors have evolved remarkably in recent years. Researches and studies in the area of microelectronics have allowed scientists to develop miniature circuits entailing sensing capability which can find use in physiological monitoring in daily routine. These developments are capable of improving health monitoring in various aspects (Fotiadis, 2015).

2.4 BIOSENSORS

Biosensors constitute a significant and special group in the science and technology of sensors. A proposed definition of what a biosensor really is, could be the following definition:

'A biosensor is an analytical device, which is useful for the detection of an analyte that combines a biological component with a physicochemical detector'

Biosensors technology's wider aim is the outcome of a digital electronic signal, which is proportional to the concentration of a chemical or a set of chemicals (Turner et al, 1987). A usual biosensor device typically consists of three components, which are presented in detail below:

The first component is the bio-recognition component, which is also often called as bio-receptor. The bio-receptor is typically a biological element with high sensitivity, which may consist of enzymes, tissues, cell receptors, antibodies and nucleic acids. The bio-receptor is a biomimetic element or a material with biological

origin that has the ability to interact with the desired analyte in the surroundings in which it is located. These biologically sensitive elements have synthetic or physical origin. Nowadays, biological engineering science is capable of creating several types of bio-receptors (Hierlemann & Hagleitner, 2003) (Hierlemann B. H., 2003) (Dr. Guiseppi) (Wang & Liu, 2011).

The bio-transducer or the detector element is the second component which may be, for example, optical, piezoelectric, electrochemical, etc. The bio-transducer is an integral component of a biosensor device. The received signal from the interaction between the analyte and the biological element must be transformed into a signal, which is easier to measure and quantify. The bio-transducer undertakes this function. More specifically, the interaction between the bio-receptor and the analyte is measured by the bio-transducer which in turn outputs a measurable signal that is proportional to the presence of the aimed analyte in the sample of interest (Hierlemann & Hagleitner, 2003) (Hierlemann B. H., 2003) (Dr. Guiseppi) (Amandeep, Minni, & Kamaljit, 2013).

The third component is the biosensor's reader device. The signal processors of a biosensor's reader device has as a primary liability the display of the final results in a user friendly way. This component constitutes the most expensive part of the biosensor device. However, this part of the biosensor device is responsible for creating a user friendly display that can include transducer and sensitive elements. Different applications require different biosensor working principles, so the biosensor reader devices are usually designed, manufactured and produced in such way that can find use in any demand (Hierlemann & Hagleitner, 2003) (Hierlemann B. H., 2003) (Dr. Guiseppi) (Amandeep, Minni, & Kamaljit, 2013).

The design and in turn the development of a biosensor has the broader goal of quick enabling, convenient testing at the point of interest where the signal was produced (Hierlemann & Hagleitner, 2003) (Hierlemann B. H., 2003) (Dr. Guiseppi). The most known biosensors are the amperometric biosensors. This type of biosensor technology has been widely studied over the last two decades and they still remain in the spotlight. Amperometric biosensors have the ability to monitor currents generated from the interaction between a biological system and an electrode (Hyung Yoo & Youn Lee, 201).

Biosensors have been on the market for many decades. In 1962 the first generation of glucose biosensors was developed by the famous scientists Clark and

Lyons. Nowadays, diabetics hold the fourth generation of glucose biosensors in their hands, which helps them, improve their glycemic profile (Hyung Yoo & Youn Lee, 201).

Thereafter, it has been realized that the ideal biosensor should meet certain requirements and properties such as high sensitivity, great selectivity, good repeatability and accuracy, rapid response, high reliability and ability to self-monitor, wide dynamic range, long service life and reusability, low cost, response regardless of physical and chemical changes and finally should be easily manufactured (Biosensors).

As it is referred to, in the beginning of chapter 2, biosensors can be applied and in turn used in everyday objects, but most of the market is driven by biomedical engineering and technology applications. The emergence of personalized medicine seems to be during the last few years the most important trend in biosensors science and technology. An everyday and well known example is the glucose biosensors that can be used from the diabetic population at home without the presence of the physicians or hospital caregivers (Fotiadis, 2015) (Turner et al, 1987) (Turner, 2013).

The picture below depicts, in detail, the parts and the elements of a biosensor device and the procedure (path) that is followed from the bioreceptor to the display of biosensors recordings:

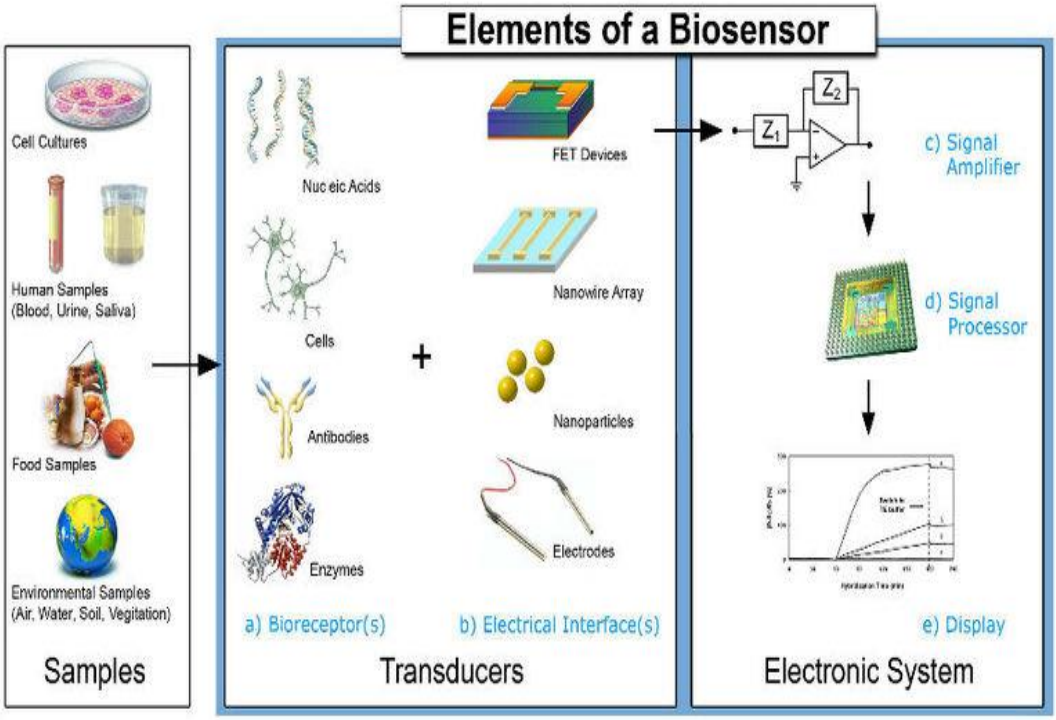


Figure 2-1 The parts that constitute a biosensor device (id-ea.org, 2014).

More precisely in biosensors operating procedures, the specific *'bio'* element recognizes the analyte of interest and the *'sensor'* element transduces the change in the biomolecule into an electrical signal which can more easily measured (Fotiadis, 2015) (Turner, 2013).

Biosensors can be classified and named either by the recognition element either by the type of the transducer. In the following sections the classification and the nomenclature of biosensors are presented in detail. Furthermore, in the following sections the major groups of the above classification are mentioned in detail.

2.5 BIORECEPTORS

The bioreceptor constitutes the part of the biosensor device which is designed to react with the specified analyte for the production of an effect that is capable of being measured and quantified by the bio-transducer. The key requirement of the bio-receptor is a high selectivity for the analyte among a wide scale base including chemical, biological and other significant components (Vo-Dinh & Cullum, 2000).

There are many types of bioreceptors which are intended for different use. They are classified according to the type of their interaction. There are interactions which involve antibodies, enzymes, nucleic acids, tissues, cellular organelles, cells or biomimetic materials (Vo-Dinh & Cullum, 2000).

2.6 BIOSENSOR READER DEVICE

The biosensor reader device is essentially an electronic system which comprises of the associated electronics or signal processors. The signal processors have as primary objective to display the results in a user friendly way (Abbot Diabetes Care, 2015).

More specifically, this electronic system includes the communication station and the software. The communication station is the interface module that transmits the data to a computer. The software is a reporting program that consists of graphics and key statistics that facilitate the analysis and interpretation of glucose standards (Abbot Diabetes Care, 2015).

In conclusion, it is clearly understood that the processor of biosensor's reader device converts the generated signal into a form that the human brain is capable of understanding. This form is may be a number, a sound or a color e.tc.

2.7 SURFACE ATTACHMENT OF THE BIOLOGICAL ELEMENTS

The design and in turn the development of a biosensor is a crucial trial. Attaching the biological elements (cells, antibodies, enzymes, etc) to the surface of the sensor still remains the most difficult part of a biosensor's development. The most simple and popular way, is the functionalization of the surface in order to suit it with the biological elements, usually using aminosilane, epoxysilane or polylysine. Subsequently, the bound biological agent may be for example, fixed by layer by layer deposition of alternatively charged polymer coatings (Pickup, Zhi, Khan, & Birch, 2008) (Barbarisi, Bechi, Innocenti, Redi, & Rosso, 2011) (Gupta & Chaudhury, 2007) (M.I.T Technology Review) (Murugesh, Vengata, Puja, & Kaliappan, 2014). There are four major ways to attach the biological component to the transducer. These four ways are physical adsorption, covalent bonding, membrane entrapment and matrix entrapment (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006).

2.8 BIOTRANSDUCERS

The biotransducer constitutes an integral and very important part of a biosensor system device. More specifically, the biotransducer constitutes the recognition-transduction component of the biosensor system (Wang J. , 2008).

A typical biotransducer consists of a biorecognition layer and a physicochemical transducer, which operate together for the conversion of the received biochemical signal to an electronic signal. Generally, by transducer we mean the device that transforms one form of energy into another. A usable output could be a chemical, optical, mechanical or electrical signal (Wang J. , 2008) (Dorf, 2006) (Smith, 2000).

The biorecognition layer usually contains enzymes or binding proteins like antibodies. It is also worth noting that the biorecognition layer can be comprised of oligonucleotide sequences, subcellular fragments, single whole cells, small numbers of cells on synthetic scaffolds, animal tissues or plant tissues (Wang J. , 2008).

The other part of a typical biotransducer provision is the physicochemical transducer. This part is in intimate and controlled contact with the biorecognition layer. The biological element interacts with the analyte, leading to a physicochemical change which is produced within the biorecognition layer, in turn this measurement is

recognized by the physicochemical transducer and produces an electrical signal that is proportional to the concentration of the analyte (Wang J. , 2008).

The physicochemical transducer can be electrochemical, optical, electronic, gravimetric, pyroelectric or piezoelectric. Based on the type of the biotransducer, biosensors can be classified as mentioned in the following subsections.

2.8.1 Electrochemical Biotransducers

By electrochemical biotransducers we mean all the biotransducers that contain a biorecognition element which selectively interacts with a target analyte and in turn produces an electrical signal. This signal should be proportional to the concentration of the target analyte (Wang J. , 2008) (Dr. Kriz, 2005).

In fact the electrochemical biosensors operating procedure is based on measuring an electrical magnitude such as current or voltage. Typically, the bioelement is an enzyme or an antibody and the mutant is an electrode or an electrochemical provision. Electrochemical biosensors constitute the most common biosensors, especially when the bioelement is an enzyme. The most commonly used types are amperometric and potentiometric. The most popular and widely used are the glucose biosensors (Dorf, 2006) (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006).

Furthermore, a great advantage of electrochemical sensors is that they have the ability to operate in ambient temperatures between -50^{o}C and +50^{o}C, without the need of any external heating. Consequently, their power requirements can be extremely low (Dorf, 2006).

Nevertheless, there are many and different approaches that can be used for the detection of electrochemical changes during a biorecognition event including amperometric, potentiometric, conductometric and impedimetric (Wang J. , 2008) (Dr. Kriz, 2005). Different needs require different approaches suitable with the proper technology to identify accurately the desired target.

2.8.1.1 Amperometric biotransducers

Amperometric transducers are often encountered in biosensors systems. By amperometric transducer we mean the transducer in which the concentration of the substance of interest is determined by an electric current which is produced between

two electrodes immersed in the test solution when one of the electrodes is kept at a selected electric potential with respect to the solution (Wang J. , 2008) (Dr. Kriz, 2005) (Amperometric Transducer, 2003 - 2015).

The whole idea for example, is that an enzyme works as the bio-recognition element or reagent and an amperometric transducer device relays the signals from the enzymatic reactions to the microprocessor unit (Dr. Kriz, 2005).

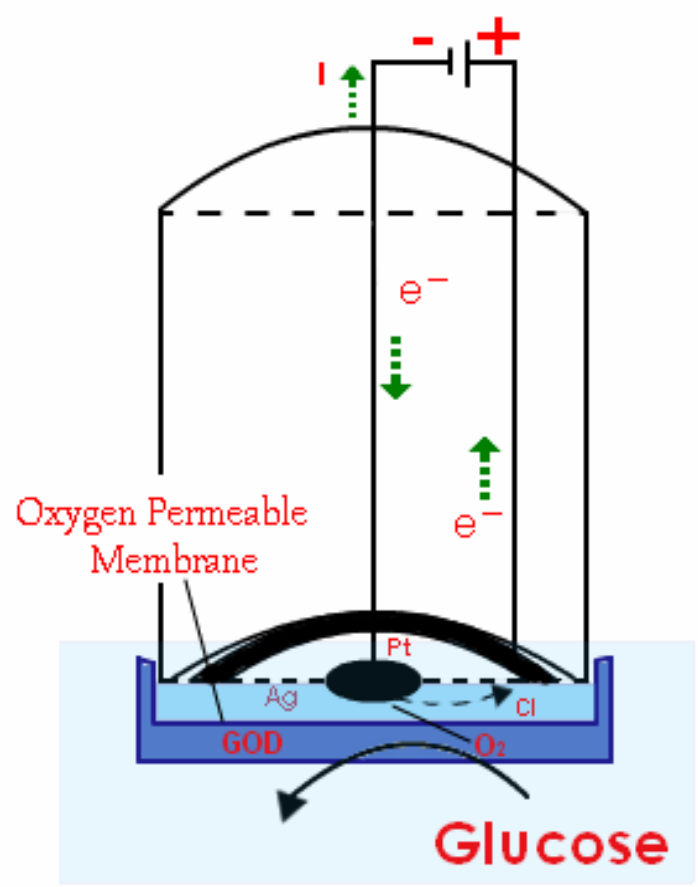


Figure 2-2 Amperometric glucose biosensor (Amperometry, 2016).

The amperometric transducers are useful for the detection in current as a result of electrochemical reduction or oxidation. More specifically, the bio-receptor molecule is immobilized on the working electrode. The potential between the two electrodes (working electrode and reference electrode) is fixed at a rate and then the current is measured with respect to time. The applied potential is the driving force for the electron transfer reaction. The producing current is a direct measure of the rate of the electron transfer. The current mirrors the reaction that appears between the bio-receptor molecule and the analyte and is limited by the mass transport rate of the analyte to the electrode (Wang J. , 2008) (Dr. Kriz, 2005) (Grieshaber, McKenzie, Voros, & Reimhult, 2008) (Wikipedia The Free Encyclopedia, 2015).

In conclusion, the amperometric method is the most common method of electrochemical detection. In the specific biochemical recognition, reactions involved both detectable elements and specifically redox enzymes such as glucose oxidase. Field's potential is maintained at a constant level to allow the oxidation or reduction of the analytical element. The measurable current can be mathematically defined and also can be measured by Faraday's law as referred to below in Equation. 2.1:

$$m = \left(\frac{Q}{F}\right)\left(\frac{M}{z}\right), \tag{2.1}$$

Where, *m* is the mass of the substance liberated at an electrode in grams, *Q* represents the total electric charge passed through the substance, *F* is the Faraday constant which is calculated $F = 96485\,C \times mol^{-1}$, *M* is the molar mass of the substance, *z* is the valiancy number of ions of the substance altered (Strong, 1961).

2.8.1.2 Potentiometric biotransducers

Potentiometric biosensors are useful for the measurement of a potential or change accumulation of an electrochemical cell. An ion selective electrode and a reference electrode usually constitute the comparison of a potentiometric biotransducer. The membrane of the ion selective electrode selectively interacts with the charged ion of interest. This interaction causes the accumulation of a change potential compared to the reference electrode. The reference electrode provides a constant half-cell potential that is unaffected by analyte concentration (Novell, Parrilla, Crespo, Rius, & Francisco, 2012) (Orna & Stock, 1989) (Grieshaber, MacKenzie, Voros, & Reimhult, 2008).

Furthermore, potentiometric biosensors find a wide usage in glucose biosensors technology. Their working principle is simple, when a ramp voltage is applied to an electrode in solution, an electrochemical reaction allows current flowing (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006). In potentiometric glucose biosensors the above reaction mirrors the concentration of blood glucose.

In order to measure the electromotive force or the potential between the two electrodes when insignificant current flows between them, the best solution is a high impedance voltmeter.

The potentiometric response is governed by the equation of Nernst in which the potential is proportional to the logarithm of the concentration of the analyte (Novell, Parrilla, Crespo, Rius, & Francisco, 2012) (Orna & Stock, 1989).

2.8.1.3 Impedimetric biotransducers

Biorecognition events are capable of causing resistive and capacitive changes. Electrochemical impedance spectroscopy intervenes and measures these changes which have a biorecognition event origin. Typically, a small amplitude sinusoidal electrical stimulus is applied, causing current to flow through the biosensor (Juttner & Lorenz, 1991) (Wikipedia, The free encyclopedia). The resistive and capacitive components of impedance are determined from in phase and out of phase current responses (Juttner & Lorenz, 1991) (Wikipedia, The free encyclopedia). The interfacial impedance between the electrode and solution present changes resulted of the analyte binding. An impedance analyzer can be used to control and apply the stimulus as well as measure the impedance changes (Wikipedia The Free Encyclopedia, 2015) (Juttner & Lorenz, 1991) (Wikipedia, The free encyclopedia).

According to the above, it is clearly understood that impedimetric biosensors are based on the immobilization of an antigen between two electrodes which act as capacitor's reinforcements. In turn, the antigen connects to the antibody in the sample and then a change appears in the impedance (capacitance) of the capacitor (Biosensors) (Wikipedia The Free Encyclopedia, 2015).

2.8.1.4 Conductometric biotransducers

Conductometric sensors involve measuring the change in conductive properties of the sample solution. The interaction between the biomolecule and the analyte can change the concentration of the ionic species, therefore leading to a change in the solution by electrical conductivity or current flow (Wikipedia The Free Encyclopedia, 2015) (Wikipedia, The free encyclopedia) (Muhammad-Tahir & Alocilja, 2003).

Two metal electrodes are separated at a certain distance and an AC potential is applied across these electrodes. This applied potential cause's current flow between

the electrodes. During a biorecognition event the ionic composition changes, using an ohmmeter is capable to measure the change in conductance (Muhammad-Tahir & Alocilja, 2003).

2.8.2 Optical Biotransducers

Optical biotransducers constitute an integral part of optical biosensors devices. More specifically, they are important because they find use in the significant stage of signal transduction. The use of photons in this type of bio-transducers aims at collecting information which is related to the analyte. These are highly sensitive, highly specific, small in size and cost effective (Borisov & Wolfbeis, 2008).

The detection method of the optical biotransducers depends upon the enzyme that can convert the analyte into a product which is either oxidized or reduced at the working electrode (Ligler, Frances, Taitt, & Chris, 2002). The principle of evanescent field detection usually finds use in an optical biosensor system as the transduction principle and constitutes one of the most sensitive detection methods (Abel, Weller, Duveneck, & Widmer, 1996).

Furthermore, biotransducers based on optical methods, are becoming increasingly popular day by day, due to the development of optical fibers. Optical properties can be changed by luminescence, ultraviolet-visible absorption, internal reflection spectroscopy and laser light scattering (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006).

2.8.2.1 Luminescence method

Luminescence method constitutes a method that is useful for the development of an optical biosensor. By luminescence method we generally mean the method that allows us to measure doses of ionizing radiation (Wikipedia: The free encyclopedia, 2010).

Furthermore, a well-known example of a luminescence based method is the combination of chemiluminescence and fluorescence in a competitive immunoassay. Chemiluminescence occurs by the oxidation of certain substances. It produces visible light in the cold and in the absence of any exciting illumination that is in the dark, in turn a fluorescent substance absorbs this light and changes it into a light with another wavelength. This occurs for example, when a chemiluminescent-labelled antigen

binds to a fluorescent bind antibody. Hence, the ratio of the original chemiluminescent versus labelled antibodies. One knows the number of added labelled antibodies, so the concentration of unlabeled antibodies can be calculated (Fotiadis, 2015) (Munnikes, 2006).

2.8.2.2 Total internal reflection

Total internal reflection constitutes an optical detection method which is frequently used in biosensors technology. Total internal reflection methods or TIR as it is usually called, based on the complete reflection of a ray of light that hits a less dense medium (Fotiadis, 2015) (Munnikes, 2006).

According to Snell's law at first it is clearly observed that the transmitted light beam vanishes for angles larger than the critical angle θ_c. In turn, according to quantum mechanics science, there is a finite probability for a photon, which should be reflected, to reach the classically forbidden medium. Thus, an exponentially decaying wave on the other side is observed. This evanescent wave can be used in an immunoassay, where the antigens are located at the surface in the less dense medium (Fotiadis, 2015) (Munnikes, 2006).

2.8.3 Pyroelectric Biotransducers

The first half of the word pyroelectric refers to the Greek word *'πυρά'* which means fire. The whole idea behind pyroelectric biosensors is the generation of an electric current resulted by a temperature change. This change induces a polarization in the substance, which in turn produces a dipole moment in the direction of the temperature gradient (al., 1990).

As a result, there is a net voltage across the material. This net voltage can be calculated by the equations 2.2 and 2.3.

$$\Delta V = \omega PAr\Delta T(1+\omega^2\tau_E^2)^{-\frac{1}{2}} \quad (2.2)$$

$$\tau_E = rC \quad (2.3)$$

Where, V is Voltage, ω is the angular frequency of the modulated incident, P is the pyroelectric coefficient, A is the area of film, r is the resistance of the film, C is the capacitance of the film, τ_E is the Electrical time constant of the detector output

2.9 GLUCOSE BIOSENSORS

A definition of what could be called glucose biosensor might be the following:

'By glucose biosensor we mean the device that identifies quickly and precisely the levels of glucose from the surroundings in which it is located.'

A glucose biosensor has the ability to produce an electronic signal that is proportional to the glucose concentration where is located. There are several types of sensors, which can be categorized according to the recording method (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009).

The picture below depicts an example of a glucose biosensor companied with its transmitter:

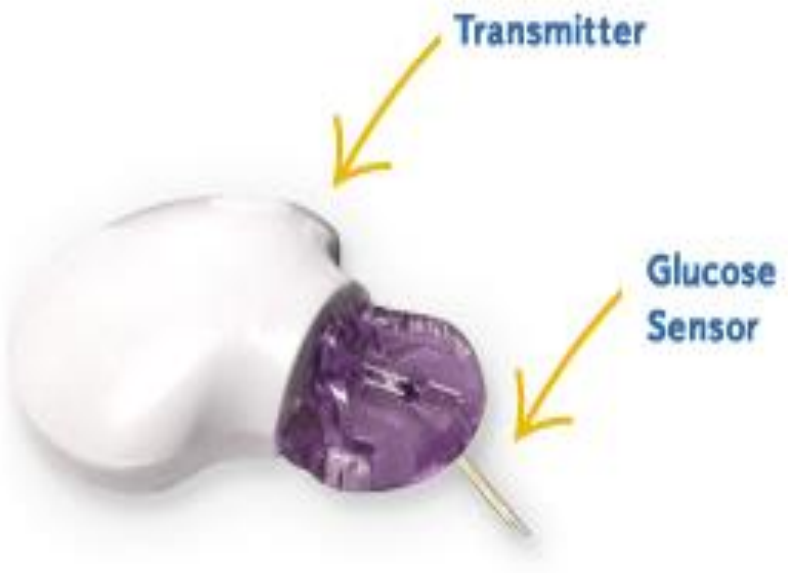


Figure 2-3 Enzymatic blood glucose biosensor (Karmell, 2011).

The route (path) that is followed by the process of recording is described as seen below:

- The analyte diffuses from the solution on the sensor surface.

- It reacts with organic sensor material.
- This reaction changes the properties on the surface of the bio-transducer.
- This change of properties is converted into an electronic signal.

The picture below depicts the route (path) that was described previously in the sections above.

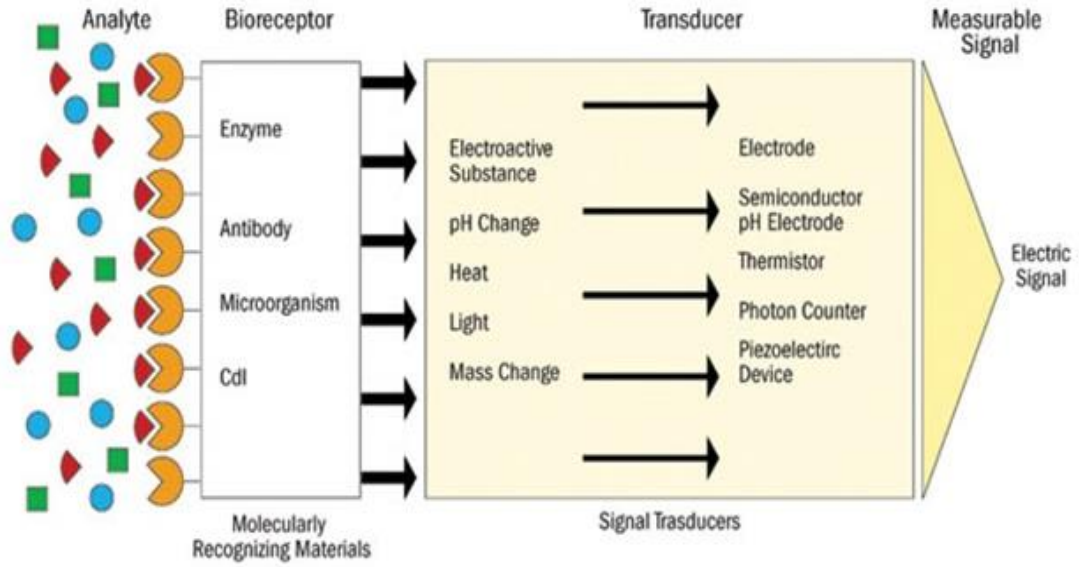


Figure 2-4 The Path that is followed by a glucose monitoring biosensor device (Fisitech - Introduction to Biosensor, 2011).

2.9.1 Classification of Glucose Biosensor Types by Placement and Measurement

Glucose biosensors are divided in two major categories which are '*in vivo'* and '*in vitro'*. Both of them are useful for the diabetic population but their differences are many, including the way they operate, the way they monitor glucose and even the way they are used in the diabetic's daily routine.

Since Clark's invention back in 1962 Glucose biosensors technology have made significant improvements over the last decades. Nowadays, there are several options for blood glucose monitoring, including several types of sensors. The four major types by the placement and usage are subcutaneous, epidermal, continuous and non-continuous glucose biosensors.

A representative example that reflects the differences of glucose biosensors, which will be described in detail below, is that in fact the differences in functionality

and efficiency have been linked with the differences between the photographic camera and the video camera (Didaggelos & Iliadis, 2008).

In Vitro and In Vivo biosensors and their operations and uses are discussed further in the following sections.

2.9.1.1 In vitro biosensors

The nomenclature *'in vitro'*, deriving from the Latin language, refers to the studies which are performed with cells or biological molecules that in turn are studied outside their normal biological context. Whereas, in-vitro biosensors are the sensors that take place in a test tube or outside a living organism in general. The biosensor uses a biological element (enzymes, tissues, cells, etc) which is capable of recognizing or signaling the biochemical changes in solution. Then the bio-transducer converts the biochemical signal to a quantifiable signal (Didaggelos & Iliadis, 2008).

In-Vitro biosensors are widely used for glucose monitoring. These sensors operate with discontinuous measurements and within portable electronic devices are useful for self-monitoring blood sugar for people with diabetes mellitus or in hospitals where it can be measured using capillary or venous blood (Didaggelos & Iliadis, 2008).

However, In-Vitro biosensors can provide useful information for blood sugar levels at a given time (Didaggelos & Iliadis, 2008).

2.9.1.2 In vivo biosensors

The nomenclature *'in-vivo'*, deriving from the Latin language, refers to the studies in which the effects of various biological entities are tested on whole, living organisms. So, according to the definition above, by In-Vivo biosensors we mean all the sensors that operate on a living organism. Following the aforementioned, it is clearly understood that In-Vivo biosensors are very useful for discontinuous or continuous measurements. The continuous monitoring of glucose levels using In-Vivo biosensors can provide information on the variation of glucose levels during the day and help to make the right decisions in relation to food intake and treatment to be followed (Didaggelos & Iliadis, 2008) (Iverson, 2007).

By In-Vivo biosensors we mean all the biosensors that operate inside the body. However, biocompatibility still concerns the design and development of an In-Vivo

biosensor. In addition, the long-term interaction with the body during an extended period of using the device is another concern. The third concern is failure. If there is a failure, the device must be removed and replaced, causing loss of money and in some cases additional surgery (Didaggelos & Iliadis, 2008) (Kotanen, Gabriel Moussy, Carrara, & Guiseppi-Elie, 2012).

Finally, it is worth noting that in order to use the electrochemical sensors for in-vivo measurements, they must be biocompatible, solid and nontoxic. In-Vivo biosensors are very useful for application on continuous glucose monitoring within the body (Fragkou, 2006).

2.9.2 Continuous Blood Glucose Monitoring Biosensors

Diabetes is a metabolic disorder with chronic characteristics and high demands. Blood glucose monitoring is a procedure which gives the upper hand in the diabetes treatment and care. Blood glucose monitoring constitutes a vital piece of a comprehensive management plan. This procedure may be intermittent or continuous and achieved with a helping hand from glucose biosensors.

As diabetes mellitus has been increasing across the world, the need for innovative diabetic management biosensor devices has increased the adoption of continuous glucose monitoring (CGM) devices. Continuous glucose monitoring market globally is focused on applications which can provide accurately and continuous glucose measurements in order to find use in home glucose monitoring. Medtronic, Dexcom, Animas Corporation, Omnipod are some of the major players in the continuous glucose monitoring market.

However, Continuous glucose monitoring biosensors are divided in two major categories including invasive and non-invasive biosensors.

2.9.2.1 Advantages of continuous glucose monitoring

Continuous glucose monitoring has many advantages which are presented in detail below:

- It detects four times more severe exacerbations of glucose compared to the self-monitoring of blood glucose.
- Continuous glucose monitoring can also reduce significantly A1c levels when is compared only with the fingersticks method.

- Another important advantage is the accuracy of recording continuous glucose demonstrated by a number of published studies and allows adjustment of the patient in the corresponding treatment.
- Continuous recording of glucose can also show us the change in levels over time and not just momentary.
- Finally, continuous glucose monitoring gives early warning of an upcoming hypoglycemic or hyperglycemic event (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009) (Medtronic.com).

2.9.2.2 Disadvantages of continuous glucose monitoring

Continuous glucose monitoring has some disadvantages too. The most important include:

- Inexperience on the use of continuous recording.
- Improper calibration may lead to inaccurate results.
- Measurements in the conventional way on the finger are not completely removed.
- Glucose levels are recorded in the subcutaneous tissue rather than into the blood resulting in deviation in 15 minutes relative to that in the blood (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009) (Medtronic.com).

2.9.3 Invasive Biosensors

Invasive biosensors take place in the tissue for their exposure to the interstitial fluid or the fluid conveyed out of the body. More specifically, invasive blood glucose measurement methods referred to capillary blood concentration measured by chemical analysis and recently by photometric analysis too. The most widely used method for measuring the concentration of capillary blood is the so called *'finger stick'*. Using invasive biosensors we are capable of measuring glucose by an absolute concentration (Didaggelos & Iliadis, 2008).

Biosensors science is called to give a solution to serious issues for invasive methods such as biocompatibility and interferences in biosensor environment. By biocompatibility we refer to the body's reaction with the implanted biosensor or biosensor's reaction with the body. Interferences in biosensor's environment may

cause non-specific adsorption or it is possible to affect the biosensor's stability and lifetime (Fragkou, 2006).

Subcutaneous enzymatic biosensor and microdialysis glucose biosensor are the most popular invasive glucose biosensors for continuous monitoring and are presented in detail below:

2.9.3.1 Subcutaneous enzymatic biosensors

Subcutaneous enzymatic sensors use electrochemical methods and they are the most commonly used sensors. Typically, subcutaneous enzymatic sensors include an electrode which at its end comprises an enzyme. The sensors take place in the subcutaneous tissue either in the form of a needle catheter penetrating the skin or fully implantable (Didaggelos & Iliadis, 2008).

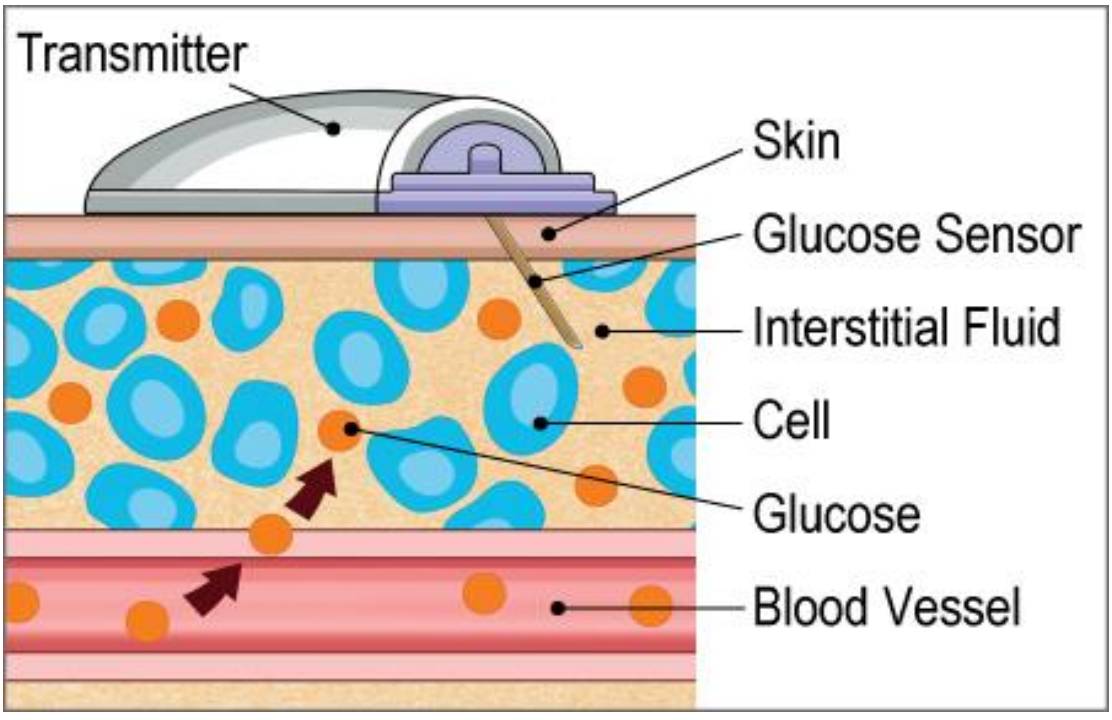


Figure 2-5 Example of subcutaneous continuous glucose monitoring biosensor device (Medtronic - Sensors and Trasmitters, 2016).

Enzymes selectively react with a chemical substance to modify it, usually as the first step in a chain of reactions to release energy. The reaction, which takes place behind enzymatic glucose biosensors, is the selective reaction of glucose oxidase (enzyme) with glucose. An enzymatic reaction can be sensed by measuring the rise in

temperature associated with the heat of reaction or by the detection and measurement of reaction by-products. In the example of glucose, the reaction can be sensed by measuring the local dissolved peroxide concentration. This can be achieved via an electrochemical analysis technique called amperometry which was described in the sections before (Dorf, 2006).

The idea behind subcutaneous enzymatic biosensors is that a tiny glucose sensing device (biosensor) is inserted just under the skin. In turn, the biosensor measures glucose in the tissue and sends information to a pager-sized device. Then an average glucose value is automatically recorded every 5 minutes. Finger stick method and regular meter are needed to calibrate. In addition, for better prevention when glucose is out of target, an alarm sounds (American Diabetes Association).

2.9.4 Non-invasive Biosensors

In contrast to invasive biosensors, non-invasive biosensors are not based on blood reception but their glucose detection capability is based on other body fluids or the correlation of glucose with other sizes, more easily measurable. Non-invasive biosensors are mainly optical or cutaneous sensors. Using non-invasive biosensors, measurements aren't carried out with sufficient accuracy (Didaggelos & Iliadis, 2008).

The difficulties in developing non-invasive blood glucose biosensors are mainly related to the skin structure and the presence of many different substances with similar characteristics in the blood. More specifically, the skin is laminated and each layer has a different structure. The stratum corneum and the adipose tissue do not contain glucose, in contrast to the epidermis and dermis which contain glucose. Furthermore, the roughness of the skin leads to a high specular reflectance.

In conclusion, optical, reverse ionization and tissue impedance spectroscopy are the three major categories of non-invasive biosensors. These major categories are presented in detail below:

2.9.4.1 Optical biosensors

The idea behind the optical method is that it is carried out, by focusing a beam of light somewhere in the organization and the light absorption by the skin depends on

the chemical constituents of the skin such as water, fat, glucose etc (Didaggelos & Iliadis, 2008).

The glucose concentration is determined by changes in the intensity of the wavelength and polarization of the transmitted optical signal. The usual optical methods include Raman spectroscopy, scattered light measurements and infrared spectroscopy (Didaggelos & Iliadis, 2008).

Figure 2-6 Optical glucose biosensor device by Echo Therapeutics Inc (Hoskins, Non-Invasive Diabetes Technology: Still Dreaming, 2012)

Over the last decades, the need for non-invasive biosensors has been increasing day by day. C8 Medisensors by San Jose of California made a biosensor device which is based on optical methods. More specifically, this device uses light for the detection of glucose molecules under the skin via interstitial fluid. The sensor monitors the vibrations of the molecules which show readings on a small portable monitor worn on the skin underneath clothing. It is worth noting that this device received the CE mark approval 5 years ago (Hoskins, Healthline.com, 2012).

Figure 2-7 Optical glucose biosensor by C8 Medisensors (Napodano, 2013).

2.9.4.2 Raman spectroscopy

Raman spectroscopy is a special spectroscopic technique applied to research and study data and information regarding the structure of molecules, ions and crystals. More specifically, this technique has its base on the use of a laser light in order to induce oscillation and rotation of one solution. The molecules vibration affects the emission of scattered light.

Furthermore, Raman spectroscopy, which provides sharper and less overlapped spectra compared to NIR, fixed wavelength lasers at a relatively low cost, can also be used (Kafiul, 2013).

However, there are two major disadvantages using this method. The first one is the long spectral acquisition times and the second disadvantage is the problem of the interference related to other compounds (Kafiul, 2013).

2.9.4.3 Reverse iontophoresis

During the reverse ionization or iontophoresis, a stable low-power electric current is applied to the skin, causing movement of glucose and interstitial fluid

transporting thereof out of the skin. The measurement of glucose is electrochemically achieved by reacting glucose oxidase (Didaggelos & Iliadis, 2008).

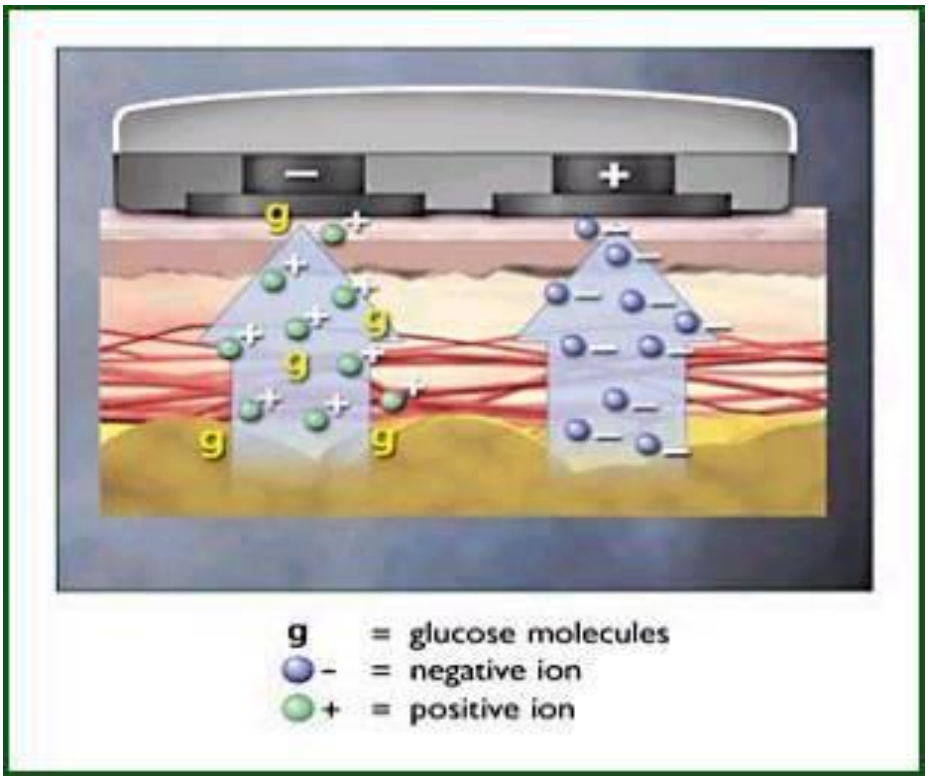


Figure 2-8 Reverse Iontophoresis (Saidin, Rozlin, & Mohd Mazdek, 2002).

Glucose is transported by electro osmotic flow and in turn is collected primarily at the cathode electrode. Regarding the blood glucose monitoring, this procedure results in a lower glucose concentration but in a much cleaner sample (Rozlin, Mohd Mazdek, & Saidin, 2002).

2.9.4.4 Tissue impedance spectroscopy

During this method biological fluid is extracted. We have a production of glucose measurements in real time. The tissue impedance varied, by alternating the current, which is related to the glucose concentration. This method is considered an unreliable method (Didaggelos & Iliadis, 2008).

There are two main reasons that this method is considered unreliable. Firstly, some problems remain to be clarified, such as the effect of body water content and of dehydration. Secondly, some diseases affecting the cell membranes can also have some kind of influence that needs to be evaluated (Kafiul, 2013).

2.9.5 Intermittent Blood Glucose Monitoring Biosensors

Diabetes mellitus disease has high demands during the everyday life of a diabetic patient. Blood glucose monitoring is a very important process and gives the upper hand to the diabetic population for the management and treatment of this metabolic disorder. Before continuous blood glucose monitoring appeared, glucose meters, including glucose biosensors with discontinuous character, were in the arsenal of the diabetic population.

However, most of the diabetic population continues using these biosensors. These biosensors mainly have an electrochemical origin and are well suited for addressing the requirements of home blood glucose monitoring in diabetes disease. As it has referred before, diabetes has many requirements, so home blood glucose devices must be of extremely high quality, because they are called upon, daily, to diagnose potentially life threatening conditions (Wang J. , 2008) (Fotiadis, 2015) (Georga et al, 2014).

The majority of blood glucose meters for glucose monitoring at home, rely on disposable screen-printed enzyme electrode test strips. As depicted in picture 2-9, test strips consist of an electrode system and a hydrophobic layer which constitutes the place of drawing the blood (Wang J. , 2008) (Fotiadis, 2015) (Georga et al, 2014).

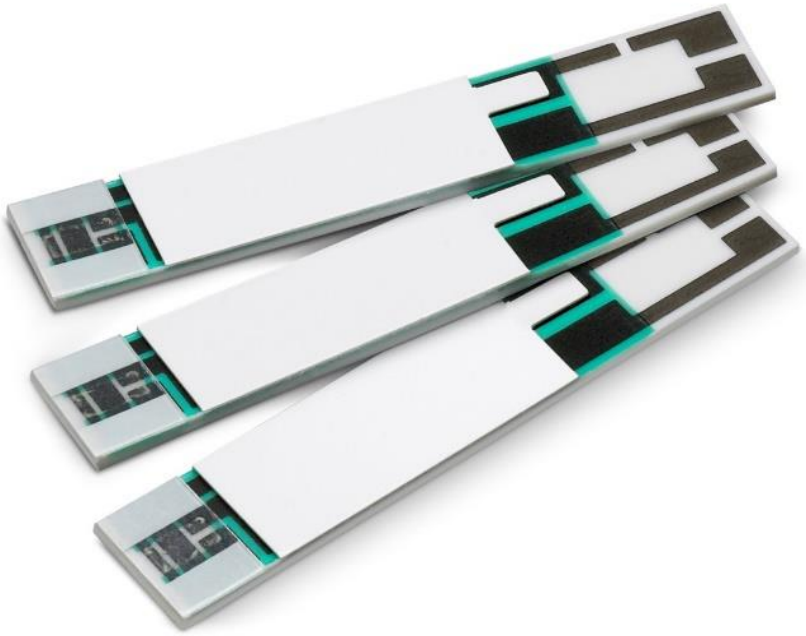


Figure 2-9 Test strips useful for blood glucose monitoring (Scheiner, 2013)

Furthermore, the screen printing technology involves printing patterns of conductors and insulators onto the surface of planar solid substrates. These are based on pressing the corresponding inks through a patterned mask. Each of these strips contains the printed working electrode fitted with the necessary reagents and the reference electrode. The reagents of the working electrode are commonly dispensed by an ink-jet printing technology. Also, two other electrodes (a counter electrode and an additional working electrode) might be included. A variety of membranes is often incorporated into the test strips and along with surfactants, it is used to provide a uniform sample coverage and separate the blood cells (Wang J. , 2008) (Fotiadis, 2015) (Georga et al, 2014).

In conclusion, glucose meter, with non-continuous blood glucose biosensors, have flooded the market over the last decades. The first device of this type was launched by Medisense Inc. in the United Kingdom during 1987 and since then over forty different commercial strips and pocket sized monitors have been introduced for home blood glucose monitoring. During the last decade the entire burden has fallen on the blood glucose continuous monitoring market (Wang J. , 2008) (Fotiadis, 2015) (Georga et al, 2014).

2.10 ELECTRODES

An electrode is an electrical conductor which finds use in making contact with a nonmetallic part of a circuit. The word electrode is of Greek origin, from the words (ήλεκτρο) meaning amber from which the word electricity is derived and the word (οδός) meaning a way (Weinberg, 2003).

An electrode can serve as an anode or as a cathode in an electrochemical cell. The anode refers to the electrode at which negatively charged particles (electrons) leave the cell and oxidation occurs as a result of this escape and the cathode refers to the electrode at which electrons enter the cell and reduction occurs. Each electrode can operate either as the anode electrode either as the cathode electrode, depending on the current's direction through the cell (Weinberg, 2003).

Electrodes are useful since they provide a current through nonmetal objects and in turn altering them in numerous ways and to measure conductivity for numerous proposes. There are several types of electrodes including electrodes for fuel cells,

physiological monitoring in biomedical research, grounding, and cathodic protection etc.

In biomedical engineering and specifically in biosensors technology, the most widespread electrodes are the electrodes for chemical analysis using electrochemical methods (Durst, Baumner, Murray, Buck, & Andrieux, 1997).

In blood glucose monitoring, electrodes constitute an integral component of a biosensor device. An example of electrode useful for blood glucose measurements is depicted in the figure below:

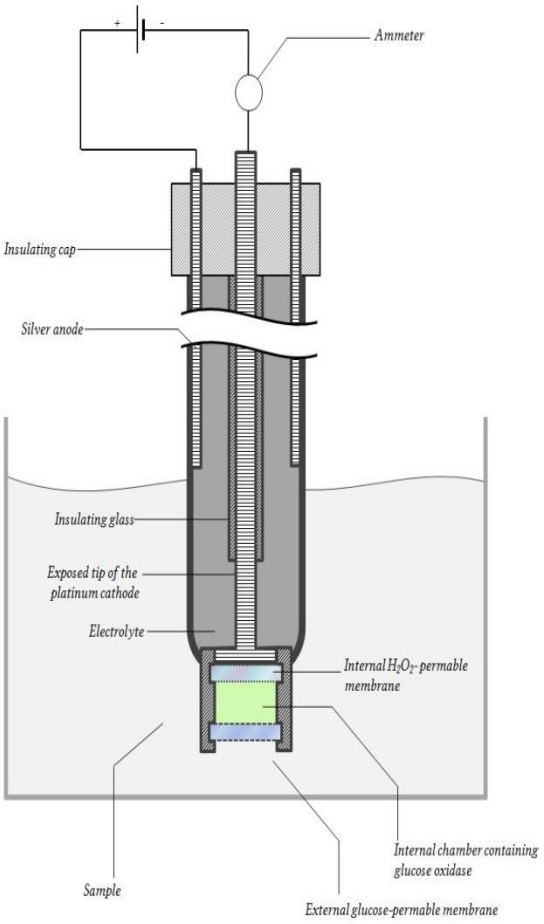


Figure 2-10 Structure of an electrode (Deranged Physiology - Amperometric Measurement of Glucose, 2013 - 2016)

2.11 ANALYTES

In biosensor's technology the term analyte always comes up. This significant term refers to the measured substance by the biosensor. The term analyte is often referred to as a substrate too, which is another nomenclature for the same term. In a biochemical process, biosensors have the ability to analyze any substance that is

consumed or produced. The bioreceptor (biorecognition element) should be characterized by high selectivity to the analyte (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006).

Furthermore, there are several types of analytes that can be measured from biosensors and in turn, form the base of the design, development and manufacture of the respective biosensors. Analytes can be anorganic compounds such as gases, ions and heavy metals, or organic compounds such as aminoacids, proteins, urea and glucose (Fotiadis, 2015) (Munnikes, 2006) (Mohanty et al, 2006).

According to the above it is clearly understood that analytes are one of the most important parts in the biochemical process, as they constitute the substance that is measured by the biosensors.

2.12 GLUCOSE OXIDASE

The blood glucose biosensor, which uses the enzyme glucose oxidase in order to break blood glucose down, is the most famous biosensor. The glucose oxidase enzyme, also known as notatin, is an oxide-reductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. The glucose oxidase is produced by insects and also displays antibacterial activity when oxygen and glucose are present (Wong, Wong, & Chen, 2008).

Glucose oxidase is widely used in order to determine the free glucose within the body fluids. It is also used in vegetal raw material and in food industry. However, it also presents many applications in biotechnologies, biochemistry and nanotechnologies (Ghoshdastider, et al., 2015).

The most known and commercial application of glucose oxidase is in enzymatic glucose biosensor devices, which an electrode is used in order to take up the electrons needed for the oxidization of blood glucose and in turn produce an electronic current that is proportional to glucose concentration. This is the technology behind the disposable glucose sensor strips used by the diabetic population to monitor serum glucose levels (Ghoshdastider, et al., 2015) (Blandford, 2013) (Cass, et al., 1984).

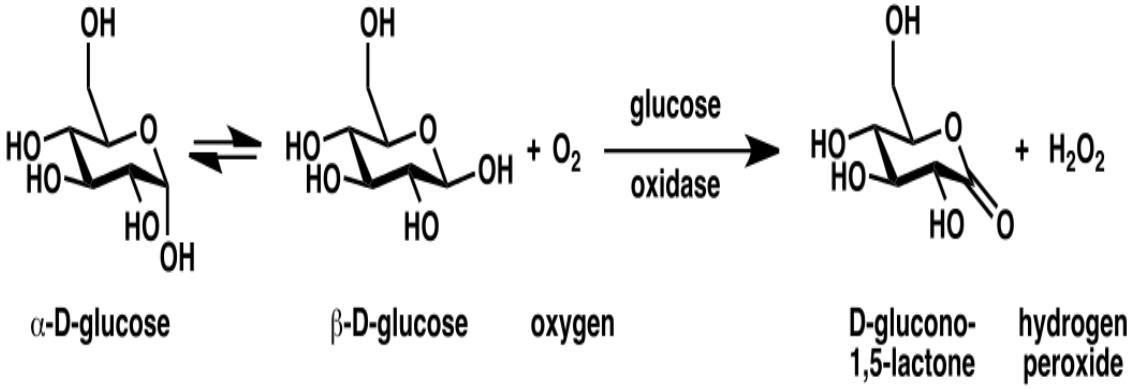


Figure 2-11 Reaction which is catalyzed by glucose oxidase (Wong et al, 2008)

2.13 BLOOD GLUCOSE MONITORING

Blood glucose monitoring is a popular way of testing the concentration of blood glucose. Blood glucose monitoring constitutes a very important and integral part of the daily routine for the diabetic population. The most popular way to measure the blood glucose concentration is by piercing the skin, usually on the finger, drawing blood, then applying the blood to a chemically active disposable test-strip we can read the blood glucose concentration (Holt, 2008).

Physicians advise the diabetic population on the appropriate monitoring regime for their condition. The diabetic population, including all type 1 diabetes and many type 2 diabetes, usually test their blood glucose concentration 3-10 times per day, on the other hand the diabetic population with usual type 2 diabetes test their blood glucose concentration at least once a day. This is due to the fact that can understand the effectiveness of their prior insulin dose and in turn to help them determine their next insulin dose (Holt, 2008).

Improved technology for measuring blood glucose is rapidly changing the standards of care for the diabetic individuals. Blood glucose monitoring reveals individual patterns of blood glucose changes and gives a helping hand to the diabetic population about many daily routines, such as meal planning, activities and, as mentioned before, what time of day to take medications (Holt, 2008).

Nevertheless, biosensor devices, used for continuous glucose monitoring, have appeared only just recently. The most popular continuous monitoring sensors are for subcutaneous use. They are inserted subcutaneously, usually in the abdomen or on the upper thigh, because these are the areas that are less likely to receive shocks or be affected by clothing. The biosensor is typically a small flexible electrode for the

introduction of which an importer called SenSerter is used (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009).

In conclusion, we understand that with blood glucose monitoring we can maintain blood glucose within the target range which constitutes the primary goal. In addition, we are in the position to maximize learning and participation within this procedure and prevent the highs and lows more easily.

In the picture below an example of blood glucose monitoring with the classic method with fingersticks is depicted.

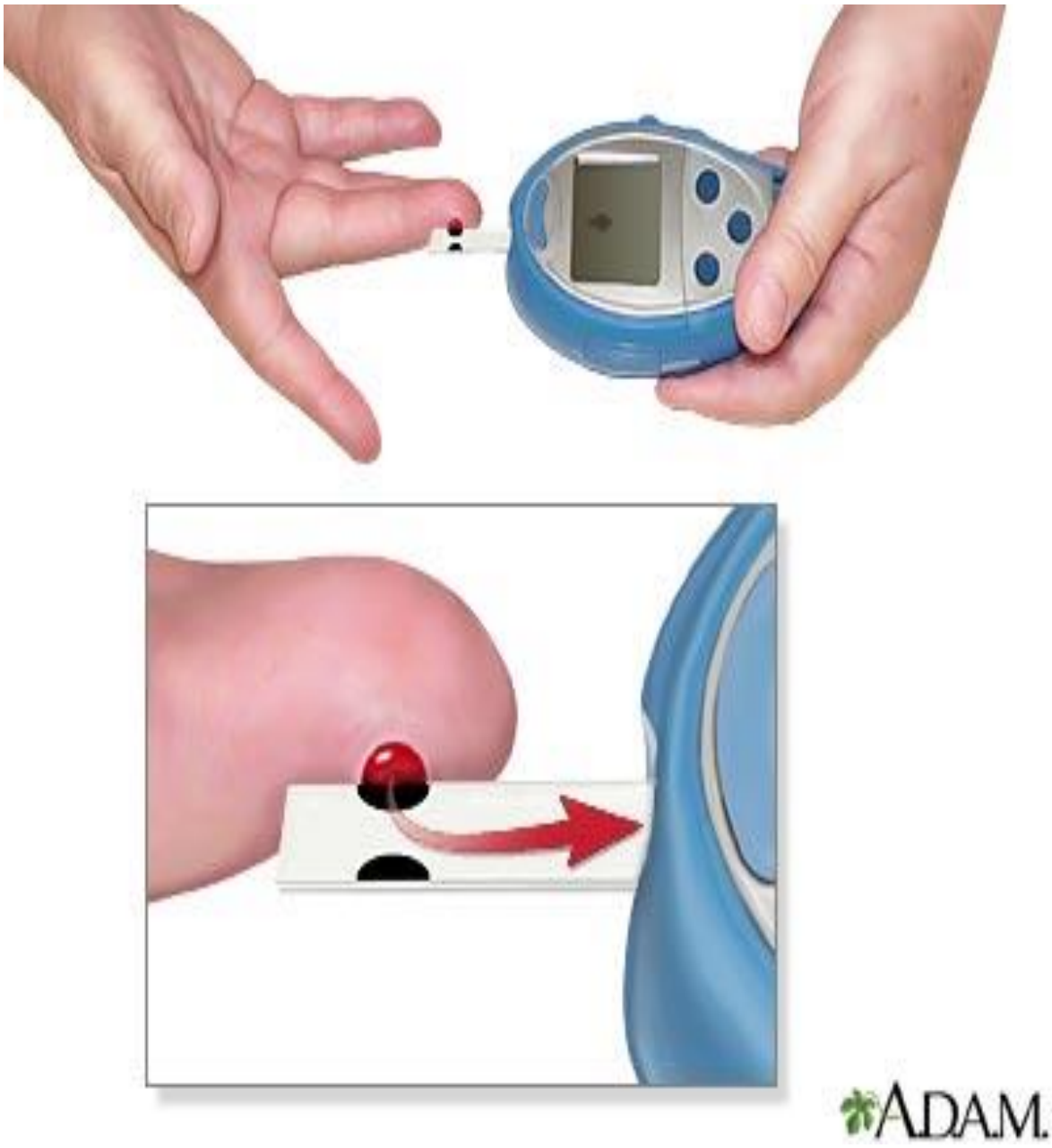


Figure 2-12 Blood glucose monitoring with the fingerstick method (Hendrick Health System, 2015)

The following picture depicts a biosensor device for continuous glucose monitoring connected to an insulin pump which is placed on the abdomen.

Figure 2-13 Blood glucose biosensor connected to insulin pump (Medtronic, 2016)

The biosensors for continuous glucose monitoring should be replaced approximately every three to six days, depending on the specifications of the respective manufacturers. Their expiration date ranges from six to nine months from the date of manufacture. They should also be preserved at temperatures of $2^{o}C$ - $27^{o}C$ (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009) (Medtronic.com).

Nowadays, it is possible to send and transfer all the recorded data to a computer. The transmitter, which is connected to the sensor collects data after encoding the values of glucose, it sends this information wirelessly to the insulin pump. The pump displays the glucose values (Didaggelos & Iliadis, 2008) (Gerasimidi - Vazaiou, 2009) (Medtronic.com).

Another important parameter which concerns all types of biosensors and devices is that they should bear the CE marking which symbolizes conformity to all the obligations incumbent on manufacturers for the product by virtue of European community provisions providing for its affixing (Internal Market, Intustry, Entrpreneurship and SMEs).

2.14 GLUCOSE METERS

By glucose meters or more commonly glucometers we mean those medical devices which are useful in order to measure the levels of blood glucose. As glucose meters can also be used strips of glucose paper when they are dipped into a substance and in turn measured on the glucose chart. A glucose meter is a very important device with daily use in order to monitor blood glucose concentration at home (Medtronic.com) (Diabetic Seniors-Informational Resource for seniors with diabetes, 2014).

More specifically, by piercing the finger with a lancet or something sharp, a small drop of blood is obtained and in turn is placed on a special test strip. Then the meter 'reads' the blood and in turn calculates the blood glucose level. The glucometer then displays the level in units of mg/dl (Medtronic.com) (Diabetic Seniors-Informational Resource for seniors with diabetes, 2014).

In conclusion, according to the market globally, glucose monitoring devices are expected to be more than 18$ billion by 2017 and this number seems to have a growing rate in the next following years.

2.15 WEARABLE SYSTEMS FOR BLOOD GLUCOSE MONITORING

In recent years, diabetes mellitus has shown a growing rate which in combination to daily diabetic needs requires the active and constant attention and participation of physicians, diabetic population and their caregivers too. With the passing of time, diabetes care has evolved, presenting new therapeutic methods and technologies.

Nowadays, more emphasis is given on wearable medical devices for continuous blood glucose monitoring by several medical corporations and companies. As mentioned earlier, the diabetic population plays an active role in the daily life of diabetes, having to properly manage themselves medically and their lifestyle factors too, which in turn are directly related to glucose metabolism (Georga, Protopappas, Bellos , & Fotiadis, 2014).

Figure 2-14 GlucoWatch by Cygnus constitutes an example of a wearable glucose biosensor system (Endotext, 2013)

The technology behind blood glucose monitoring presents a high increasing rate during the passing of time and the idea of an automated closed-loop system in type 1 diabetes mellitus is now at the threshold of the market. With the broader aim being the creation of a fully closed loop system, current approaches aim towards overnight glucose control in order to reduce in minimum the risk of nocturnal hypoglycemia. The technological achievements in continuous glucose monitoring (CGM) and the evolution of continuous subcutaneous insulin pumps could probably be contributed to a more complete (efficient and safe) therapy scheme (Georga, Protopappas, Bellos , & Fotiadis, 2014).

Furthermore, in insulin treated patients self-blood glucose monitoring allows the assessment of short term glycemic status and it is also related to a better long term glucose control, as it is reflected in lower HbA1c levels and fewer acute complications in type 1 diabetes mellitus. Nowadays, all commercial devices have an internal memory and most of them are connected to the user's personal computer through a USB port, where the measurements can be accessed and in turn analyzed with the use of data management tools accompanied with the device (Georga,

Protopappas, Bellos , & Fotiadis, 2014) (American Diabetes A.Standards of medical care in diabetes, 2013) (Cobelli, Renard, & Kovatchev, 2011).

Figure 2-15 Contour Next USB by Bayer constitutes an example of commercial glucose monitoring device having USB (Medtronic - Minimed Product, 2016)

According to American Diabetes Association (ADA), continuous glucose monitoring could probably be a supplemental tool in order to monitor the blood glucose levels at home in those individuals with hypoglycemia unawareness and/or frequent hypoglycemic episodes in contrast to intermittent glucose monitoring which does not have the ability to capture the temporal variations in 24h glucose levels, especially during the night, when blood glucose concentration is seldom measured (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Klonoff, 2005).

Furthermore, as mentioned earlier, invasive methods in continuous glucose monitoring have been focused on subcutaneously implantable biosensors that measure the blood glucose concentration within the body fluids, giving an average value of 1-5 minutes. An example is the Guardian Real Time by Medtronic and Freestyle Navigator II by Abbot which have the ability to inform diabetics in real-time of their subcutaneous glucose levels, trends and rate of change, enabling real-time decisions to be made by the diabetics themselves (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Battelino, et al., 2011).

However, it is clearly understood that wearable systems for continuous blood glucose monitoring have many benefits and improve the diabetic's life day by day. These wearable devices are connected to insulin delivery pumps, which are designed and developed in order to supply a continuous flow of basal insulin at a customizable rate and at the same time allowing diabetics the on-demand administration of supplementary bolus doses (Georga, Protopappas, Bellos , & Fotiadis, 2014). Of course the ideal project is the closed-loop system (artificial pancreas) which ideally could provide a 24hr automatic control of insulin delivery, targeting to achieve tight glucose control and minimizing the risk of hypoglycemia's appearance. The evolution of smartphone technologies allows the realization of an automated, portable closed-loop system for overnight glucose control (Georga, Protopappas, Bellos , & Fotiadis, 2014).

In conclusion, the accomplishment of a fully closed-loop system depends on its hardware components, as well as on the control algorithm. Another demand is the design and development of more accurate and reliable glucose sensors and devices including efficient wireless communications systems (Georga, Protopappas, Bellos , & Fotiadis, 2014). The future seems to be brighter, giving hope regarding the development of new products that give the diabetic the upper hand to manage and control diabetes mellitus properly.

2.16 MOBILE APPLICATIONS FOR BLOOD GLUCOSE MONITORING

In recent years, mobile applications or mobile apps, as they are usually referred to, have flooded several aspects of our lives making our daily routine more comfortable. A proposed definition about mobile apps could be the following:

"A mobile application or mobile app is a type of application software designed to run on a mobile service, such as a smartphone or a tablet computer."

The development of Mobile applications frequently serves to provide users with similar services to those accessed on PC computers. Mobile applications are in general small, individual software units with limited function. The use of this software has been popularized by companies and corporations like Apple Inc. Apple Inc. via its App Store, sells thousands of applications for the iPhone, iPad and iPod. Web apps, online apps, iPhone apps or smartphone apps constitute mobile applications too (Janssen, 2010 -2015).

Figure 2-16 Diabetes mobile applications for iPhone (Plant Advancing Canadian Manufacturing - Diabetes management tool turns smartphones into glucose monitors, 2015)

Mobile applications are one step away from the integrated software systems generally found on PC computers. Instead of each app providing limited and isolated functionality, such as a game avoided multitasking due to limited hardware resources of the early mobile devices, their specificity is now part of their desirability because they allow individuals to choose what their devices are able to do. The idea behind mobile apps is to take applications which are PC-based and in turn port them to a well suited mobile device (Janssen, 2010 -2015). The evolution of mobile Health and the appearance of new methods and technologies in mobile computing and wireless communications have led to the design and development of more efficient mobile health applications (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Janssen, 2010 - 2015).

Figure 2-17 The iWatch is a useful smart watch for blood glucose monitoring using mobile apps (Vincent, 2014).

A use has been found for these advanced mobile health applications in self-monitoring diabetes and providing feedback. The wider aim of mobile diabetes health is the design and development of intelligent products that have the ability to communicate unobtrusively in order to assist diabetics with the management of diabetes (Georga, Protopappas, Bellos , & Fotiadis, 2014). Mobile apps are so important because in combination with smartphones they are capable of handling multi-parametric daily data for medication, dietary habits, physical activity, weight determination and blood pressure (Georga, Protopappas, Bellos , & Fotiadis, 2014). This multi-parametric character constitutes the spearhead of mobile diabetes Health science. In practice, it is proved that many mobile interventions have shown positive effects on diabetic's glycemic control. The existence of many differences between one manufacturer to another is definitely noteworthy and this constitutes a small barrier for the appraisal of mobile applications results.

Mobile diabetes applications constitute part of mobile health science which is the science where medicines are practiced and public health supported by mobile devices (Adibi, 2015). Health monitoring systems provide patients with effective means for tracking and displaying the most significant variables for self-managing diabetes (Georga, Protopappas, Bellos , & Fotiadis, 2014). The most common approach regarding mobile diabetes applications involves the specification of food type and quantity utilizing a built-in food database and the subsequent automatic estimation of food composition. In a similar way, information about patient's physical activity including type and time are also registered, approached through the use of suitable databases (Georga, Protopappas, Bellos , & Fotiadis, 2014).

Furthermore, automatic data transfer functionalities are capable of improving the quality of self-monitoring and supporting the patient's long term engagement. According to a recent systematic review of mobile applications in diabetes mellitus, the communication between glucose meters and mobile phones in 87,5% of the studies were accomplished by methods including Bluetooth, physical wire to the phone or even infrared signaling (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Holtz & Lauckner, 2012). An example appearance of these functionalities is Glooko by Glooko Inc. which constitutes an iPhone databased diabetes management system that simplifies data downloading through the use of a cable compatible with 19 different blood glucose meters (Georga, Protopappas, Bellos , & Fotiadis, 2014).

Data visualization constitutes a significant component of health monitoring systems. The graphical representation of blood glucose levels, which is included in many mobile diabetes intervention studies and in the majority of commercial applications, should foster the identification of glycemic trends with respect to time (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Baron, McBain, & Newman, 2012). Another significant part of self-monitoring procedure constitutes the knowledge regarding upcoming activities. Many mobile applications, which are often called reminders too, are capable of collecting data regarding upcoming activities and reminding the user about them at the right time (Georga, Protopappas, Bellos , & Fotiadis, 2014) .

Nowadays, within the scientific community and the diabetic population too, it is common knowledge that daily self-monitoring constitutes one of the most important parts of diabetes management and health maintenance. Therefore, the scientific community places emphasis in the analysis of daily data in real-time. Consequently, patients can be provided with supportive feedback related to diabetes management. A healthcare team of experts through a mobile diabetes intervention has the ability to review each patient's data and in turn support patients about customized decisions (Georga, Protopappas, Bellos , & Fotiadis, 2014). Another feature of mobile diabetes applications is that it has an educational supporting character, including information about the nature of this metabolic disorder and its effective management. The latest mobile applications message patients that target not only at improving medication adherence but also at promoting healthy behaviors, giving well-structured advice according to international health organizations. An example of these apps is Tactio Health by Tactio Health Group which has the ability to create a weekly activity program recommended by the World Health Organization (W.H.O) and at the same time to display the diabetic's weekly performance (Georga, Protopappas, Bellos , & Fotiadis, 2014) (Arora, Peters, Agy, & Menchine, 2012).

In conclusion, mobile apps, as mentioned earlier, constitute a significant part of blood glucose monitoring systems especially in wearable devices. Real time recording, keeping track of diabetes, as well as information concerning the diabetic's lifestyle, give the upper hand in self-monitoring and improving the quality of the diabetic population lives. It is proved that both mobile medical devices and mobile applications actually improve glycemic control of diabetes. Nevertheless, correct and reliable information, education and right usage of mobile health applications

constitute parameters for a better management of diabetes disorder by the diabetic population and their clinical experts.

2.17 FUTURE OF GLUCOSE METERS AND GLUCOSE BIOSENSORS

It is expected that within the next decade, simple glucose meters may be replaced with continuous glucose sensor devices for all of the diabetic population. This evolution of the glucose meters technology, will most likely decrease complications found in people with diabetes by limiting problems associated with hyperglycemia and hypoglycemia (Abbot Diabetes Care, 2015) (Medtronic.com).

Researches and studies have shown that a disposable tear biosensor could become a reality. Glucose detection methods for clinical tear studies include liquid chromatography-electrospray ionization mass spectroscopy, high performance capillary electrophoresis with pulsed amperometric detection, enzymatic colorometry and enzymatic fluorometry (Bishop, La Belle, Vossler, Patel, & Cook, 2010).

The tear glucose device design and development include contact lens sensors with changing optical properties corresponding to glucose concentration. Polymer materials will have the premium role during the development of tear glucose biosensors. In theory this creative approach offers continual measurement. The tear glucose sensor offers the accuracy of electrochemical detection associated with the fabrication reproducibility of commercial screen-printed sensors (Bishop, La Belle, Vossler, Patel, & Cook, 2010).

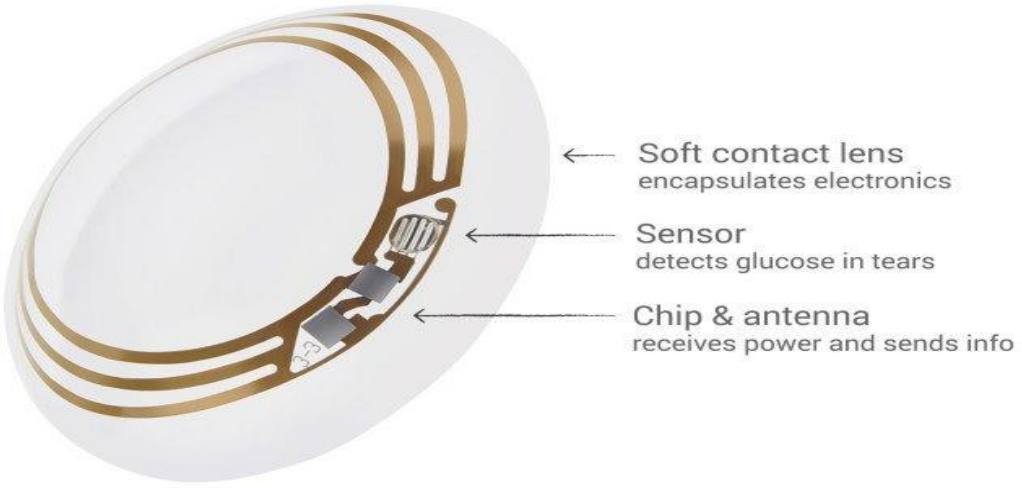


Figure 2-18 Tear glucose biosensor (Tenderich, Helathline - NewsFlash: Google Is Developing Glucose-Sensing Contact Lenses!, 2014)

During the last 7 years, advantages in cellular data communications technology have enabled the design and development of blood glucose meters that have the ability to directly integrate cellular data transmission capability. This method enables the individual to both transmit glucose data to the medical caregiver and in turn receive direct guidance from the physician or the caregiver on the screen of the glucose meter (Abbot Diabetes Care, 2015) (Medtronic.com).

2.18 VOICE OF THE MARKET

As mentioned before, glucose biosensors technology has been making strides and has been evolving more and more each time with a high growth rate. The broader aim of this evolution is the improvement of the diabetic's quality of life. Needle free blood glucose monitoring constitutes a high demand of diabetics as pricking fingers is painful, while minimal invasive methods cause some discomfort to user.

Another demand of the diabetic market is the convenient, continuous and low cost of devices. That means that new glucose biosensor devices should be easy to operate within a short installation and measuring time. Continuous glucose monitoring operation is also capable of providing a wider picture of the glucose rate. Traditional glucose meters have a low cost in contrast to the subcutaneous biosensors devices which are in higher cost.

In conclusion smart wearable devices, suited with recordable data and analysis capabilities seem to gain more ground every day in the diabetic's market voice (Ho, Hao, Yu, & Jian An, 2015).

2.19 ARTIFICIAL PANCREAS

The artificial pancreas is an upcoming technological achievement which is being developed in order to help the diabetic population to automatically control their blood glucose levels. The wider aim is to provide the substitute endocrine functionality as it becomes in the case of a healthy pancreas (Artificial Pancreas Project Research).

The pancreas has several important exocrine and endocrine functions, but it is the lack of insulin production which constitutes the driving force to design and develop a substitute such as artificial pancreas. While the current state of insulin replacement therapy has the advantage of its life saving capability, the task of

manually managing the blood sugar level with insulin alone is arduous and inadequate (Artificial Pancreas Project Research).

The achievement of the artificial pancreas has managed to reach important goals. The first achievement is the improvement of insulin replacement therapy until glycemic control becomes practically normal, avoidance of the complications of hyperglycemia. The second achievement is to ease the burden of therapy for the insulin dependent (Artificial Pancreas Project Research) (diabetes.co.uk).

The artificial pancreas consists of several parts include a biosensor, making continuous measurements of the glucose which continuously determines glucose. It also includes a pump with the potential of continuous infusion of insulin. An algorithm controls the rate of the administration of insulin, based on the measured glucose signal (Hyung Yoo & Youn Lee, 201) (Artificial Pancreas Project Research) (Artificial Pancreas Information on MedicineNet.com).

The insulin pump is linked to the continuous measurement system, which, with the help of suitable computer software, the right amount of insulin is provided at the right time, as in the pancreas of individuals who do not suffer from diabetes mellitus. The functional combination of glucose sensor, computer and insulin pump that results in providing the necessary insulin is what is called a ‘closed circuit’ (Artificial Pancreas Project Research) (Artificial Pancreas Information on MedicineNet.com).

3 MATERIALS AND CLINICAL PROTOCOL

3.1 ABSTRACT

Diabetes mellitus is a metabolic disorder with chronic characteristics, causing problems to the diabetic population's daily life and routine. At the same time it affects the individual's quality of life and reduces their life expectancy up to ten years. Studies and researches have shown that with the right help (treatment and guidance) and attention diabetics can greatly improve their quality of life. Nowadays, the evolution of science has given diabetics and their physicians the upper hand in diabetes treatment and management. Many corporations have placed great emphasis in the design and development of products and devices that aid in the daily management and control of diabetes. This is also reflected in the market, where these kind of products are promoted by the respective companies on an everyday basis. Devices like these are used for the successful conduct of this research, which took place in the University Hospital of our city.

Chapter 3 begins by analyzing how the equipment is used during the process of this research. Special emphasis has been given to each of the device's characteristics, benefits and way of operation. More specifically, these devices are: (i) Fitbit Flex, (ii) Empatica E4, (ii) Medtronic iPro 2 and (iv) BodyMedia SenseWear Armband. These devices aim to provide us with clear and reliable measurements about patient's physical activity and measurements of the variation of glucose during the two weeks of the research. The second part of chapter 3 deals with the procedure of the survey and the following protocol during the research process. The protocol was approved by the Scientific Committee of Ioannina University Hospital. Also in chapter 3 the economic data of this thesis is presented in detail. The study was performed among Caucasian Greek adults with type 1 diabetes mellitus between the ages of 18 and 65 years old. The duration time of the study was two weeks for each individual. In the first week, patients wear continuous glucose monitoring (CGM) devices (iPro 2) and physical activity is prohibited. In the second week, physical activity is performed in combination with the continuous glucose monitoring. A co-primary outcome will be the difference in glucose variability between the two weeks of the research.

In conclusion this research's objective is to collect measurements for the, variation of glucose in combination with or without physical activity. Subsequently

the data is processed by giving us clear conclusions that could be used to improve the management and treatment of diabetes and thus improve the patient's quality of life.

3.2 DEVICES

Since 1962, where Professor Leland C. Clark developed the first and most widespread used commercial biosensor, scientists have seen an impressive improvement in biosensor technology. This improvement is reflected in the diabetic's daily life, giving them the upper hand in addressing the adverse symptoms and side effects of diabetes. Every year many corporations present new biosensor devices to the market, with new capabilities and applications, fitting them with accelerometers, oximeters and glucose biosensors. Some of these devices found use in our research giving us a better understanding of the nature and behavior of diabetes in combination with physical exercise or lack of. These devices are described in detail in the following sections.

3.2.1 Medtronic iPro2 CGM System

Medtronic's iPro2 constitutes a continuous glucose monitoring system which provides us with 24hour readings regarding the fluctuation of glucose, something that can lead healthcare professionals and physicians to gain a more complete insight into the diabetic population's glycemic profiles and in turn help the diabetic individuals improve their glycemic control and their quality of life. This well-designed device consists of five components: the digital recorder, the docking station, the dock USB cable, the wall powered adapter and three cleaning plugs (Clinical Management For Diabetes Healthcare Professionals, 2016). Figure 3-1 depicts iPro2 continuous glucose monitoring system with its docking station:

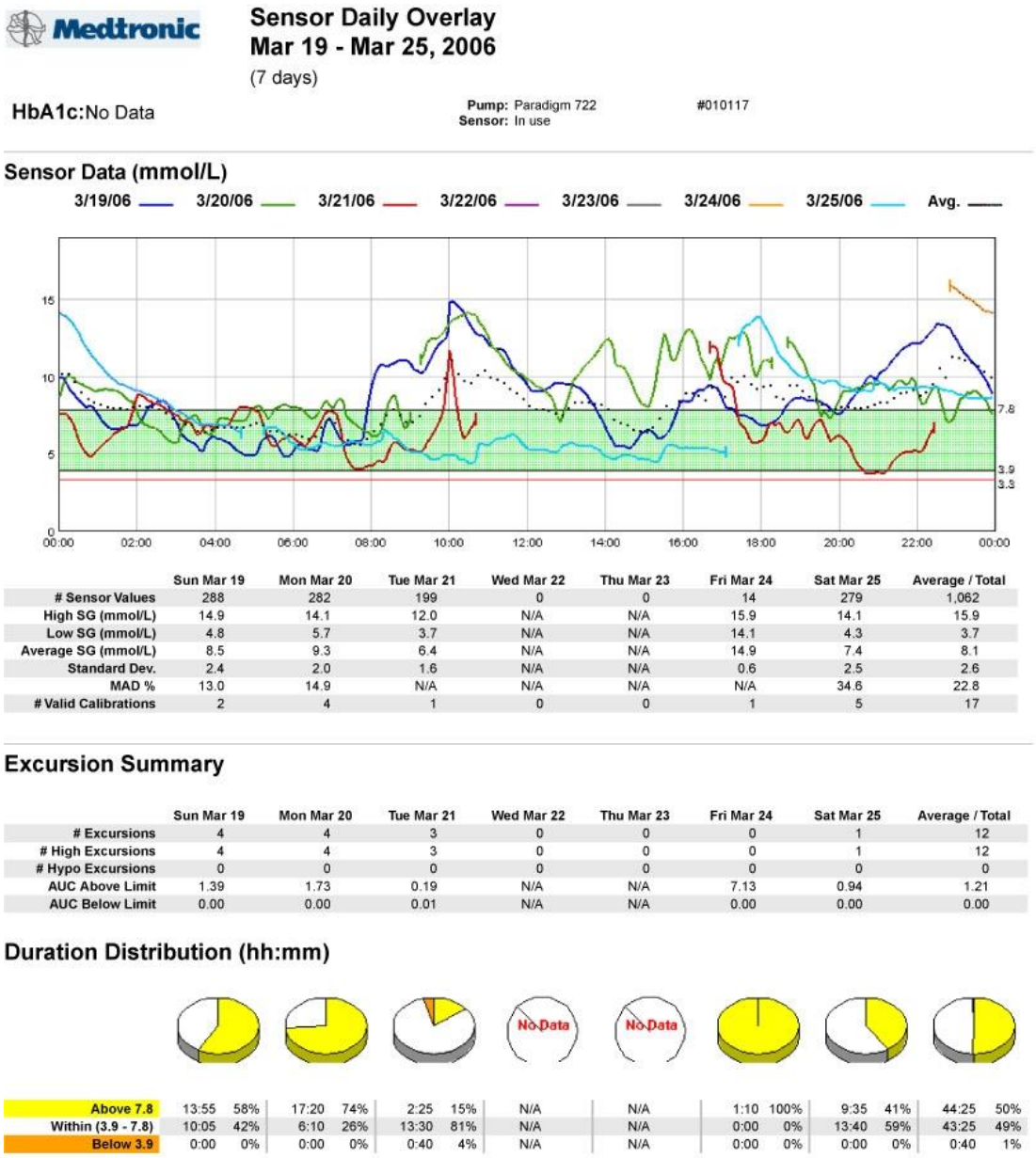
Figure 3-1 iPro2 and its docking station
(Clinica Santa Maria - Diabetologia, 2010)

More specifically, the iPro2 collects and stores data through a glucose biosensor. In turn, the user can upload the data into the iPro2 software (CareLink iPro Therapy Management Software for Diabetes) and generate reports as well as store the collective data. When the iPro2 is connected to an inserted glucose biosensor, a green light flashes on the device. Of course, this function allows the device to be charged, something that can be achieved with the docking station. The iPro2 docking station outside of the charging function, allows us to upload the data into the Software. The three lights (white, green, red) of the dock provide status information. Through the USB cable the data is transferred from the dock to the computer. The iPro2 also includes cleaning plugs, which can provide a water-tight seal to protect the connector on the iPro2 (Clinical Management For Diabetes Healthcare Professionals, 2016).

Figure 3-2 Medtronic iPro2 connected with a glucose biosensor (Monitoring Glyukozy, 2016)

Furthermore, as mentioned earlier, the iPro2 requires software to upload and in turn, display the collective data. CareLink constitutes an internet based system which is designed to help users obtain information from all the diabetes management tools, like glucose meters, insulin pumps and continuous glucose monitoring devices. This software gives a helping hand in organizing the collective data in charts, graphs and friendly readable tables (Clinical Management For Diabetes Healthcare Professionals, 2016). These reports can help the individuals to identify trends and other information that can lead to improved management for better control (Clinical Management For Diabetes Healthcare Professionals, 2016).

In conclusion, the iPro2 was a useful tool in our hands in order to yield good and accurate results, during the course of the measurement process. It is also worth noting that none of the individuals complained that the iPro2 bothered them in some way. In combination with CareLink, the iPro2 constitutes a very easy to use device which can become a part of the individual's daily life, bringing tangible results.



Medtronic

Sensor Daily Overlay
Mar 19 - Mar 25, 2006
(7 days)

HbA1c: No Data

Pump: Paradigm 722 #010117
Sensor: In use

Sensor Data (mmol/L)

3/19/06 3/20/06 3/21/06 3/22/06 3/23/06 3/24/06 3/25/06 Avg.

	Sun Mar 19	Mon Mar 20	Tue Mar 21	Wed Mar 22	Thu Mar 23	Fri Mar 24	Sat Mar 25	Average / Total
# Sensor Values	288	282	199	0	0	14	279	1,062
High SG (mmol/L)	14.9	14.1	12.0	N/A	N/A	15.9	14.1	15.9
Low SG (mmol/L)	4.8	5.7	3.7	N/A	N/A	14.1	4.3	3.7
Average SG (mmol/L)	8.5	9.3	6.4	N/A	N/A	14.9	7.4	8.1
Standard Dev.	2.4	2.0	1.6	N/A	N/A	0.6	2.5	2.6
MAD %	13.0	14.9	N/A	N/A	N/A	N/A	34.6	22.8
# Valid Calibrations	2	4	1	0	0	1	5	17

Excursion Summary

	Sun Mar 19	Mon Mar 20	Tue Mar 21	Wed Mar 22	Thu Mar 23	Fri Mar 24	Sat Mar 25	Average / Total
# Excursions	4	4	3	0	0	0	1	12
# High Excursions	4	4	3	0	0	0	1	12
# Hypo Excursions	0	0	0	0	0	0	0	0
AUC Above Limit	1.39	1.73	0.19	N/A	N/A	7.13	0.94	1.21
AUC Below Limit	0.00	0.00	0.01	N/A	N/A	0.00	0.00	0.00

Duration Distribution (hh:mm)

Above 7.8	13:55	58%	17:20	74%	2:25	15%	N/A		N/A		1:10	100%	9:35	41%	44:25	50%
Within (3.9 - 7.8)	10:05	42%	6:10	26%	13:30	81%	N/A		N/A		0:00	0%	13:40	59%	43:25	49%
Below 3.9	0:00	0%	0:00	0%	0:40	4%	N/A		N/A		0:00	0%	0:00	0%	0:40	1%

Figure 3-3 CareLink Daily Overview Table and Report
(Medtronic - Minimed Products, 2016)

3.2.2 BodyMedia SenseWear System

BodyMedia SenseWear constitutes a wearable device which has the ability to collect and analyze continuous and accurate physiological and lifestyle data. BodyMedia SenseWear system consists of three components, the SenseWear armband, the Optional SenseWear Display and the SenseWear Software. This device's software and hardware are highly accurate and easy to use. The whole idea

behind this wearable device is that regular exercise and physical activity continue to constitute very significant factors in preventing serious medical conditions like obesity, cardiovascular disease, diabetes mellitus and other chronic disorders (BodyMedia SenseWear System, 2013).

Figure 3-4 BodyMedia SenseWear Armband
(Best Fitness Tracker Reviews, 2013)

Furthermore, SenseWear's hardware consists of two parts. The first part is the SenseWear armband, which is small, thin and very comfortable to wear. It has a memory capacity of about 28 days under steady use and its battery power lasts about 5-7 days under steady use. It collects physiological data at a rate of 32 times/second from sensors including Heat Flux, Galvanic Skin Response (GSR), 3-axis accelerometer and finally skin temperature. The second part is the SenseWear Display (optional), which provides immediate, up to the minute, data regarding the amount of moderate and vigorous activity done, calories burned and steps taken. It also allows patients to monitor their progress throughout the day and indicates in real time when their goals have been met and finally, it helps motivate patients towards activity goals allowing them to have a wider picture of their lifestyle (BodyMedia SenseWear System, 2013).

Figure 3-5 BodyMedia SenseWear's sensors (Edwards, 2011)

In regards to the SenseWear software, a PC based software application helps patients to have an easy way of viewing how their lifestyle habits affect their daily life. It also has the ability to graph the raw data for specified time periods, including speed and distance, customize the collection rate of each sensor channel and finally specify activity levels and set their thresholds (BodyMedia SenseWear System, 2013).

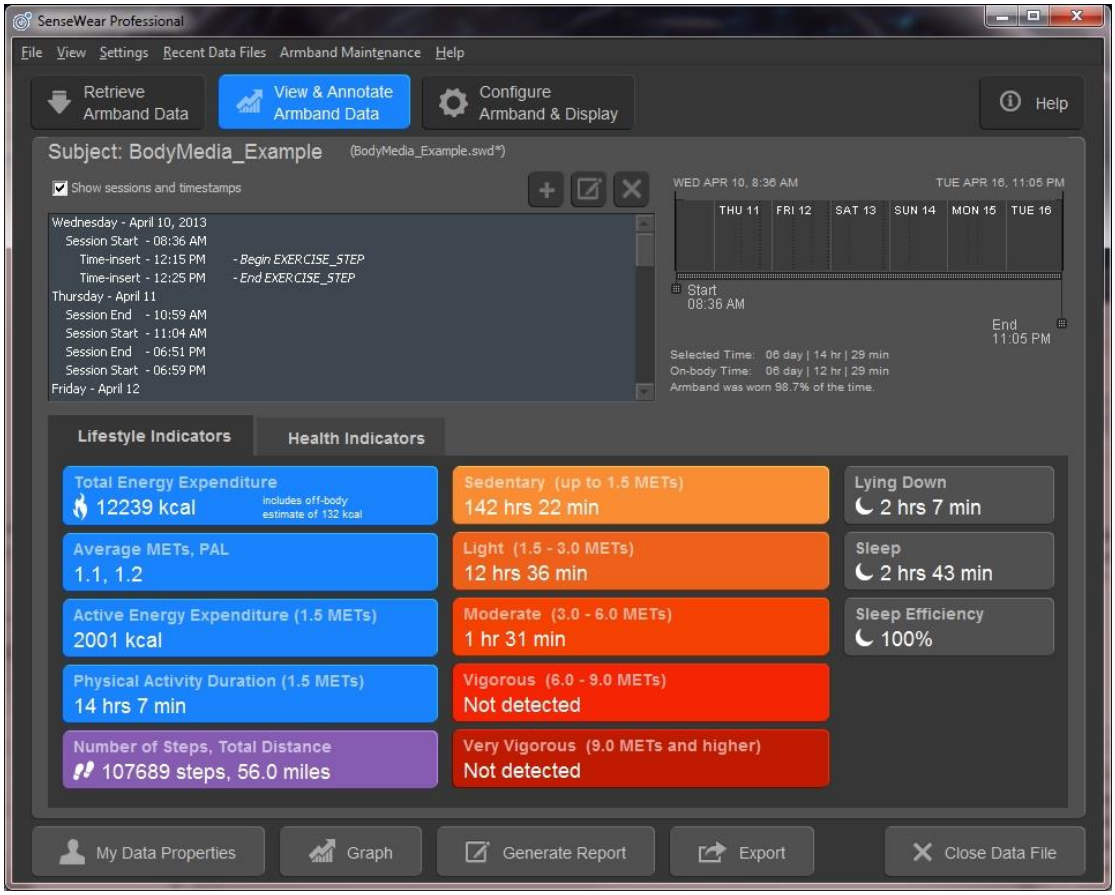


Figure 3-6 BodyMedia SenseWear applications (BodyMedia Review: Evolution of the Newest BodyBugg Calorie Counter Armband, 2015)

In conclusion BodyMedia SenseWear constitutes a wearable device which helps us understand how everyday habits affect our daily life. In our research this wearable device gave a helping hand in order to have a better understanding of the correlation between diabetes mellitus and physiological activity and in turn to reach conclusions regarding how physiological activity affects diabetic individuals (BodyMedia SenseWear System, 2013).

3.2.3 Empatica E4 Wristband

Empatica E4 Wristband constitutes a wearable wireless device designed for continuous, real time data acquisition in daily life. This wearable device uses several types of physiological sensors including accelerometer, photoplethysmography sensor and electrodermal activity sensor. More specifically, E4 Wristband consists of a photoplethysmography sensor which measures blood volume pulse (BVP), a 3-axis accelerometer which has the ability to capture motion-based activity, an electrodermal activity sensor (EDA) which is used to measure sympathetic nervous system arousal

deriving features which are related to stress. Furthermore, this wearable device presents another two amazing features which are the ability to tag events and correlate them with physiological signals and to read peripheral skin temperature (Empatica E4 Wristband, 2015).

Figure 3-7 Empatica E4 Wristband characteristics
(E4 wristband technical specifications, 2016)

Moreover, from the hardware's point of view this device constitutes the result of years of research and development with the wider aim of being the most accurate and comfortable wearable device. E4 Wristband hardware specifications include smart Bluetooth technology (low consumption and short range communication technology), flash memory with 60+ hours of data storage, splash resistant materials with band made of polyurethane, case from polycarbonate and glass fiber and finally lenses from polycarbonate and silicon (Empatica E4 Wristband, 2015).

Nevertheless, it is worth knowing that the E4 can store data in its internal memory. The data can later be downloaded via USB through the Empatica Manager.

The E4 wristband has the ability to connect to a smartphone or desktop computer via Bluetooth. Both modes can upload the data recorded in Empatica's secure cloud platform. Then users can easily access their data (Empatica E4 Wristband, 2015).

In conclusion, Empatica E4 wristband constitutes one of the most accelerate and well-designed wearable devices. Vital signs and movement detection consists the major features of this wearable device with a well-designed lifestyle character helping our research in combination with the other devices for better treatment and management of diabetes mellitus (Empatica E4 Wristband, 2015).

Figure 3-8 Empatica E4 mobile application (Empatica.com, 2016)

3.2.4 Fitbit Flex

The Fitbit Flex constitutes a wearable device which has the ability to track movement 24 hours a day. Fitbit's Flex debut in the market of wearable monitoring devices was in May 2013. This well designed device tracks movement including the number of steps taken in one day, the travelled distance and the most active minutes. It also calculates the calories that the user consumes during the day. Fitbit flex is

typically worn on the wrist just like a wristwatch. A simple display of 5 led lights indicates the movement measurements during the day and vibrates to indicate that user's goal has been reached. The lights also indicate the battery level which lasts 5-7 days and takes 1-2 hours to charge. Charging the battery is achieved with the included specialized USB charger (fitbit flex, 2015).

Figure 3-9 Fitbit Flex (Diaz, 2014)

Furthermore, Fitbit Flex includes sleep patterns too and has sync functions just like several other wearable devices of Fitbit Inc. In addition, this wearable device constitutes one of the most water resistance wearable trackers of the market. Fitbit Flex's measurements are visible through a mobile application on a smart phone's screen and on windows too (fitbit flex, 2015).

Figure 3-10 Fitbit Flex Dashboard (Martin, 2013)

Measurements are depicted as shown on the dashboard in the figure 3-10 above. Fitbit Flex consists of two separate parts, the sensor and the strap. The sensor part consists of 3 axis-accelerometer and a vibration motor. Furthermore, the syncing is achieved automatically and wirelessly using Bluetooth 4.0 wireless technology. Syncing requires internet connection within a range of 20 feet. From the perspective of materials science, the flexible wristband is made of flexible durable elastomer material, similar to that used in many sports watches (fitbit flex, 2015).

In conclusion, Fitbit Flex, in combination with other several devices in this thesis, is used to help us extract conclusions about a patient's activity and to help us learn how this activity affects the diabetic's daily life.

3.3 PROCEDURE

3.3.1 Introduction

Continuous glucose monitoring constitutes a useful tool helping us to achieve a better estimation of the glucose variability (GV) in the diabetic population through

time. Wide scale uses have been found for continuous glucose monitoring in diabetics with type 1 diabetes mellitus. The patients are usually combined with insulin pumps (Standards of medical care in diabetes, 2015) (Rizos, Ntzani, & Fotiadis, 2015). Continuous glucose monitoring provides important information about glucose fluctuation, helping to calibrate the rate and correction of insulin infusion through the pump by the individuals themselves, their caregivers or physicians. Continuous glucose monitoring constitutes an essential part of several insulin pumps too. These devices are usually guided by the real-time data of glucose levels in hypoglycemic situations (sensor augmented pumps). Beyond type 1 diabetes mellitus, continuous glucose monitoring often finds use in pregnant women with gestational diabetes. Gestational diabetes is a type of diabetes which appears during pregnancy by a percentage of women, whose symptoms and undesired complications are similar to diabetes type 2. Furthermore, continuous glucose monitoring finds use in type 2 diabetic patients with poor glycemic control, profound glucose ups and downs, and frequent or unexplained hypoglycemic symptoms. The route for the desired, eagerly anticipated closed-loop biosensor device systems is achieved through the incorporation of continuous glucose monitoring applications. Based on the data provided by continuous glucose monitoring, dietary habits and lifestyle of the diabetic population, physicians usually customize insulin treatment and give advice and guidance towards a certain lifestyle and dietary changes and modifications (Rizos, Ntzani, & Fotiadis, 2015).

Physical activity constitutes one of the most significant factors for glucose variability beyond the dietary habits and patient's lifestyle. The intensity and type of physical activity are crucial determinants, which affect blood glucose regulation. Normally among the healthy population the control of glucose homeostasis during exercise is dictated by a complex interaction between numerous determinants including hormones, the nervous system, and various molecular regulators within the skeletal muscles and the liver. In contrast, the control of glucose homeostasis during exercise is extremely challenging for insulin-treated patients as insulin levels cannot change rapidly and appropriately in response to exercise (Rizos, Ntzani, & Fotiadis, 2015) (Riddell & Perkins, Exercise and glucose metabolism in persons with diabetes mellitus: perspectives on the role forcontinuous glucose monitoring, 2009) (Riddell & Perkins, 2006) (Yardley, et al., 2012). During prolonged moderate-intensity aerobic exercise the failure of insulin levels to decrease at the onset of exercise causes a

reduction in glucose concentration, whereas during anaerobic exercise there could be an increase in blood glucose levels due to catecholamine elevation which are not offset by the increase in insulin availability (Rizos, Ntzani, & Fotiadis, 2015) (Yardley, et al., 2012) (Riddell & Perkins, 2009) (Sigal, et al., 1994). This exercise-induced hyperglycemia can last for some minutes or a large period of time (hours) after the end of the physical activity and is capable of compromising the overall glycemic control and the subsequent exercise performance. It is worth noting that, many sporting activities are a combination of both aerobic and anaerobic sequences and patterns (Rizos, Ntzani, & Fotiadis, 2015) (Guelfi, Jones, & Fournier, 2007). Following physical activity and regardless of the exercise motif, the insulin dependent diabetics are prone to late-onset post-exercise hypoglycemia appearing or lasting for several hours following the end of physical activity (Rizos, Ntzani, & Fotiadis, 2015) (Riddell & Perkins, Exercise and glucose metabolism in persons with diabetes mellitus: perspectives on the role forcontinuous glucose monitoring, 2009) (McMahon, et al., 2007) (Ertl & Davis, 2004). Furthermore, it is possible that physical activity can hide some of the hypoglycemic symptoms like sweating, dizziness and weariness. This exercise procedure results in the fact that diabetic individuals are frequently unable to sense hypoglycemia during the physical activity or after the physical activity period (Rizos, Ntzani, & Fotiadis, 2015).

Existing tools assessing physical activity are questionnaires as well as objective monitoring devices. Questionnaires are cheap and easy to administer, but suffer from recall bias, floor effects (the lowest response category is too high for many respondents) and are unreliable for walking, one of the most predominant activities in adults (Rizos, Ntzani, & Fotiadis, 2015) (Harris, et al., 2009) (Jorstad - Stein, Hauer, Becker, & et al, 2005) (Tudor - Locke & Myers, 2001). Motion sensors (pedometers and accelerometers) are the most well-known and well validated devices that provide objective physical activity measurements. Pedometers are cheap and have the ability to measure step count but not intensity, therefore they cannot distinguish the difference between walking speeds or types of exercise when the legs are not exercised (Rizos, Ntzani, & Fotiadis, 2015) (Riddell & Perkins, Exercise and glucose metabolism in persons with diabetes mellitus: perspectives on the role forcontinuous glucose monitoring, 2009) (Deiss, et al., 2006). Accelerometers measure the body's acceleration in one or more directions continuously for long periods and some of them can also record step counts. The magnitude and the intensity of the acceleration

provide an output, the activity counts per unit of time, which is used to distinguish the difference between different walking speeds and intensity levels. Another useful way for the estimation of total energy expenditure is heart rate monitoring. Generally, there is no difference when heart rate monitoring was compared to doubly labelled water (the gold standard for the assessment of total energy expenditure), although the individual differences were major as shown by the standard deviation of the mean, ranging from -17% to 52% (Rizos, Ntzani, & Fotiadis, 2015) (Westerterp, 2009) (Livingstone MB, et al., 1992) (Livingstone , et al., 1990). Heart rate monitoring is also applied as an indicator of activity intensity and in combination with body movement as a measure of physical fitness (Rizos, Ntzani, & Fotiadis, 2015) (Rennie, et al., 2005) (Plasqui & Westerterp, Accelerometry and heart rate as a measure of physiacl fitness: proof of concept., 2005) (Plasqui & Westerterp, Accelerometry and heart rate monitoring as a measure of physical fitness: cross-validation, 2006). Heart rate monitoring is an objective method affected by more determinants than physical activity. Thus, it could be a useful indicator for the activity of sympathetic nervous system, which also regulates glucose levels (Rizos, Ntzani, & Fotiadis, 2015) (Westerterp, 2009).

Insulin degludec is currently the basal insulin with the longest half-life and it has been reported that it decreases the glucose variability throughout the day, compared to insulin glargine, the most popular basal insulin. Adult patients with type 2 diabetes mellitus on basal insulin have attained a certain way of life including more stable dietary habits, as well as restricted and specific physical activity patterns. They represent the ideal group to see the effect of physical activity on glucose variability depending on the basal insulin they use, as well as to test the performance of the various types for physical activity measurements (Rizos, Ntzani, & Fotiadis, 2015).

Currently, there are few reports on the effect of physical activity on glucose ups and downs assessed by continuous glucose monitoring with several results which depend on the type and intensity of exercise, the age of the population studied and the type of diabetes (Rizos, Ntzani, & Fotiadis, 2015) (Gillen, et al., 2012) (Maran, et al., 2010). Although the existing evidence suggests that continuous glucose monitoring could reduce the glycated haemoglobin (HbA1c) in type 1 diabetic adults (Rizos, Ntzani, & Fotiadis, 2015) (Szypowska, Ramotowska, Dzygalo, & Golicki, 2012) (Tamborlane, et al., 2008) and may lower the number and time spending during hypoglycemic events in type 1 diabetic children, there is a gap of knowledge

regarding the effect of physical activity and the type of monitoring devices, as well as what parameters of these devices correlate best with glucose variability. This kind of data would be valuable for the development of algorithms applied to technically supported insulin infusion systems for type 1 diabetics, in order to attain better glucose control and avoid undesired hypoglycemic situations. Thus, the dream of the closed-loop system would become a reality much sooner (Rizos, Ntzani, & Fotiadis, 2015).

The primary target is to objectively measure the effect of physical activity on glucose ups and downs assessed by continuous glucose monitoring and to study what data correlates best with glucose variability in type 1 diabetic patients. Furthermore, we tried to find the role of heart rate measurement not only as an indicator of physical activity, but also as a surrogate marker of sympathetic nervous system activation, which plays a key role for glucose homeostasis in these diabetic patients (Rizos, Ntzani, & Fotiadis, 2015) (Brage, et al., 2007).

3.3.2 Patients and Methods

3.3.2.1 Study design and participants

The study was performed among Caucasian Greek adults with type 1 diabetes mellitus aged 18-63 years. Seven patients were involved in this study, and all of them did not suffer from a concomitant medical illness that would limit in any way their physical activity. Eligible subjects were advised to avoid swimming (due to technical failure) and record the time and duration of specific activities (e.g. road biking, elliptical, stair climbing, incline walking). The eligible subjects were instructed to wear the CGM system along with the devices for physical activity and heart rate monitoring for 7 consecutive days. During that period, all subjects were obliged to abstain from any structured exercise program (only walking at their usual pace was permitted). Then, all subjects had to continue the exercise once a day. If any of the individuals had not already performed a certain exercise in his everyday life, he was instructed to walk for more than 30 minutes with a walking speed of more than 5 km/hour once daily. The follow up for each participant will stop 14 days later.

Pregnant women, patients with previous stroke, pulmonary disease, peripheral arterial disease, any condition related to movement disability, renal (serum creatinine levels greater than 1.5 mg/dl) or liver disease (transaminase levels greater than the

upper normal values) were excluded from the study. Diabetics on exclusively anaerobic exercise programs were also excluded from the study.

All male subjects were advised to eat 1600 calories daily and all women 1400 calories daily of a predefined choice of foods for the first 7 days. Then, they had to repeat the same dietary pattern for the next 7 days. The participants had to record their food intake, the total calories and the carbohydrate content (in grams) of each meal. Information on insulin regime (i.e. type of insulin, injection dosage and time) was also recorded on a daily basis using a specially designed paper diary.

3.3.2.2 Monitoring devices

We used the Medtronic iPro2, a CGM system approved both for the US and the European market. It allows the collection of data in a blinded way in order to better evaluate the patient treatment. (Medtronic.com). This system does not require calibration during its use; instead the calibration data is entered retrospectively, thus allowing elimination of the bias from patient interaction. The results can be stored and reviewed later by the health care professional. The patient provides the list of glucose measurements, the meals and exercise time. This system claims better accuracy with 11% mean absolute relative difference compared to 16% from the other CGM device by the same Company, the Guardian RT (Medtronic.com) (Nardacci, Bode, & Hirsch, 2010) (Keenan, Cartaya, & Mastrototaro, 2010) (Mastrototaro, Shin, Marcus, & Sulur, 2008). The CGM sensor is calibrated when blood (and interstitial) glucose are in a steady state (glucose levels changing less than 2 mg/dl/min) (Buckingham , et al., 2006).

The enhanced BodyMedia SenseWear System was used to assess the physical activity of the participants, which includes the SenseWear Armband, the SenseWear Software and optionally the SenseWear Display device (Andre, et al., 2006). The SenseWear Armband collects physiological data at a rate of 32 times/second from the following sensors: (i) a 3-axis accelerometer which measures motion and steps taken, (ii) a galvanic skin response (GSR) sensor which measures the electrical conductivity of the skin, (iii) a sensitive electronic thermometer which measures the surface temperature of the body and (iv) a heart flux sensor which measures the rate at which heat is dissipating from the body. The SenseWear software records and analyses physiological parameters and uses algorithms to report daily movement, calories

burned, degree of physical activity and steps taken. Participants were instructed to wear the device on the back of the upper arm, on their non-dominant hand, while awake and to remove it for water activities such as swimming. The system's output was initialized and downloaded using the SenseWear software, including raw data from sensors, as well as comprehensive reports on energy expenditure, MET levels, number of steps and total distance, physical activity levels and duration (including sedentary, light, moderate vigorous and very vigorous levels), sleep duration and efficiency, lying down time and On/Off body time. Data was included if the subject had accumulated a minimum of 10 hours of valid activity recordings per day for the whole study duration (14 days) (Hagstromer, Troiano, Sjostrom, & Berrigan, 2010) (Trost, McIver, & Pate, 2005).

Participants also wore the Fitbit Flex wearable activity tracker (fitbit flex, 2015) (Withings Inspire Health, 2009 - 2015). An integrated 3-axis accelerometer allows both devices to track movement 24 hours a day with a sampling rate of 1 minute. The recorded data consists of steps taken, calories burned, distance travelled and active minutes. Fitbit Flex is typically worn on the wrist like a wristwatch and can sync automatically and wirelessly to leading smartphones and computers using low consumption and short range communication Bluetooth wireless technology.

Heart rate monitoring was performed with the Empatica E4 Wristband, a wearable wireless device designed for continuous, real-time data acquisition in daily life (Garbarino, Lai, Bender, Ricard, & Tognetti, 2014). The E4 Wristband has four embedded sensors: (i) a photoplethysmography sensor which measures blood volume pulse (BVP) from which heart rate (HR), heart rate variability and other cardiovascular features may be derived, (ii) a 3-axis accelerometer which captures motion – based activity, (iii) an electrodermal activity (EDA) sensor (GSR sensor) which measures sympathetic nervous system arousal to derive features related to stress, engagement and excitement and (iv) an infrared thermopile which reads peripheral skin temperature. The E4's custom PPG sensor illuminates the skin and measures the light reflected, where each cardiac cycle appears as a peak of light absorption. Moreover, through a proprietary artifact removal technique, which is based on a combination of multiple wavelengths, E4 attenuates noise even when there are no repetitive movements that affect the sensor. The data output consists of four time series i.e. EDA (also known as GSR), BVP, acceleration, HR and temperature. The data recorded are seamlessly uploaded in Empatica's secure cloud platform.

Physical activity logs were completed with the participant's recordings of their activities at the end of each day. An activity list was provided for the participant on the daily log sheet and the broad categories included: household activities, lawn/garden activities, volunteer/occupational, care of others, transportation, walking, dancing, sports, conditioning, and inactivity. Participants had to record how long each activity was performed. MET values were assigned to each activity and multiplied by the number of minutes each activity performed resulting in MET/min. All physical activity logs were checked by the researcher and reviewed with each participant to ensure completeness. When there are obvious discrepancies between the three objective monitoring devices of physical activity, then the accordance of their outcome with the report of the questionnaires has to be checked (Ainsworth, et al., 2000).

3.3.2.3 Outcomes

The primary outcome will be considered the difference of glucose variability (GV) between the first 7 days when structured physical activity is prohibited compared to the last 7 days when structured physical activity is performed. A co-primary outcome will be the correlation of physical activity data with glucose variability recorded by the continuous glucose monitoring systems (CGMS). Secondary outcomes will be the associations of various parameters assessing glycemic variability with different variables of physical activity/heart rate monitoring.

Glycemic variability and glucose exposure will be assessed by measurements such as: (i) the coefficient of variation (CV), which represents the SD divided by the mean glucose from the CGM expressed as a percentage), (ii) the mean, (iii) the SD and (iv) the IQR (Standl, Scnell, & Cariello, 2011) (Cameron, Donath, & Baghurst, 2010) (Bergenstal R. , et al., 2013).

3.4 ECONOMIC COST OF THE RESEARCH

The economic cost of the present thesis was a significant factor for its achievement. Several parts of the research require a high economic budget in order to accomplish it in high level regarding the quality of the received data, the accuracy during the data analysis and finally the outcome of high quality results and conclusions.

Regarding the medical devices used for continuous blood glucose monitoring, it is worth noting that at the end of each week of the protocol the patient's sensor was replaced from a new one. Also in order to ensure that problems during the placement of the devices will not arise, 6 devices of iPro2 were in our arsenal. It is enough to think that each of them has a current market cost near to 1,200€, also every sensor has a current cost of 50€. Of course the consumables products like tapes have an additional cost of 2.5€ for each tape. Regarding the economic cost of the physiological monitoring devices there were certain differences. More specifically, Fitbit Flex has a current cost near to 100€ in the market with no additional costs and 6 of them were in use during the present research. This is a reason that this product is very attractive for the population who want to monitor their sport performance.

Furthermore, SenseWear Armband is another device which was useful in order to monitor continuously the physiological activity. This device has a current price near to 100€ in the market. During the present thesis 4 SenseWear device were used. Also this device has no additional costs. Finally the Empatica E4, which was another useful device for physiological monitoring was found wide scale use during the measurements procedure. This device has a current price near to 1,600€ in the market and 2 of them were in our medical device arsenal. This device with no additional costs was the most expensive device of the program. Of course there were lower costs of consumables too, like papers for the patient's diaries.

In conclusion, from the above it is clearly understood that the accomplishment of this survey required a high economic budget. This economic cost covered from the University of Ioannina. Finally as high as the economic cost was, the results fully met our goals.

4 DATA PROCESSING AND ANALYSIS

4.1 ABSTRACT

Diabetes mellitus constitutes a metabolic disorder with chronic characteristics. Glucose plays a key role in diabetes mellitus and ensures that it is well controlled and managed and in turn it improves the individual's quality of life. On the other hand, suboptimal and poor glycemic control may lead to undesired conditions such as hyperglycemic or hypoglycemic episodes, which may constitute a threatening factor for the patient's life.

In the last decade big steps have been taken in the sector of proper utilization of the data obtained from diabetic patients. Continuous glucose monitoring devices appeared in the market as 'Deus ex Machina', providing information about the patient's glycemic control around the clock and not in isolated moments. In turn, this data can potentially reveal the missing parts of a patient's glucose profile and with optimal utilization can improve the control and management of the disease. On the other hand, underutilization of glucose data may contribute to a poor glycemic control factor among diabetic patients. In order to achieve optimal utilization of glucose data, scientists and researchers have introduced some specific standards and measures in the analysis of this data.

Chapter 4 deals with the data processing and analysis which was taken from the seven individuals during this research. The first part of chapter 4 presents the measures and standards that deal with the overall view of a patient's glycemic profile, then an analytical description of the measures that is used in this research is made. The second part of the research deals with the pure part of the processing and analysis of the glucose data and the way that this analysis was achieved.

In conclusion, chapter 4 constitutes a very significant part of this research trying to explain the measures and methods that were used in order to analyze each patient's glucose data and along with chapter 5 reveal the missing parts of their glucose profile giving a key message about the fluctuation of glucose and the correlation between the fluctuation of glucose and physical exercise.

4.2 GLYCEMIC CONTROL

Diabetes mellitus requires the constant attention and vigilance of the individuals and their caregivers to properly manage, with tangible results, their glycemic control. It is also worth noting that the correct and complete information about the physiology of the disease, their condition and the latest news about diabetes, plays a key role in the course of the individual's health profile. Continuous glucose monitoring facilitated the effort to achieve a better understanding regarding the weak points of each patient and in turn to help them improve them. The optimal use of CGM devices can potentially lead to the desirable results and improve the patient's glycemic control.

However, despite the latest biomedical engineering improvements, CGM devices and insulin pumps, significant weakness between individuals remains. In most cases, this is due to suboptimal glycemic control which is often a result of poor adherence to prescribed insulin regimens (Bergenstal M. , et al., 2013). Studies and researches have shown that missing insulin doses, avoiding insulin at the start of the meal and not working to refine insulin to carbohydrate ratios at each meal are also correlated with higher HbAc1 levels (Bergenstal M. , et al., 2013). Many individuals have revealed the fear that avoiding insulin doses keeps them away from hypoglycemic events. This is due to the fact that, from the beginning of their disease, diabetics learn that hypoglycemic conditions are potentially dangerous, generating physical problems which in turn affects consciousness and humiliates them socially. It has been proven that frequent hypoglycemic events increase the fear of low glucose levels in diabetics (Bergenstal M. , et al., 2013). This open secret was revealed in our research as well, firmly verifying the above. During the two weeks of the program, many individuals revealed that they prefer to be in a hyperglycemic condition than in a hypoglycemic, frequently trying to avoid their insulin doses.

In conclusion, methodical strategies, in combination with constant and correct information, can potentially give a helping hand in order to understand glucose patterns and accommodate therapy intensification while reducing the frequency and fear of hypoglycemia, can probably improve the individual's glycemic control (Bergenstal M. , et al., 2013).

4.3 STANDARDIZATION OF GLUCOSE REPORTS AND ANALYSIS

Continuous glucose monitoring has the ability to produce, at a staggering pace, a large amount of information, often confusing an inexperienced individual's judgment (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). So, after the development of CGM devices and their mass introduction to the market, the physicians and the individuals were called upon to read and interpret the received data, a new need appears. This is the need for a systematic approach, in order to explain and interpret the CGM received glucose data (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). The scientific community heard the call and in response tries to standardize the glucose profile summary measurements and the value of a uniform glucose report, in order to help physicians, researchers and the diabetic population (Bergenstal M. , et al., 2013). There are a large number of measures available in order to give an overall glycemic control profile of an individual. Of course, some measures stand out more among others, regarding their validity and their solvency in terms of the results that can lead to how these results are able to help us understand the received data more easily and in turn formulate the patient's glycemic profile.

Some other measures lie in restrictions and are unable to lead to tangible results, causing the scientific community to disagree, in some cases, about their validity and solvency. A well-known example is the HbA1c as a sole measure, though it constitutes a significant part of diabetes management (Bergenstal M. , et al., 2013). This is due to the fact that glycated hemoglobin has the inability of diurnal glucose patterns characterizations, which constitute critical factors to understand safe, effective and timely insulin adjustment and updated clinical decision making (Bergenstal M. , et al., 2013). It is known that, red blood cells live three months more or less, so in this way glycated hemoglobin is considered to reflect, in general, mean blood glucose over this period of time and in turn HbA1c doesn't constitute a good indicator of day to day diabetes control (Matthaei, Assessing the Value of the Ambulatory Glucose Profile in Clinical Practice, 2014). In the non-diabetic population HbA1c levels range closely to 5,7%, but among diabetics these levels rise to 7% (Spero, 2016). It is also worth noting that, individuals who probably present similar HbA1c levels, doesn't necessarily mean that they present similar patterns of

glucose excursions and rates of hypoglycemia around the clock (Bergenstal M. , et al., 2013) (Matthaei, Assessing the Value of the Ambulatory Glucose Profile in Clinical Practice, 2014).

Furthermore, it is clearly understood that if we want to evaluate an individual's status, common definitions and measures are required, thus making clinical decisions more informed (Bergenstal M. , et al., 2013). In this way, the need of standardization of clinical terms and measurements allows a very accurate assessment of diabetics and their comparisons of status progress every time they visit their clinician. In addition, standardization can potentially make an individual's care and clinical research more efficient (Bergenstal M. , et al., 2013). In this survey, based on the above, we tried to take into consideration some measures that help us understand better each patient's glycemic profile. These measures that we used in our research are described in detail in the next sections and subsections.

4.3.1 Target in Range (TIR)

Glucose presents a fluctuating character in diabetic patients, often with indistinct patterns generating undesired conditions. The diabetic individual is considered wise enough to check the glucose levels in his blood regularly during the day. Of course the use of CGM devices has highly improved this procedure, but what is the correct value range and when does it need adjustment?

According to the American Diabetes Association, the normal glucose levels for people without diabetes are within the limits of 70 – 99 mg/dl before meal, but for a person with diabetes are 70 – 130 mg/dl before meal and less than 180 mg/dl after meal (Spero, 2016). It is always wise to remember that the wider aim in diabetes mellitus is to keep glucose levels as close to normal as possible (Spero, 2016). According to Ambulatory Glucose Profile (AGP) the selected target range of glucose levels for diabetic individuals is 70 – 180 mg/dl (Bergenstal M. , et al., 2013). In this research it was considered that the ideal target range of glucose levels is 70 – 140 mg/dl. For values below 70 mg/dl the individual is in the hypoglycemic area and for values above 140 mg/dl he is in the hyperglycemic area (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). The expression of TIR can be as a percentage (%) of readings in range or in hours per day in range, which both of them

are included in this survey. The choice of these two outcome metrics held because they are simple, direct and easy for a patient to understand and to also have a continuing follow up for observation of patient's improvement (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009) (Bergenstal M. , et al., 2013).

In the present survey the time above and below target range was considered a significant and very meaningful factor too, which in combination with the measurements of normal target range will give more comprehensive results. Thus, in this direction these measurements are presented in this research as Average Hours per Day above or below the limits of 70 – 140 mg/dl and also presented as a percentage (%) of readings above or below the normal target range that we've set. These measures are presented in detail in the next subsections.

4.3.1.1 Hyperglycemia

Diabetes mellitus has the ability to lead diabetic patients to several undesired conditions. Hyperglycemia constitutes an undesired condition in which, blood glucose levels rise above the set limit of 140 mg/dl. There are several criteria about the quantifying and reporting of hyperglycemic events depending on the duration time or the frequency. Of course, the division of hyperglycemia into levels, depending on the severity of hyperglycemic events, has been proposed by several studies and researches (Bergenstal M. , et al., 2013).

In this survey it was considered wise to measure the average hours per day above the limit of 140mg/dl and the percentage of events above target range (>140 mg/dl) during each week of the program. Furthermore, another significant measure constitutes the maximum glucose value that was recorded too, during the weekly program. These three measures help the quantification simply and directly and in turn the reporting of hyperglycemic events in order to improve the glycemic profile of the individual (Bergenstal M. , et al., 2013).

4.3.1.2 Hypoglycemia

The ultimate goal of diabetes management is the decrease of possible long and short term complications, in order to improve the patient's quality of life (Rodriguez - Gutierrez, et al., 2016). As in the case of hyperglycemia, in hypoglycemia too,

glucose values appear outside of the specified limits. The difference is that, hypoglycemic events appear when glucose values fall under the specified limit of 70 mg/dl leading the individual to undesired conditions.

Furthermore, the lower the glucose levels are from the limit of 70 mg/dl, the more severe hypoglycemia is characterized and in turn leading to more severe undesired complications. Studies and researches have suggested the division of hypoglycemia into levels, depending on the severity of hypoglycemic events, something that has been proposed in the case of hyperglycemia (Bergenstal M. , et al., 2013). According to the American Diabetes Association (ADA) the definition of hypoglycemia is when the glucose values fall under the limit of 70 mg/dl something that was found to be in agreement in this survey too (American Diabetes Association, 2013) (American Diabetes A.Standards of medical care in diabetes, 2013).

In the present research, in order to quantify and report hypoglycemia, it was considered wise to measure the percentage of values below the limit of 70 mg/dl during the weekly program for each week. Another performed measurement was the average hours per day of hypoglycemia. This measurement helps quantify the average time which the individual spent under hypoglycemic conditions during each week of the program. The minimum glucose value was also recorded for each of the two weeks (Bergenstal M. , et al., 2013).

Finally, even the not so serious hypoglycemic events cause disruptions in the diabetic's daily life and the more serious the hypoglycemic events, the more serious their consequences. Severe hypoglycemia presents high correlation with cardiovascular events, cognitive impairment, dementia, even death (Rodriguez - Gutierrez, et al., 2016). Hypoglycemia still remains the greatest barrier in insulin therapy intensification in type 1 diabetics (Eran, Thorne, Kara, Moshe, & Dassau, 2014). The improvement of glycemic control reducing hypoglycemic events remains the wider aim of diabetes management (Bergenstal M. , et al., 2013).

4.3.2 Glucose Exposure

Diabetes mellitus is a disease which has the ability to force the diabetic individuals to make several changes in their lifestyle and daily routine even from the beginning of the metabolic disorder diagnosis. As mentioned before, diabetics should

systematically check their glucose levels during the day, but the need of accurate, simple and easy reading results remains high.

These checks are highly correlated with glucose exposure and in this research it has been considered that an evaluating option in order to report glucose exposure could be the mean glucoses of all readings. The reason of this choice is because the mean of all readings as a measurement remains a simple and easy way for the patient to understand his condition and also presents the best correlation with HbA1c something that is important for clinicians and researchers too (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). In addition, some individuals reveal that mean glucose exposure for specific periods of time gives them a helping hand to evaluate the food effects, their physical activity and their insulin adjustment (Bergenstal M. , et al., 2013).

Furthermore, in this research the iPro2 gave us the ability to produce a review which includes the mean of all readings in each week of the procedure. This was found useful in the section of analysis because it also gave us the opportunity to verify our results and in turn find deviations about the results, as it was considered wise to present the mean of all glucose readings on a weekly basis too, in this survey.

4.3.3 Glycemic Variability

Glycemic variability operates as a hypoglycemic predictor factor both in type 1 and type 2 diabetes mellitus, but still remains with serious unanswered questions related to diabetes management (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009) (Kourtoglou, 2013) (DeVries, 2013). There is a strong belief that glycemic variability may be related to the pathogenesis of complications in diabetic population (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). This is of great interest to the diabetic population, clinicians and researchers, but the confusion about its definition, identification and its clinical implications still punishes the scientific community (Kourtoglou, 2013).

However, studies and researches have shown that if the individual wants to improve the overall mean glucose or his HbA1c levels, then he should work to reduce

high glycemic variability, with wider aim the minimum risk of hypoglycemia (Bergenstal M. , et al., 2013). Several measures have been proposed in order to characterize glycemic variability (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). Standard deviation (SD) and Coefficient of variation (CV) constitute the most popular measurements and most common in use for reporting the glycemic variability (Bergenstal M. , et al., 2013) (DeVries, 2013). In this survey Standard deviation (SD), Coefficient of variation (CV) and Interquartile range (IQR) gave the upper hand to report the glycemic variability. All of these measures are presented in detail in the next subsections.

4.3.3.1 Standard Deviation (SD)

Standard deviation constitutes a measure that can be used for the quantification of the amount of variation or dispersion of a set of data. The interpretation of a low standard deviation explains that the data points tend to be close to the mean, but on the other hand the interpretation of a high standard deviation explains that the data points tend to spread out from the mean (Bland & Altman, 1996). For the quantification of glycemic variability from Continuous Glucose Monitoring (CGM), SD was proposed as the most reasonable measure because all the others are related in a linear matter to this statistical method (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). Moreover, another advantage of SD is that it has the ability to be divided in addition to total SD at least in other eight subdivisions like SD within days, SD between days and SD between time points (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009).

Furthermore, SD total presents a high degree of correlation with all the other SD divisions. SD also has the ability to combine information on glycemic variability from a matrix of different sources (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). In this research it was considered wise to use the total SD for each week of each patient and at the same time the SD between days. The total SD includes all the data from all days and all time points of day (Rodbard, Interprentation of Continuous Glucose

Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). The reason that SD was selected is because of its easy use and the correlation with other important factors despite the abnormal distribution of the glucose data. Of course, its dependence from mean glucose and in turn from HbA1c still remains a disadvantage (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009).

4.3.3.2 Coefficient of Variation (CV)

Coefficient of Variation (CV) constitutes a very useful measure of dispersion of a probability distribution or frequency distribution. More commonly it is expressed as a percentage and is interpreted as the ratio of the SD to the mean (Everitt, 1998). So it is clearly understood that the coefficient of variation is closely related to SD (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). More specifically, CV in its percentage version is expressed from standard deviation (SD) as it follows in the next equation:

$$(\%\mathrm{CV}) = [(\sigma \times 100) / \mu] \tag{4.1}$$

Where, σ is the SD and μ is the mean of observations (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009) (Everitt, 1998) (Bergenstal M. , et al., 2013).

Coefficient of variation has been proposed as one of the best measures for the characterization of glycemic variability due to fact that it is relatively more constant than other measures irrespective of mean glucose or HbA1c level (Bergenstal M. , et al., 2013) (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). This is very important because it is a well-known fact that HbA1c levels have the ability to vary in diabetes mellitus type 1 (Bergenstal M. , et al., 2013). Furthermore, another significant factor for measuring CV is the high correlation of risk of hypoglycemic events with (%CV) that has been observed (DeVries, 2013) (Rodbard, Hypo- and hyperglycemia in relation to the mean, standard deviation, coefficient of variation, and nature of the glucose distribution, 2012) (Bergenstal M. , et al., 2013).

In conclusion, CV constitutes a classical and simple measure in order to report glycemic variability (Rodbard, Hypo- and hyperglycemia in relation to the mean, standard deviation, coefficient of variation, and nature of the glucose distribution, 2012). This is the basic reason that CV was chosen among other measures in this survey. On the other hand, CV presents similar limitations as well as SD, something which is not surprising, if someone considers the correlation of CV with SD (Rodbard, Interprentation of Continuous Glucose Monitoring Data: Glycemic Variability and Quality of Glycemic Control, 2009). Furthermore, its display in a visual fashion presents some difficulties (Bergenstal M. , et al., 2013). This is something that is observed in iPro2 Daily overlay report table from CareLink. The correlation of CV with hypoglycemic events made the measuring of CV very important in our research.

4.3.3.3 Interquartile Range (IQR)

The interquartile range constitutes a measure of statistical dispersion, using more commonly as a measure of variability which is based on dividing a data set into quartiles (Upton & Cook, 1996). It is a significant factor in order to measure glycemic variability and does not depend on the assumption of normal distribution. More specifically, IQR is presented as the 50% of glucose values expressed from the difference between 75^{th} and 25^{th} percentiles of glucose values (Bergenstal M. , et al., 2013). It constitutes the most visually understandable way for the expression of glycemic variability in SMBG, as well as in visualization of data delivered from CGM too (Bergenstal M. , et al., 2013).

Furthermore, IQR has the ability to generate a very reliable aggregate measure of glycemic variability. The larger the interval is, the clearer the picture offered by IQR (Bergenstal M. , et al., 2013). The shortest time period for reliable reporting of IQR is 14 days, which is also the time period of each individual in our survey too (Matthai, et al., 2014) (Matthaei, Assessing the value of the Ambulatory Glucose Profile in clinical practice, 2014). Another advantage of IQR is that it has the ability to allow one to easily visualize the time of day or relationship to a meal or medication that there presents high glycemic variability, which may be a clinical indicator requiring special attention (Bergenstal M. , et al., 2013). Studies and researches have shown that a value of SD or CV may indicate an excessive glycemic variability

problem. The IQR adds the potential clinical advantage of being able to visualize glycemic variability reliably hour by hour throughout the day (Bergenstal M. , et al., 2013).

In this research, for the better reporting of glucose variability, the data set was divided in 5 subdivisions, the 10^{th}, 25^{th}, 50th, 75th and 90^{th} percentiles. The difference between the 75^{th} and the 25^{th} percentiles represent the IQR, the 10^{th} and 90^{th} percentile track glucose excursions. The 10^{th} percentile can especially help to identify hypoglycemic events. This can be achieved by analyzing, whether the 10^{th} percentile line is within or approaching the hypoglycemic range (Matthai, et al., 2014). The 50^{th} percentile, also known as the median curve, represents the median glucose value for its time point (Matthaei, Assessing the value of the Ambulatory Glucose Profile in clinical practice, 2014). It is worth noting that when the curves of the 5 percentiles seem to be close, it is quite certain that the glycemic variability will be low (Matthaei, Assessing the value of the Ambulatory Glucose Profile in clinical practice, 2014).

This way of reporting the glycemic variability gave the upper hand to portraying overall diurnal glucose characteristics of both glycemic variability and glucose exposure. In this survey IQR, median, 10^{th} and 90^{th} percentiles constitute integral components serving as measures for the glucose values in the respective quarters in the course of the week. Each of these quartiles corresponds to a different glucose value and every quartile operates as a different measure. For a better understanding, the next figure presents all the above percentiles (measures) in detail:

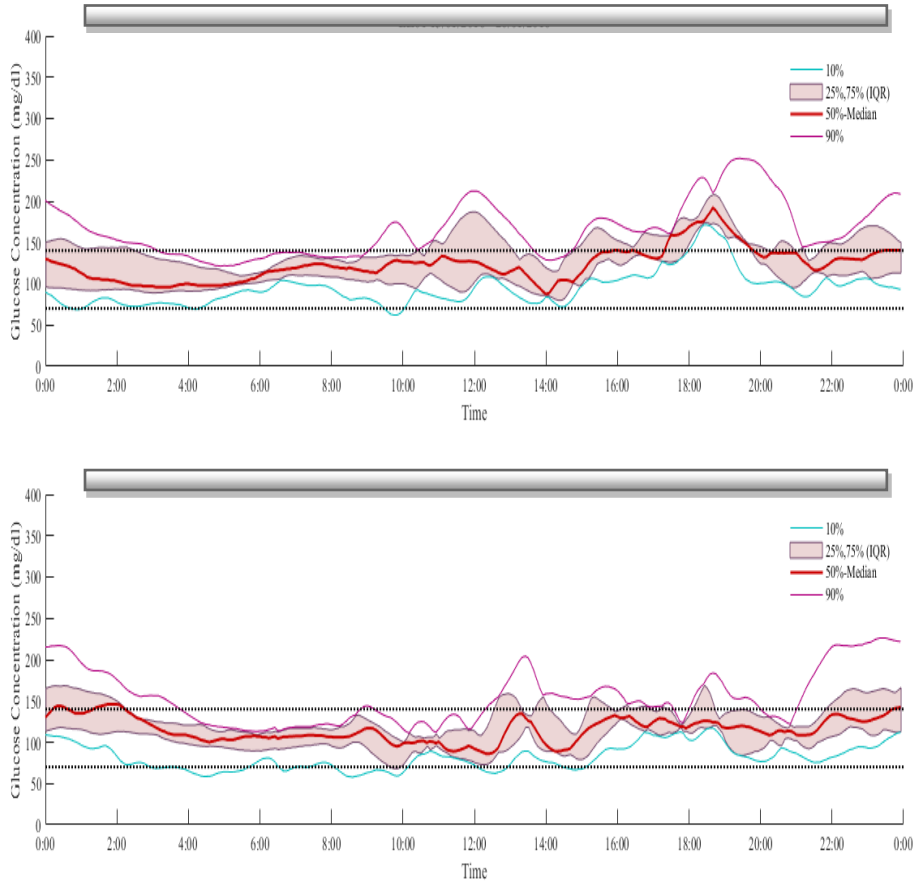


Figure 4-1 Report of IQR, Median, 10th and 90th percentiles

Figure 4-1 constitutes an analysis report example which presents each week of an individual. It represents the IQR expressed from the difference between the 75th and the 25th percentile colored in pink, Median 50% colored in red, 10th percentile colored in light blue and 90th percentile colored in purple. More specifically, for example, during the first week in figure 4-1, if the glucose concentration at 02:00 pm is taken as a reference point then the 10th percentile shows that the 10% of glucose values are below 100mg/dl, the 50th percentile (Median) shows that the 50% of glucose values are below 160mg/dl, 90th percentile shows that the 90% of glucose values are below 175mg/dl. Another instructive example, for a better understanding of the division in percentiles, may be the identification of hypoglycemic events. For example, if the curve of the 10th percentile crosses the limit of 70 mg/dl, then a moderate risk of hypoglycemic event appears, because constantly 10% of the glucose values fall in this range (Bergenstal M. , et al., 2013).

In conclusion, in this survey the division into percentiles and more specifically the IQR gave the ability to determine the appearance of an underlying pattern quicker and easier.

4.4 CORRELATION ANALYSIS

Correlation analysis was considered a wise procedure to identify, quantify and report the correlation between physical exercise and glucose concentration. In order to achieve the best results, three measures were used:

- Covariance
- Pearson's Correlation Coefficient
- Spearman's Correlation Coefficient

The above three measures are presented in detail in the next following sections.

4.4.1 Covariance

Covariance constitutes a measure that quantifies how much two variables (*x*,*y*) which present linear relationship, change together (Zaiontz, 2013 - 2016). The covariance presents a negative sign when the variables tend to show non similar behavior and a positive sign when the variables tend to show similar behavior (Zaiontz, 2013 - 2016) (Rice, 2007). Covariance can be described by the following equation:

$$\operatorname{cov}(x,y)=\sum_{i=1}^{n}\left(x_i-\bar{x}\right)\left(y_i-\bar{y}\right)/(n-1) \tag{4.2}$$

Where, $\bar{x}$ is the mean of variable *x*, $\bar{y}$ is the mean of variable *y* and $n-1$ is the number of observation minus 1 (Zaiontz, 2013 - 2016).

4.4.2 Pearson's Correlation Coefficient

Pearson's correlation coefficient constitutes a measure which describes the linear dependence between two variables. More specifically, it presents the covariance of these two variables divided by their standard deviations (SD) (Zaiontz,

2013 - 2016) (Lockyer, 1885). Equation 4.3 presents Pearson's correlation coefficient:

$$r_{(x,y)} = \frac{\mathrm{cov}_{(x,y)}}{SD_{(x)}SD_{(y)}} \tag{4.3}$$

Where *SD* represents the standard deviation of variable *y* and *x* respectively, $\mathrm{cov}_{(x,y)}$ is the covariance of the two variables. Correlation is represented within the limits of -1 and 1. The closer the result is in 1 the better the positive linear correlation is. More specifically, this means that if the variable *x* is high, the variable *y* is high too. On the other hand, the closer the result in -1 is, the better the negative linear correlation is, meaning that if the variable *x* is high the variable *y* is low. If the result is 0 then no correlation is detected between the two variables (Zaiontz, 2013 - 2016). So it is clearly understood that the correlation coefficient determines whether two variables or a set of variables are related and how strong the relationship of these variables is. In correlation analysis p-value plays a key role. To understand what the p-value represents, it is wise to consider the 'null hypothesis', meaning that the set of data doesn't have any relation (Neil & Fenton, 2012). It is a number of values from 0 to 1, showing the probability that the data would have arisen if the null hypothesis were true. The closer the p-value is to 0 the better the correlation of the variables is. A p-value within the limits of (0,01 – 0,05) shows a highly significant correlation (Neil & Fenton, 2012).

4.4.3 Spearman's Correlation Coefficient

Spearman's correlation coefficient, in contrast to Pearson's correlation coefficient, measures when the increase of one variable, leads to an increase of the other variable without requiring that increase to be represented by a linear relationship (Lehman, O'Rourke, Hatcher, & Stepanski, 2013). Using monotonic functions, the relationship between the two variables whether they are linear or not, is described.

5 DATA ANALYSIS RESULTS

5.1 ABSTRACT

Diabetes mellitus has occupied researchers for many years. It is a chronic disease which requires readiness daily. During the last two decades studies and researches have been in public regarding the impacts of diabetes mellitus during daily life activities. It is very important to understand better this metabolic disorder as there still exist many unanswered issues. One very important issue in which the present research tries to answer is the effect of physical exercise in the fluctuation of glucose concentration.

Chapter 5 tries to put some light presenting the results of both statistical and correlation data analysis. Firstly the clinical characteristics of the diabetic individuals are presented in table 5.1. Secondly all the iPro2 reports are presented in their prototype version directly from the glucose sensors. In turn the reports from SenseWear and Fitbit are presented and discussed in detail too. In continue statistical data analysis tables are presented associated with the corresponding diagrams presenting the IQR curves in detail. Finally the tables of correlation analysis are presented including Pearson's correlation coefficient, Spearman's correlation coefficient and covariance. Then all the results will be discussed in detail.

In conclusion, Chapter 5 presents the data analysis results of the present research trying to put some light regarding the impacts of physical exercise in glucose concentration.

5.2 CLNICAL AND GLYCEMIC CHARACTERISTICS

The diabetic population presents a significant growth with the passing of time. Diabetes mellitus appears with several types including type 1, type 2 and gestational diabetes. It is worth noting that glycemic profile is different for each patient but in general should follow some basic objectives. Type 1 diabetes is the most dangerous type that diabetes mellitus can hit. In order to find more accurate conclusions about the relationship between physical exercise with glucose fluctuation and GV, the glycemic and clinical characteristics of each individual is considered. The table below presents these characteristics in detail.

Table 5.1 Patients glycemic and clinical characteristics

Number of patients	7
Ethnicity (number)	Caucasian
Non Smoker (number)	7
Type 1 diabetes (number)	7
Age (years)	18 - 63
Sex (number)	2 Male/5 Female
BMI (kg/m^2)	$28{,}2 \pm 8$
Treatment with insulin (number)	7
iPro2 (number)	7
Fitbit (number)	6
Empatica E4 (number)	5
SenseWear (number)	7

5.3 GLUCOSE REPORTS FROM IPRO 2

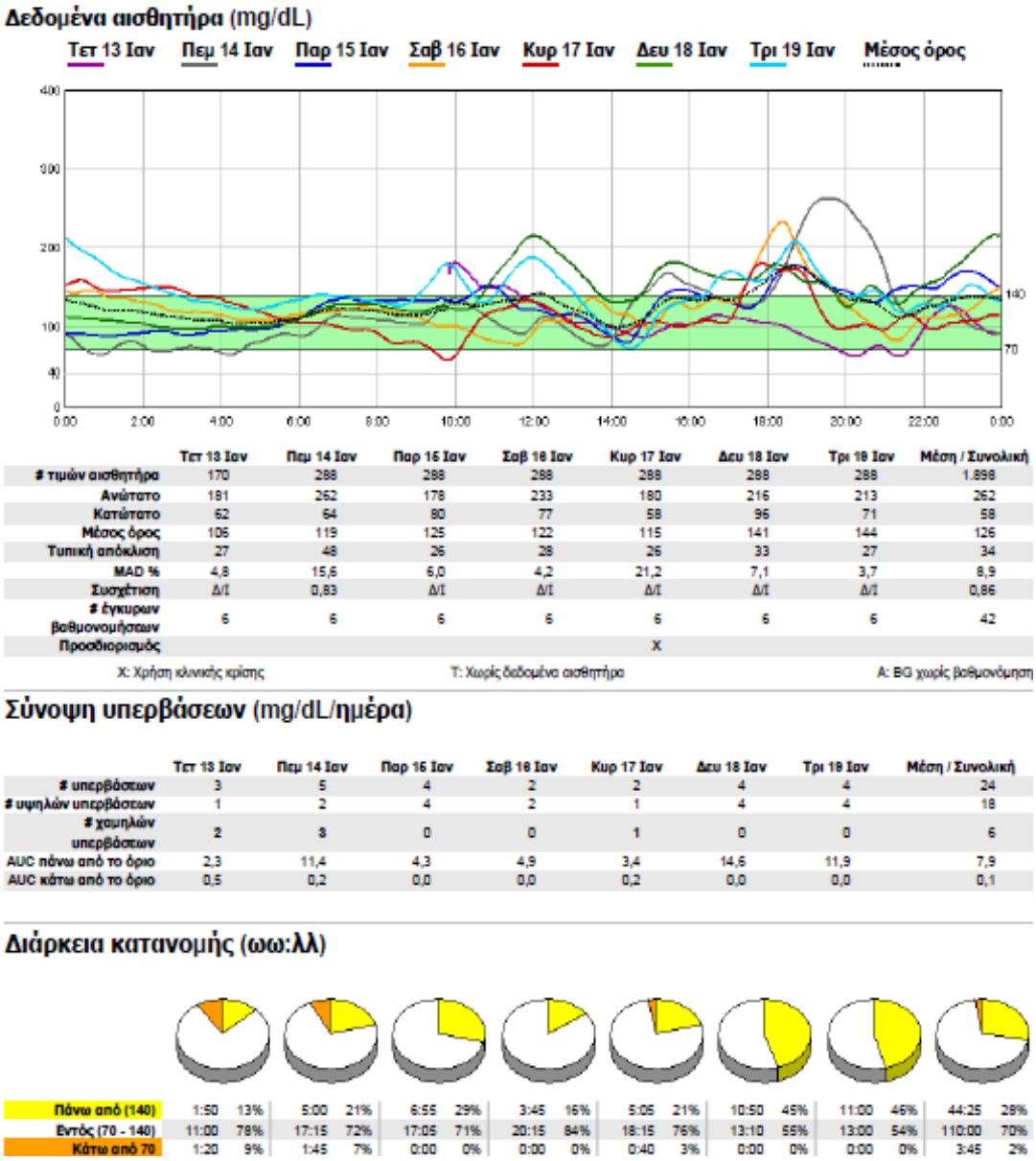
Diabetes mellitus constitutes a metabolic disorder which requires the constant attention and alert of the individuals. It has been proven by studies and researches that continuous blood glucose monitoring can reduce the frequency that diabetic complications appear.

In this survey, it was considered wise to use the iPro2 CGM system by Medtronic. CareLink (iPro2 software) was a major factor in choosing iPro 2 in order to monitor glucose during the stage of measurements in this research. CareLink gave the ability to report glucose patterns very easily and very quickly. The visualization of glucose patterns also helped users (scientists and individuals) to understand their (patients) glucose fluctuation in a very friendly way. The report of iPro2 includes the number of sensor's measurements, Standard deviation (SD), Minimum and Maximum glucose value during each day, Average glucose value per day, % Mean Average Deviation (MAD%) and the number of high and low exceedances (Medtronic.com). Last but not least, iPro 2 presents with the use of graphs, the duration allocation, both in percentages and in time duration. Some of the above measures were also taken into account in the further analysis of each individual's glucose data via our algorithms, as it will be presented in the next sections of this survey.

Finally, it is worth noting that the number of measurements during a 24h time is 288 measurements. This number arises because the sensor shows the average glucose value of the last 5 minutes and this continues every 5 minutes for all the 24h time (Medtronic.com). Equation 5.1 shows how the number of measurements arises:

$$(24h \times 60\,\text{min}) / 5\,\text{min} = 288 \tag{5.1}$$

In order to be clearly understood all of the above, the next subsections present the iPro 2 glucose daily overlay reports for each patient for each week of the program described in detail.



	Τετ 13 Ιαν	Πεμ 14 Ιαν	Παρ 15 Ιαν	Σαβ 16 Ιαν	Κυρ 17 Ιαν	Δευ 18 Ιαν	Τρι 19 Ιαν	Μέση / Συνολική
# τιμών αισθητήρα	170	288	288	288	288	288	288	1.898
Ανώτατο	181	262	178	233	180	216	213	262
Κατώτατο	62	64	80	77	58	96	71	58
Μέσος όρος	106	119	125	122	115	141	144	126
Τυπική απόκλιση	27	48	26	28	26	33	27	34
MAD %	4,8	15,6	6,0	4,2	21,2	7,1	3,7	8,9
Συσχέτιση	Δ/Ι	0,83	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	0,86
# έγκυρων βαθμονομήσεων	6	6	6	6	6	6	6	42
Προσδιορισμός					X			

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

	Τετ 13 Ιαν	Πεμ 14 Ιαν	Παρ 15 Ιαν	Σαβ 16 Ιαν	Κυρ 17 Ιαν	Δευ 18 Ιαν	Τρι 19 Ιαν	Μέση / Συνολική
# υπερβάσεων	3	5	4	2	2	4	4	24
# υψηλών υπερβάσεων	1	2	4	2	1	4	4	18
# χαμηλών υπερβάσεων	2	3	0	0	1	0	0	6
AUC πάνω από το όριο	2,3	11,4	4,3	4,9	3,4	14,6	11,9	7,9
AUC κάτω από το όριο	0,5	0,2	0,0	0,0	0,2	0,0	0,0	0,1

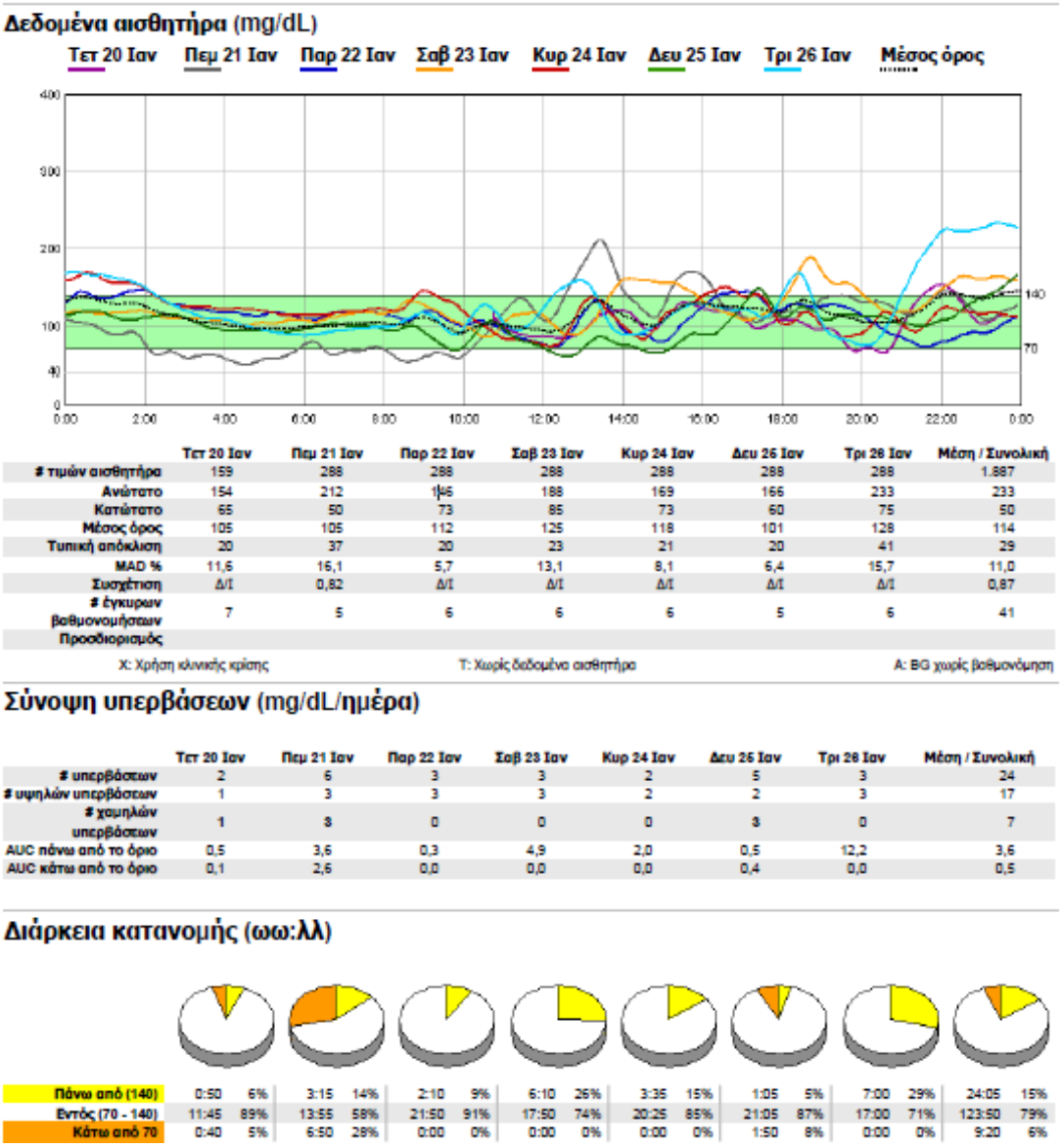
Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	1:50	13%	5:00	21%	6:55	29%	3:45	16%	5:05	21%	10:50	45%	11:00	46%	44:25	28%
Εντός (70 - 140)	11:00	78%	17:15	72%	17:05	71%	20:15	84%	18:15	76%	13:10	55%	13:00	54%	110:00	70%
Κάτω από 70	1:20	9%	1:45	7%	0:00	0%	0:00	0%	0:40	3%	0:00	0%	0:00	0%	3:45	2%

Figure 5-1 Subject 01 First Week Daily Overlay Report

Subject 01 was the first patient of the 14 days program. The report shows 288 measurements for each day, except on the first day which was 170 measurements, because the program started at 08:00 am. The Mean lies within the range limits, except on the 6th and 7th day which are 141 mg/dl and 144 mg/dl respectively.

Most of the values also lie within the range limits, something that is clearly understood from the duration allocation graph, as it is presented in the pie. More specifically, 70% of the values are within the target range, 28% over the target range and 2% down the target range.



	Τετ 20 Ιαν	Πεμ 21 Ιαν	Παρ 22 Ιαν	Σαβ 23 Ιαν	Κυρ 24 Ιαν	Δευ 25 Ιαν	Τρι 26 Ιαν	Μέση / Συνολική
# τιμών αισθητήρα	159	288	288	288	288	288	288	1.887
Ανώτατο	154	212	1||6	188	169	166	233	233
Κατώτατο	65	50	73	85	73	60	75	50
Μέσος όρος	105	105	112	125	118	101	128	114
Τυπική απόκλιση	20	37	20	23	21	20	41	29
MAD %	11,6	16,1	5,7	13,1	8,1	6,4	15,7	11,0
Συσχέτιση	Δ/Ι	0,82	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	0,87
# έγκυρων βαθμονομήσεων	7	5	6	6	6	5	6	41
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

	Τετ 20 Ιαν	Πεμ 21 Ιαν	Παρ 22 Ιαν	Σαβ 23 Ιαν	Κυρ 24 Ιαν	Δευ 25 Ιαν	Τρι 26 Ιαν	Μέση / Συνολική
# υπερβάσεων	2	6	3	3	2	5	3	24
# υψηλών υπερβάσεων	1	3	3	3	2	2	3	17
# χαμηλών υπερβάσεων	1	3	0	0	0	3	0	7
AUC πάνω από το όριο	0,5	3,6	0,3	4,9	2,0	0,5	12,2	3,6
AUC κάτω από το όριο	0,1	2,6	0,0	0,0	0,0	0,4	0,0	0,5

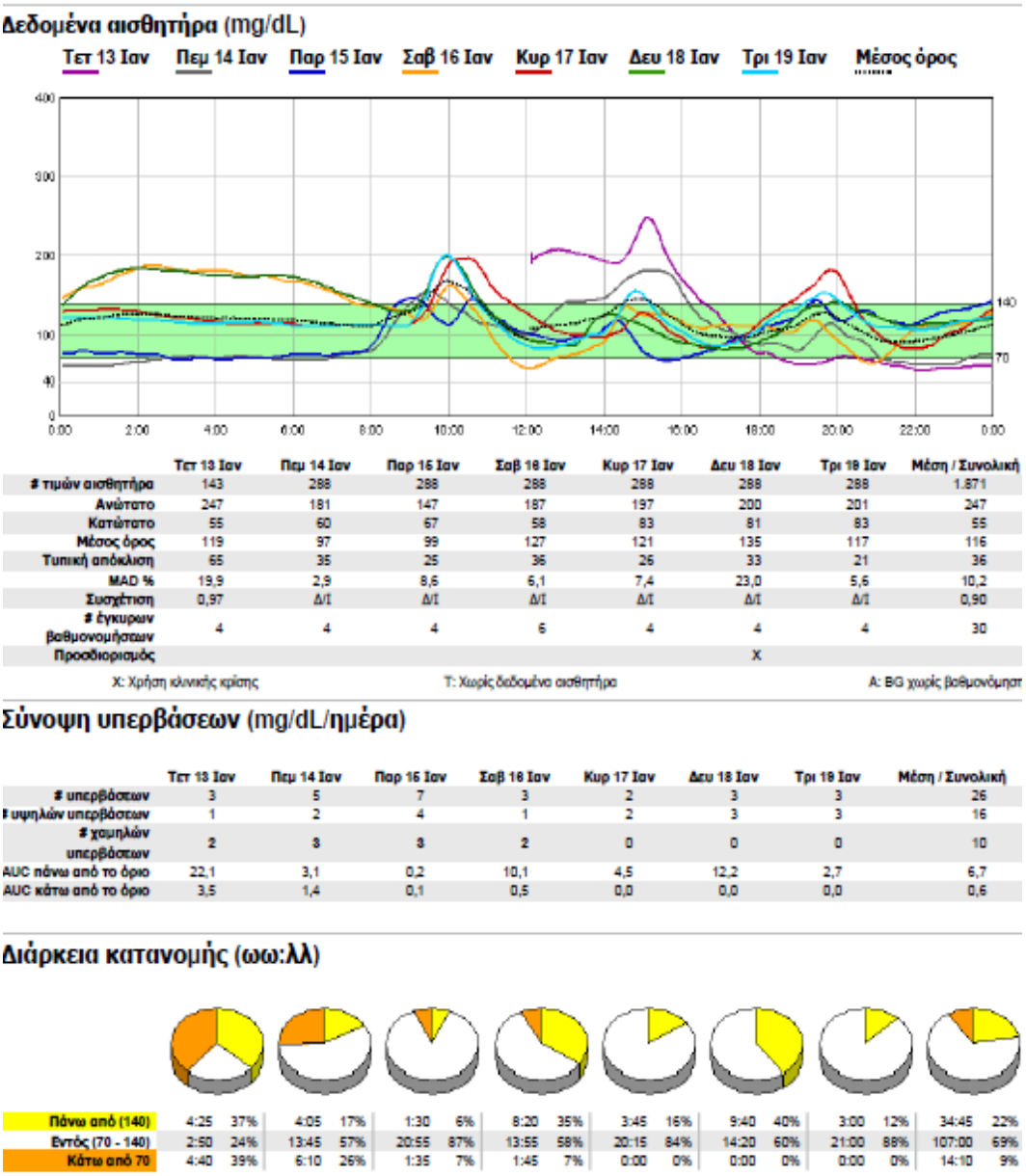
Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	0:50	6%	3:15	14%	2:10	9%	6:10	26%	3:35	15%	1:05	5%	7:00	29%	24:05	15%
Εντός (70 - 140)	11:45	89%	13:55	58%	21:50	91%	17:50	74%	20:25	85%	21:05	87%	17:00	71%	123:50	79%
Κάτω από 70	0:40	5%	6:50	28%	0:00	0%	0:00	0%	0:00	0%	1:50	8%	0:00	0%	9:20	6%

Figure 5-2 Subject 01 Second Week Daily Overlay Report

During the second week of the program, subject 01 continues having the highest percentage of the glucose values within the range limits as shown in the pie and even in higher percentage compared to the first week. Furthermore, the number of the measurements is 288 measurements except on the first day (159 measurements) that the sensor was changed. The Mean lies only within the range limits and the low excesses present a reducing picture.

Furthermore, as it is reflected in the pie graph, 15% of the values are above the target range, 6% are below the target range and 79% are within the target range. It seems that physical exercise has a positive effect on subject 01 reducing the values, resulting another 9% of the values within the target range.



	Τετ 13 Ιαν	Πεμ 14 Ιαν	Παρ 15 Ιαν	Σαβ 16 Ιαν	Κυρ 17 Ιαν	Δευ 18 Ιαν	Τρι 19 Ιαν	Μέση / Συνολική
# τιμών αισθητήρα	143	288	288	288	288	288	288	1.871
Ανώτατο	247	181	147	187	197	200	201	247
Κατώτατο	55	60	67	58	83	81	83	55
Μέσος όρος	119	97	99	127	121	135	117	116
Τυπική απόκλιση	65	35	25	36	26	33	21	36
MAD %	19,9	2,9	8,6	6,1	7,4	23,0	5,6	10,2
Συσχέτιση	0,97	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	0,90
# έγκυρων βαθμονομήσεων	4	4	4	6	4	4	4	30
Προσδιορισμός						X		

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

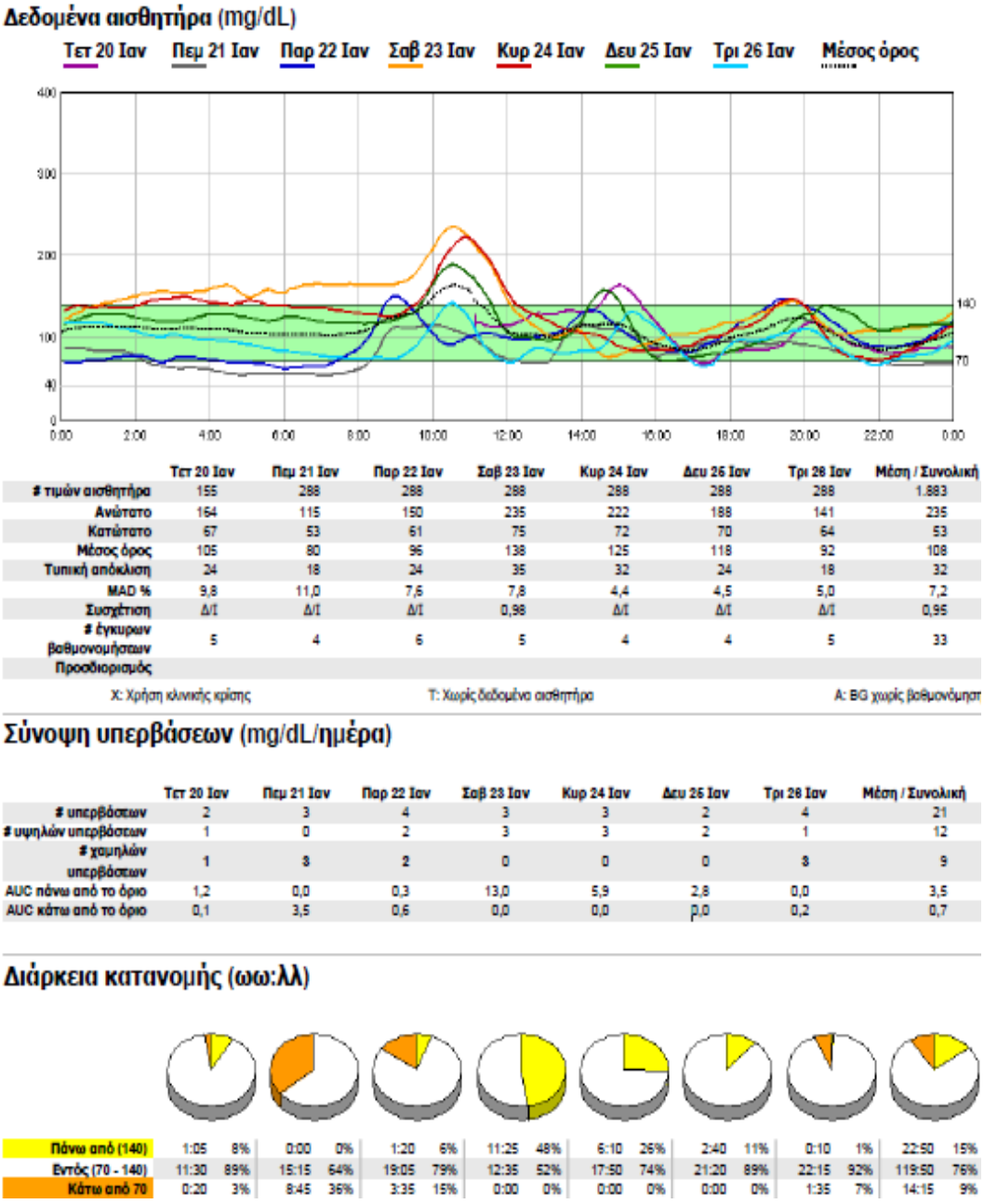
	Τετ 13 Ιαν	Πεμ 14 Ιαν	Παρ 15 Ιαν	Σαβ 16 Ιαν	Κυρ 17 Ιαν	Δευ 18 Ιαν	Τρι 19 Ιαν	Μέση / Συνολική
# υπερβάσεων	3	5	7	3	2	3	3	26
# υψηλών υπερβάσεων	1	2	4	1	2	3	3	16
# χαμηλών υπερβάσεων	2	3	3	2	0	0	0	10
AUC πάνω από το όριο	22,1	3,1	0,2	10,1	4,5	12,2	2,7	6,7
AUC κάτω από το όριο	3,5	1,4	0,1	0,5	0,0	0,0	0,0	0,6

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	4:25	37%	4:05	17%	1:30	6%	8:20	35%	3:45	16%	9:40	40%	3:00	12%	34:45	22%
Εντός (70 - 140)	2:50	24%	13:45	57%	20:55	87%	13:55	58%	20:15	84%	14:20	60%	21:00	88%	107:00	69%
Κάτω από 70	4:40	39%	6:10	26%	1:35	7%	1:45	7%	0:00	0%	0:00	0%	0:00	0%	14:10	9%

Figure 5-3 Subject 02 First Week Daily Overlay Report

Subject 02 constitutes the second individual of the program. During the first week the Mean as shown, lies only within the range limits. Subject 02 also presents few low or high excesses. Apart from the first day (143 measurements) of the program, 288 measurements correspond to each day. Most of the glucose values lie within the range limits, but on the first day values under the limit of 70 mg/dl and over the limit of 140 mg/dl have occupied the largest proportion, something that is clearly understood if someone saw the orange and the yellow parts in the duration allocation pie.



	Τετ 20 Ιαν	Πεμ 21 Ιαν	Παρ 22 Ιαν	Σαβ 23 Ιαν	Κυρ 24 Ιαν	Δευ 25 Ιαν	Τρι 26 Ιαν	Μέση / Συνολική
# τιμών αισθητήρα	155	288	288	288	288	288	288	1.883
Ανώτατο	164	115	150	235	222	188	141	235
Κατώτατο	67	53	61	75	72	70	64	53
Μέσος όρος	105	80	96	138	125	118	92	108
Τυπική απόκλιση	24	18	24	35	32	24	18	32
MAD %	9,8	11,0	7,6	7,8	4,4	4,5	5,0	7,2
Συσχέτιση	Δ/Ι	Δ/Ι	Δ/Ι	0,98	Δ/Ι	Δ/Ι	Δ/Ι	0,95
# έγκυρων βαθμονομήσεων	5	4	6	5	4	4	5	33
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

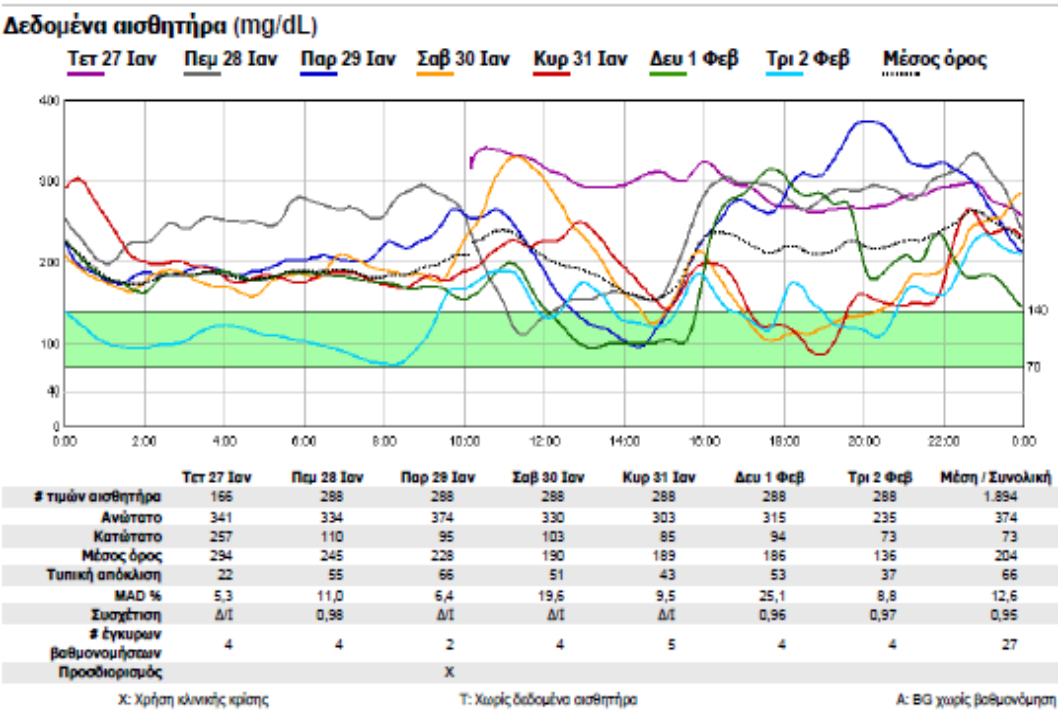
	Τετ 20 Ιαν	Πεμ 21 Ιαν	Παρ 22 Ιαν	Σαβ 23 Ιαν	Κυρ 24 Ιαν	Δευ 25 Ιαν	Τρι 26 Ιαν	Μέση / Συνολική
# υπερβάσεων	2	3	4	3	3	2	4	21
# υψηλών υπερβάσεων	1	0	2	3	3	2	1	12
# χαμηλών υπερβάσεων	1	3	2	0	0	0	3	9
AUC πάνω από το όριο	1,2	0,0	0,3	13,0	5,9	2,8	0,0	3,5
AUC κάτω από το όριο	0,1	3,5	0,6	0,0	0,0	0,0	0,2	0,7

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	1:05	8%	0:00	0%	1:20	6%	11:25	48%	6:10	26%	2:40	11%	0:10	1%	22:50	15%
Εντός (70 - 140)	11:30	89%	15:15	64%	19:05	79%	12:35	52%	17:50	74%	21:20	89%	22:15	92%	119:50	76%
Κάτω από 70	0:20	3%	8:45	36%	3:35	15%	0:00	0%	0:00	0%	0:00	0%	1:35	7%	14:15	9%

Figure 5-4 Subject 02 Second Week Daily Overlay Report

During the second week of the program the Mean continues to lie within the range limits. There are 288 measurements recorded in every other day, except on the first day (155 measurements). In addition, even more glucose values lie in the desired range limits and the high and low excesses present a reducing picture.



	Τετ 27 Ιαν	Πεμ 28 Ιαν	Παρ 29 Ιαν	Σαβ 30 Ιαν	Κυρ 31 Ιαν	Δευ 1 Φεβ	Τρι 2 Φεβ	Μέση / Συνολική
# τιμών αισθητήρα	166	288	288	288	288	288	288	1.894
Ανώτατο	341	334	374	330	303	315	235	374
Κατώτατο	257	110	95	103	85	94	73	73
Μέσος όρος	294	245	228	190	189	186	136	204
Τυπική απόκλιση	22	55	66	51	43	53	37	66
MAD %	5,3	11,0	6,4	19,6	9,5	25,1	8,8	12,6
Συσχέτιση	Δ/Ι	0,98	Δ/Ι	Δ/Ι	Δ/Ι	0,96	0,97	0,95
# έγκυρων βαθμονομήσεων	4	4	2	4	5	4	4	27
Προσδιορισμός			X					

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

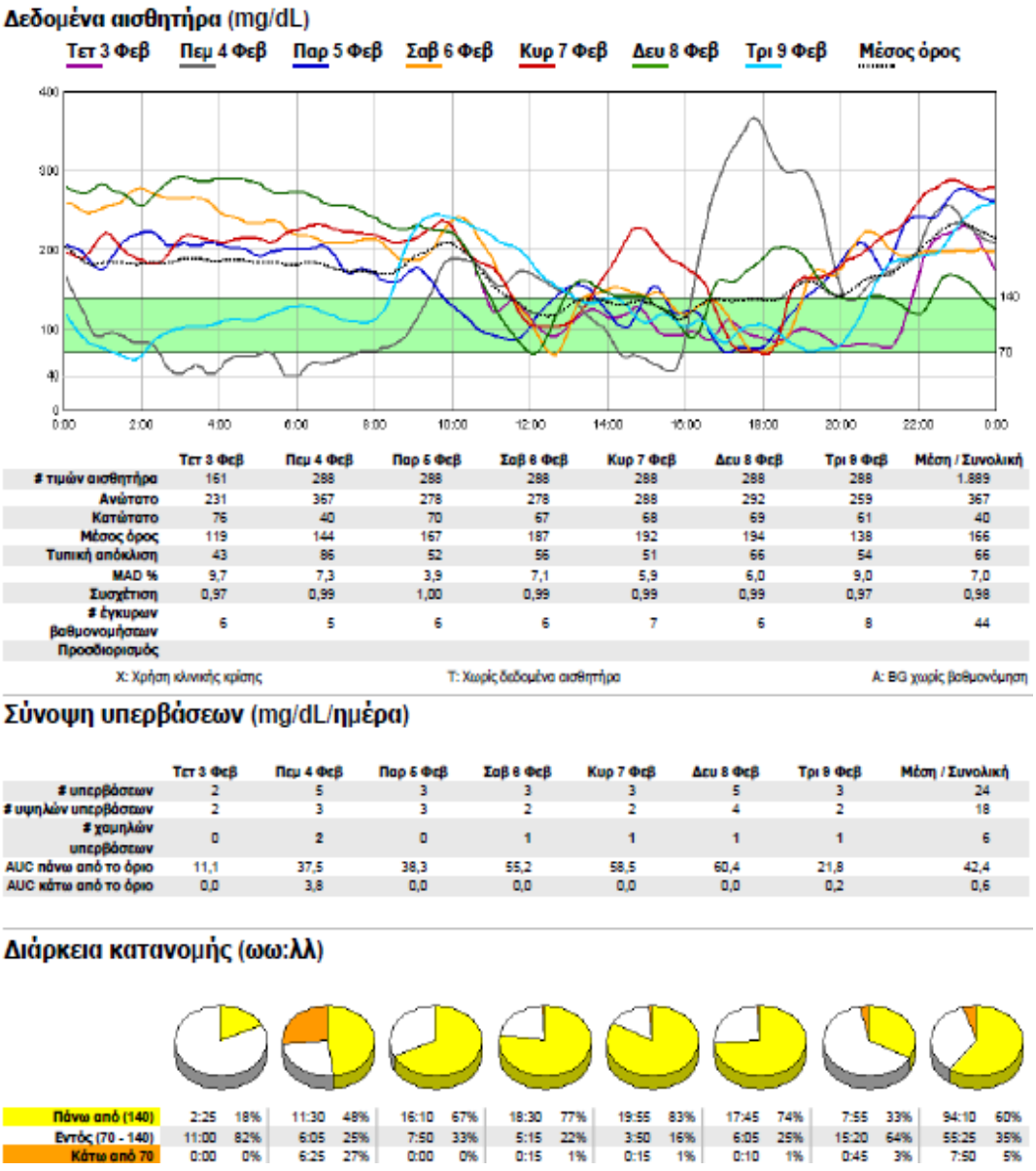
	Τετ 27 Ιαν	Πεμ 28 Ιαν	Παρ 29 Ιαν	Σαβ 30 Ιαν	Κυρ 31 Ιαν	Δευ 1 Φεβ	Τρι 2 Φεβ	Μέση / Συνολική
# υπερβάσεων	1	2	1	3	1	1	5	14
# υψηλών υπερβάσεων	1	2	1	3	1	1	5	14
# χαμηλών υπερβάσεων	0	0	0	0	0	0	0	0
AUC πάνω από το όριο	153,7	105,7	90,4	52,6	51,5	51,6	13,7	69,0
AUC κάτω από το όριο	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	13:50	100%	22:50	95%	21:35	90%	19:55	83%	21:35	90%	20:15	84%	9:15	39%	129:15	82%
Εντός (70 - 140)	0:00	0%	1:10	5%	2:25	10%	4:05	17%	2:25	10%	3:45	16%	14:45	61%	28:35	18%
Κάτω από 70	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%

Figure 5-5 Subject 03 First Week Daily Overlay Report

Subject 03 was the third patient of the program. In the first week, iPro2 report presents a Mean well above the range limits of 140 mg/dl during the whole week. The duration allocation shows that hyperglycemic values occupy most of the time daily, except the last one in which most of the values lie within the range limits. The 18% in target range and the 0% under the limit of 70 mg/dl constitute representative percentages that mirror subject's 03 hyperglycemic character. Excluding the first day which the program started, the sensor took 288 measurements per day.



	Τετ 3 Φεβ	Πεμ 4 Φεβ	Παρ 5 Φεβ	Σαβ 6 Φεβ	Κυρ 7 Φεβ	Δευ 8 Φεβ	Τρι 9 Φεβ	Μέση / Συνολική
# τιμών αισθητήρα	161	288	288	288	288	288	288	1.889
Ανώτατο	231	367	278	278	288	292	259	367
Κατώτατο	76	40	70	67	68	69	61	40
Μέσος όρος	119	144	167	187	192	194	138	166
Τυπική απόκλιση	43	86	52	56	51	66	54	66
MAD %	9,7	7,3	3,9	7,1	5,9	6,0	9,0	7,0
Συσχέτιση	0,97	0,99	1,00	0,99	0,99	0,99	0,97	0,98
# έγκυρων βαθμονομήσεων Προσδιορισμός	6	5	6	6	7	6	8	44

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

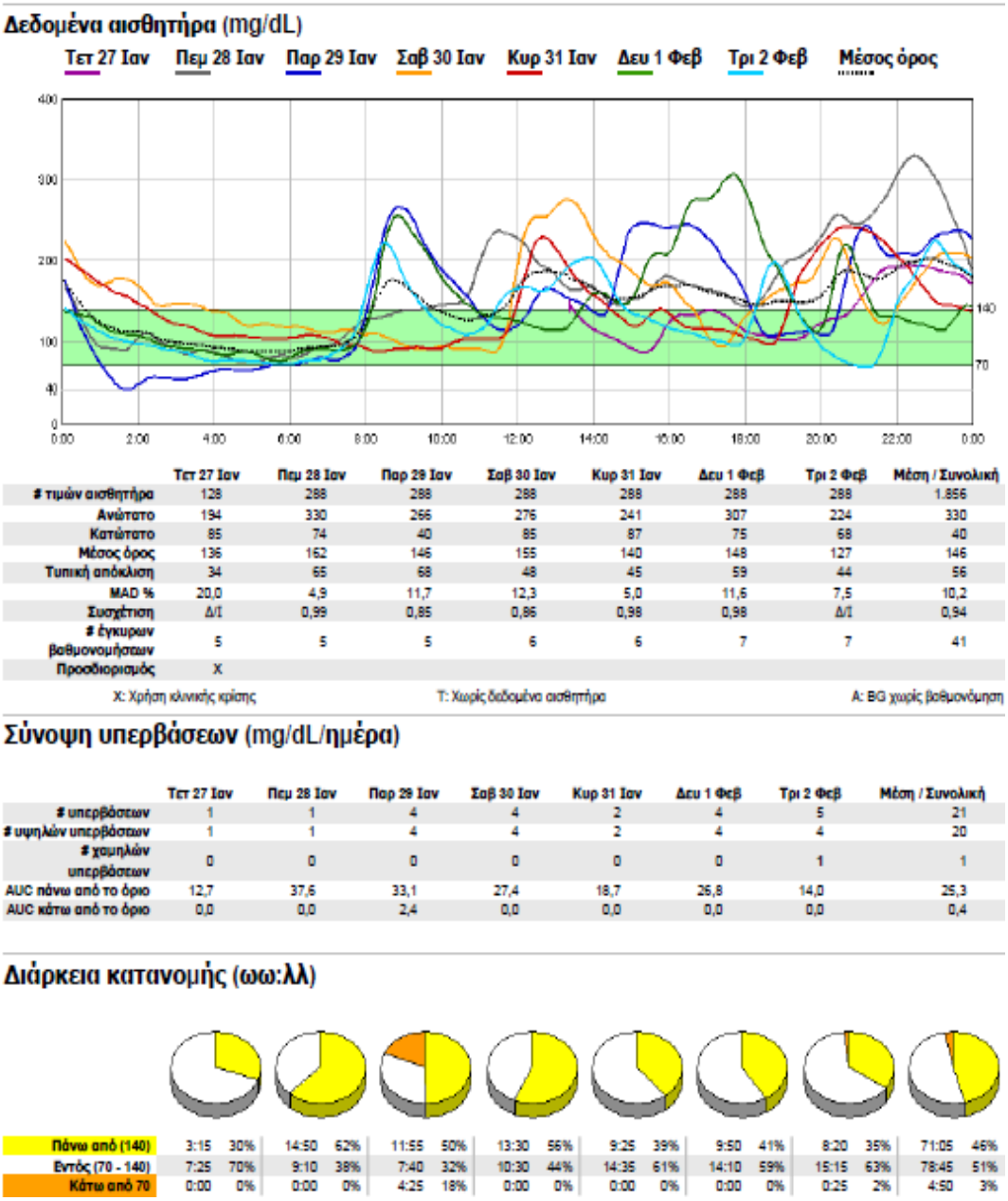
	Τετ 3 Φεβ	Πεμ 4 Φεβ	Παρ 5 Φεβ	Σαβ 6 Φεβ	Κυρ 7 Φεβ	Δευ 8 Φεβ	Τρι 9 Φεβ	Μέση / Συνολική
# υπερβάσεων	2	5	3	3	3	5	3	24
# υψηλών υπερβάσεων	2	3	3	2	2	4	2	18
# χαμηλών υπερβάσεων	0	2	0	1	1	1	1	6
AUC πάνω από το όριο	11,1	37,5	38,3	55,2	58,5	60,4	21,8	42,4
AUC κάτω από το όριο	0,0	3,8	0,0	0,0	0,0	0,0	0,2	0,6

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	2:25	18%	11:30	48%	16:10	67%	18:30	77%	19:55	83%	17:45	74%	7:55	33%	94:10	60%
Εντός (70 - 140)	11:00	82%	6:05	25%	7:50	33%	5:15	22%	3:50	16%	6:05	25%	15:20	64%	55:25	35%
Κάτω από 70	0:00	0%	6:25	27%	0:00	0%	0:15	1%	0:15	1%	0:10	1%	0:45	3%	7:50	5%

Figure 5-6 Subject 03 Second Week Daily Overlay Report

During the second week of the program, Subject 03 presents a Mean above the limit of 140 mg/dl in the five of the seven days. In addition to the first week's report, the Mean isn't well above the range limits but it presents a reducing pattern. In the second week of the program, for the first time, values under the low limit are observed. The percentage of values within the range limits has also improved something that is clearly understood from the average percentage within the range limits which in the second week is 35%.



	Τετ 27 Ιαν	Πεμ 28 Ιαν	Παρ 29 Ιαν	Σαβ 30 Ιαν	Κυρ 31 Ιαν	Δευ 1 Φεβ	Τρι 2 Φεβ	Μέση / Συνολική
# τιμών αισθητήρα	128	288	288	288	288	288	288	1.856
Ανώτατο	194	330	266	276	241	307	224	330
Κατώτατο	85	74	40	85	87	75	68	40
Μέσος όρος	136	162	146	155	140	148	127	146
Τυπική απόκλιση	34	65	68	48	45	59	44	56
MAD %	20,0	4,9	11,7	12,3	5,0	11,6	7,5	10,2
Συσχέτιση	Δ/Ι	0,99	0,85	0,86	0,98	0,98	Δ/Ι	0,94
# έγκυρων βαθμονομήσεων	5	5	5	6	6	7	7	41
Προσδιορισμός	X							

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

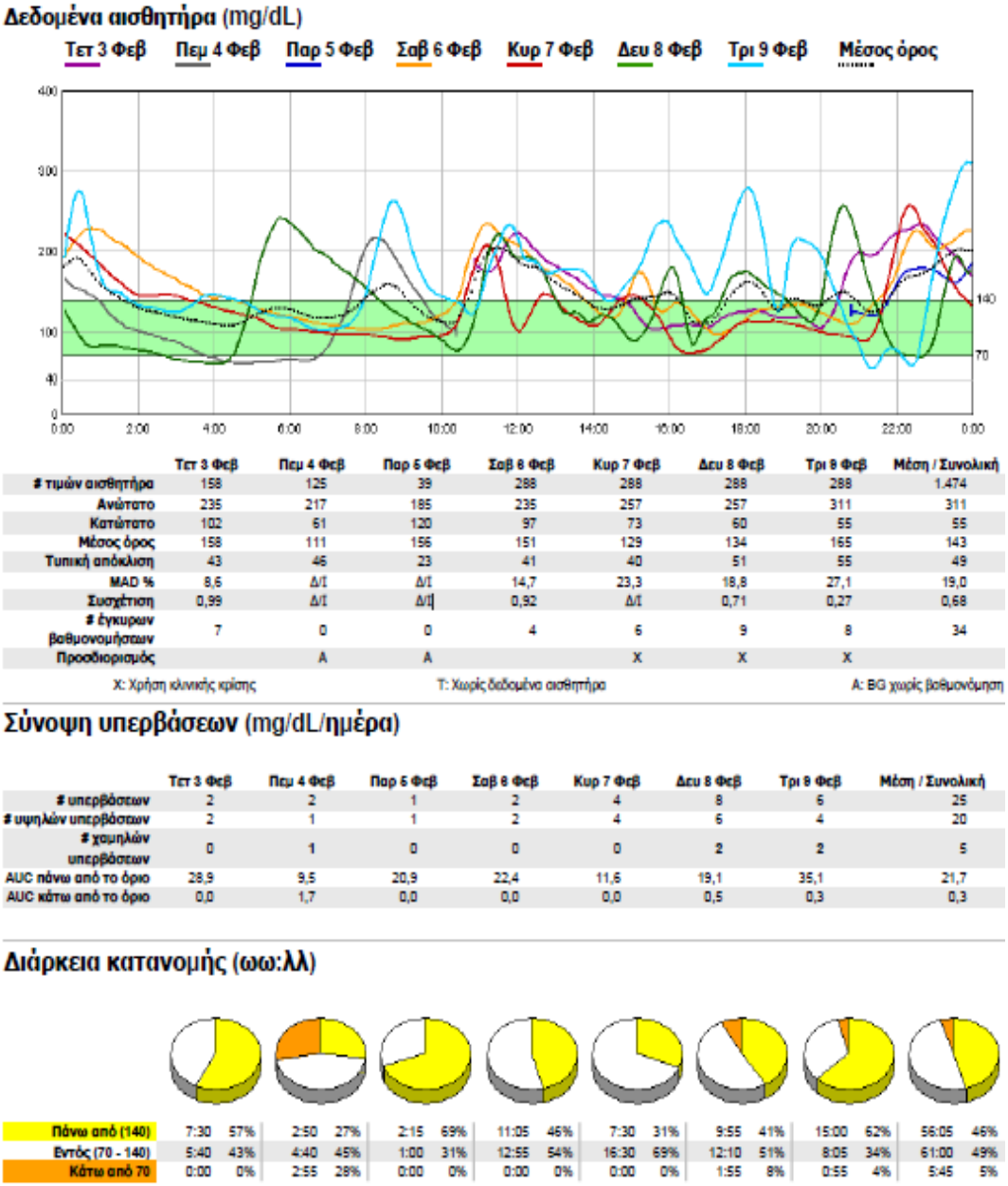
	Τετ 27 Ιαν	Πεμ 28 Ιαν	Παρ 29 Ιαν	Σαβ 30 Ιαν	Κυρ 31 Ιαν	Δευ 1 Φεβ	Τρι 2 Φεβ	Μέση / Συνολική
# υπερβάσεων	1	1	4	4	2	4	5	21
# υψηλών υπερβάσεων	1	1	4	4	2	4	4	20
# χαμηλών υπερβάσεων	0	0	0	0	0	0	1	1
AUC πάνω από το όριο	12,7	37,6	33,1	27,4	18,7	26,8	14,0	25,3
AUC κάτω από το όριο	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,4

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	3:15	30%	14:50	62%	11:55	50%	13:30	56%	9:25	39%	9:50	41%	8:20	35%	71:05	46%
Εντός (70 - 140)	7:25	70%	9:10	38%	7:40	32%	10:30	44%	14:35	61%	14:10	59%	15:15	63%	78:45	51%
Κάτω από 70	0:00	0%	0:00	0%	4:25	18%	0:00	0%	0:00	0%	0:00	0%	0:25	2%	4:50	3%

Figure 5-7 Subject 04 First Week Daily Overlay Report

Subject 04 was the fourth patient of the program. With the exception of the first day, 288 measurements were performed. The Mean constantly ranges in values near the above limit of 140 mg/dl. The duration allocation shows that the average percentage of values within the range limits was 51% with the percentage out of the limits was occupied mainly by hyperglycemic values.



	Τετ 3 Φεβ	Πεμ 4 Φεβ	Παρ 5 Φεβ	Σαβ 6 Φεβ	Κυρ 7 Φεβ	Δευ 8 Φεβ	Τρι 9 Φεβ	Μέση / Συνολική
# τιμών αισθητήρα	158	125	39	288	288	288	288	1.474
Ανώτατο	235	217	185	235	257	257	311	311
Κατώτατο	102	61	120	97	73	60	55	55
Μέσος όρος	158	111	156	151	129	134	165	143
Τυπική απόκλιση	43	46	23	41	40	51	55	49
MAD %	8,6	Δ/Ι	Δ/Ι	14,7	23,3	18,8	27,1	19,0
Συσχέτιση	0,99	Δ/Ι	Δ/Ι	0,92	Δ/Ι	0,71	0,27	0,68
# έγκυρων βαθμονομήσεων	7	0	0	4	6	9	8	34
Προσδιορισμός		A	A		X	X	X	

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

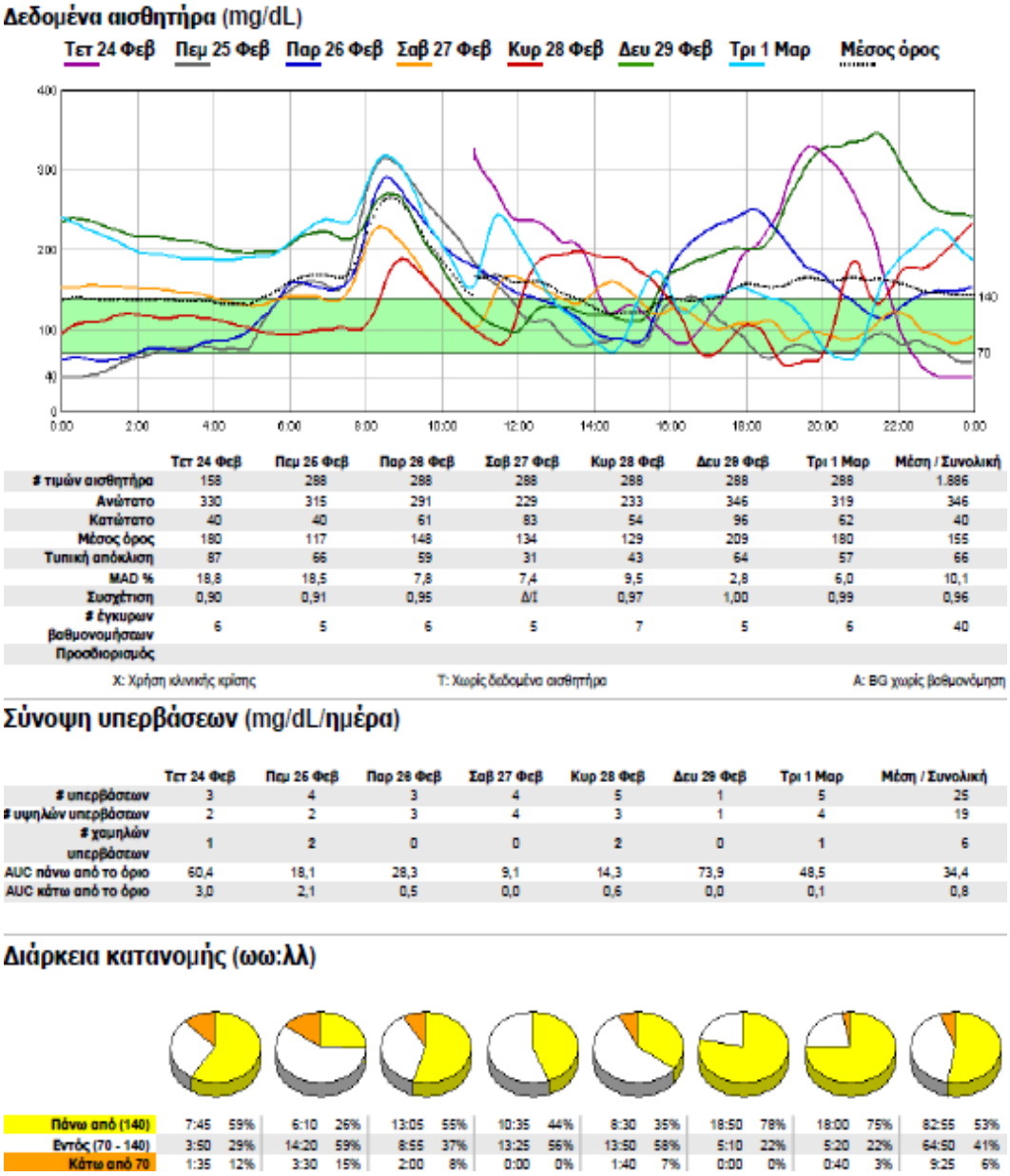
	Τετ 3 Φεβ	Πεμ 4 Φεβ	Παρ 5 Φεβ	Σαβ 6 Φεβ	Κυρ 7 Φεβ	Δευ 8 Φεβ	Τρι 9 Φεβ	Μέση / Συνολική
# υπερβάσεων	2	2	1	2	4	8	6	25
# υψηλών υπερβάσεων	2	1	1	2	4	6	4	20
# χαμηλών υπερβάσεων	0	1	0	0	0	2	2	5
AUC πάνω από το όριο	28,9	9,5	20,9	22,4	11,6	19,1	35,1	21,7
AUC κάτω από το όριο	0,0	1,7	0,0	0,0	0,0	0,5	0,3	0,3

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	7:30	57%	2:50	27%	2:15	69%	11:05	46%	7:30	31%	9:55	41%	15:00	62%	56:05	46%
Εντός (70 - 140)	5:40	43%	4:40	45%	1:00	31%	12:55	54%	16:30	69%	12:10	51%	8:05	34%	61:00	49%
Κάτω από 70	0:00	0%	2:55	28%	0:00	0%	0:00	0%	0:00	0%	1:55	8%	0:55	4%	5:45	5%

Figure 5-8 Subject 04 Second Week Daily Overlay Report

During the second week of the program, Subject 04 presents a similar behavior. The Mean ranges with values near the highest limit. The percentage of 49% of the values lies within the range limits with a small percentage of 5% under the low limit and the other 46% above the high limit.



	Τετ 24 Φεβ	Πεμ 25 Φεβ	Παρ 26 Φεβ	Σαβ 27 Φεβ	Κυρ 28 Φεβ	Δευ 29 Φεβ	Τρι 1 Μαρ	Μέση / Συνολική
# τιμών αισθητήρα	158	288	288	288	288	288	288	1.886
Ανώτατο	330	315	291	229	233	346	319	346
Κατώτατο	40	40	61	83	54	96	62	40
Μέσος όρος	180	117	148	134	129	209	180	155
Τυπική απόκλιση	87	66	59	31	43	64	57	66
MAD %	18,8	18,5	7,8	7,4	9,5	2,8	6,0	10,1
Συσχέτιση	0,90	0,91	0,95	Δ/Σ	0,97	1,00	0,99	0,96
# έγκυρων βαθμονομήσεων	6	5	6	5	7	5	6	40
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

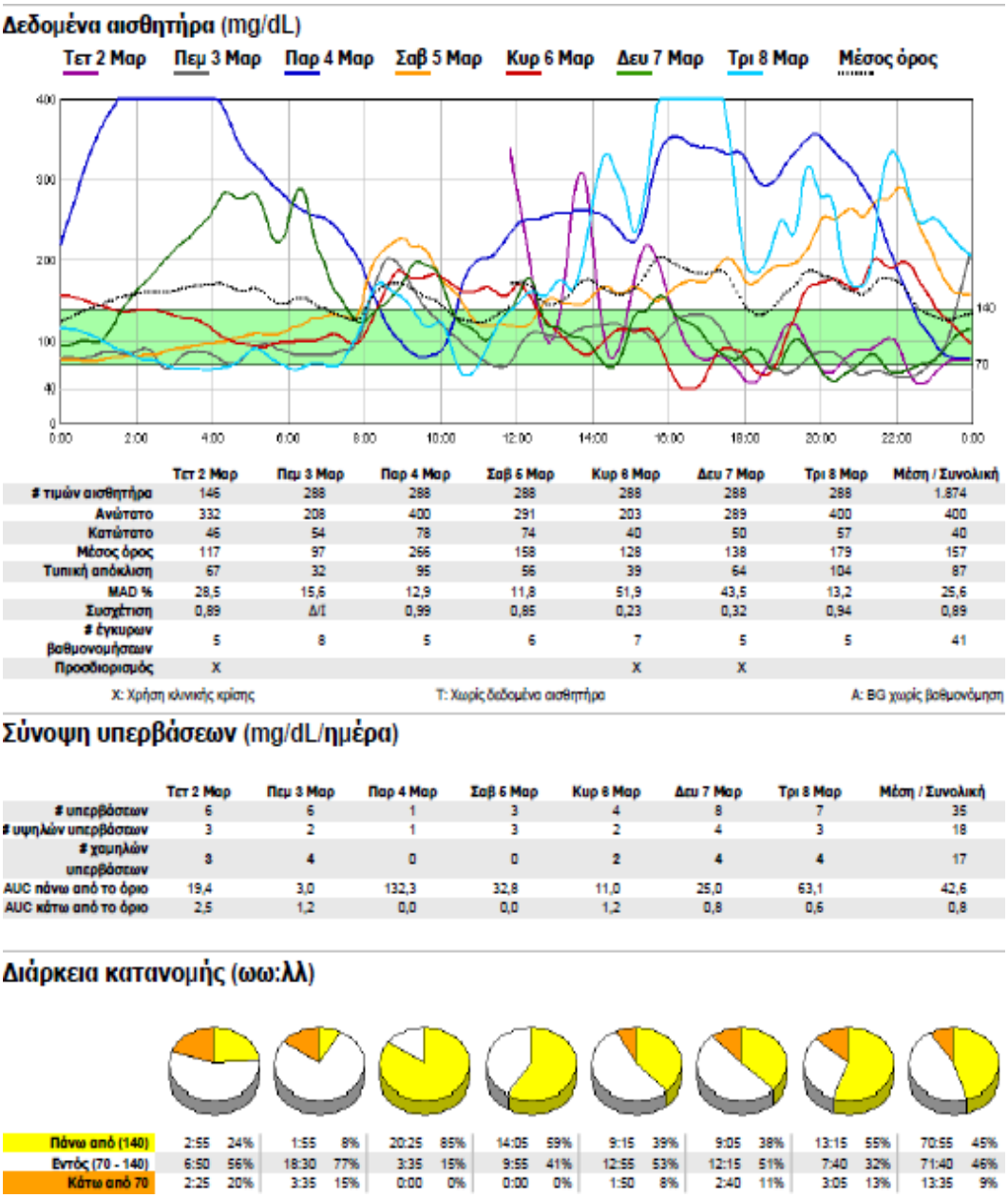
	Τετ 24 Φεβ	Πεμ 25 Φεβ	Παρ 26 Φεβ	Σαβ 27 Φεβ	Κυρ 28 Φεβ	Δευ 29 Φεβ	Τρι 1 Μαρ	Μέση / Συνολική
# υπερβάσεων	3	4	3	4	5	1	5	25
# υψηλών υπερβάσεων	2	2	3	4	3	1	4	19
# χαμηλών υπερβάσεων	1	2	0	0	2	0	1	6
AUC πάνω από το όριο	60,4	18,1	28,3	9,1	14,3	73,9	48,5	34,4
AUC κάτω από το όριο	3,0	2,1	0,5	0,0	0,6	0,0	0,1	0,8

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	7:45	59%	6:10	26%	13:05	55%	10:35	44%	8:30	35%	18:50	78%	18:00	75%	82:55	53%
Εντός (70 - 140)	3:50	29%	14:20	59%	8:55	37%	13:25	56%	13:50	58%	5:10	22%	5:20	22%	64:50	41%
Κάτω από 70	1:35	12%	3:30	15%	2:00	8%	0:00	0%	1:40	7%	0:00	0%	0:40	3%	9:25	6%

Figure 5-9 Subject 05 First Week Daily Overlay Report

Subject 05 was the fifth patient of the program. Excluding the first day of the week that the program started, 288 measurements per day were performed. Subject 05 shows a fluctuating behavior, something that is clearly understood from the Mean values of each day. The duration allocation also shows that the average percentage of 53% was in hyperglycemic values with 41% within the range limits.



	Τετ 2 Μαρ	Πεμ 3 Μαρ	Παρ 4 Μαρ	Σαβ 5 Μαρ	Κυρ 6 Μαρ	Δευ 7 Μαρ	Τρι 8 Μαρ	Μέση / Συνολική
# τιμών αισθητήρα	146	288	288	288	288	288	288	1.874
Ανώτατο	332	208	400	291	203	289	400	400
Κατώτατο	46	54	78	74	40	50	57	40
Μέσος όρος	117	97	266	158	128	138	179	157
Τυπική απόκλιση	67	32	95	56	39	64	104	87
MAD %	28,5	15,6	12,9	11,8	51,9	43,5	13,2	25,6
Συσχέτιση	0,89	Δ/Ι	0,99	0,85	0,23	0,32	0,94	0,89
# έγκυρων βαθμονομήσεων	5	8	5	6	7	5	5	41
Προσδιορισμός	X				X	X		

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

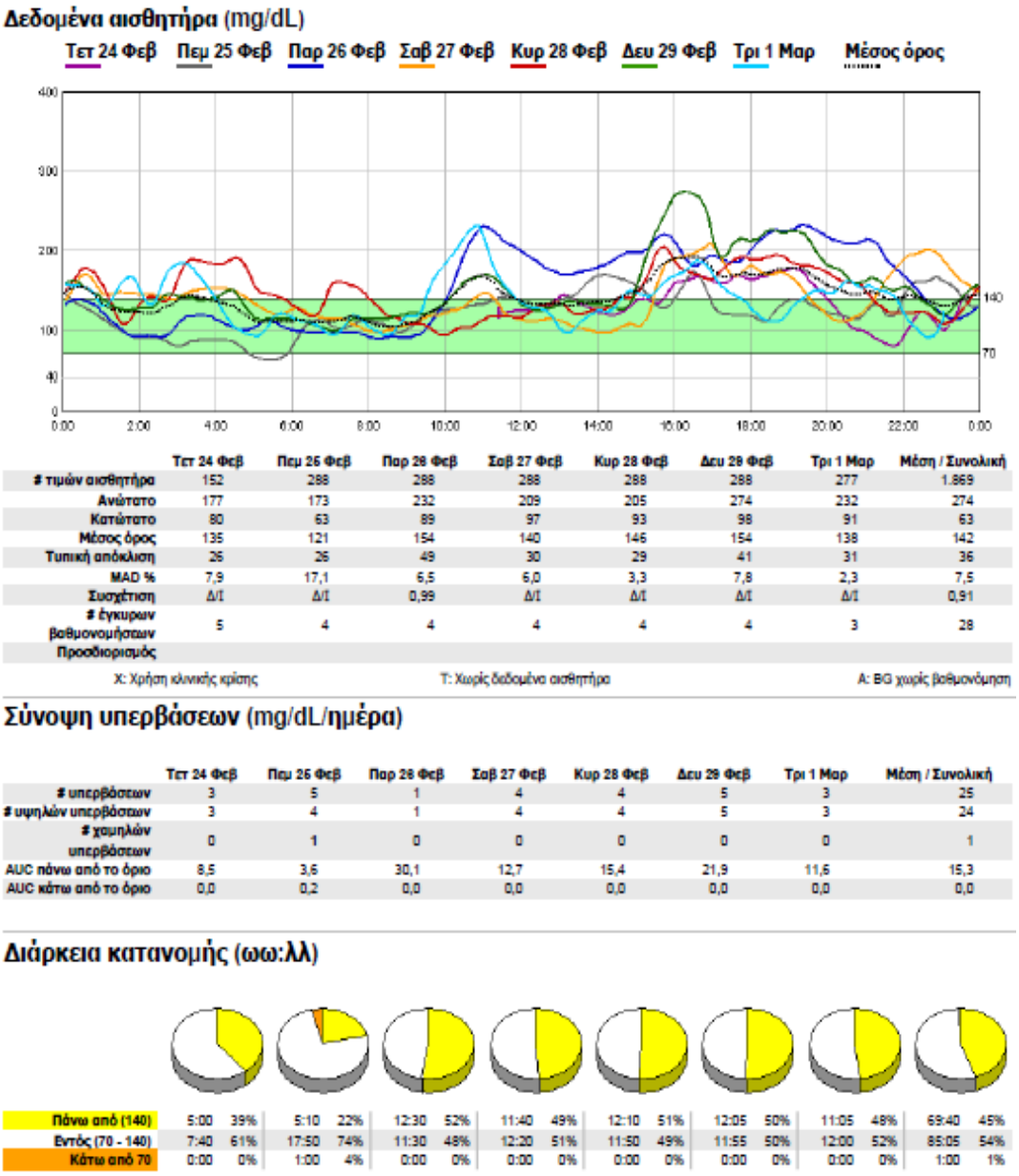
	Τετ 2 Μαρ	Πεμ 3 Μαρ	Παρ 4 Μαρ	Σαβ 5 Μαρ	Κυρ 6 Μαρ	Δευ 7 Μαρ	Τρι 8 Μαρ	Μέση / Συνολική
# υπερβάσεων	6	6	1	3	4	8	7	35
# υψηλών υπερβάσεων	3	2	1	3	2	4	3	18
# χαμηλών υπερβάσεων	3	4	0	0	2	4	4	17
AUC πάνω από το όριο	19,4	3,0	132,3	32,8	11,0	25,0	63,1	42,6
AUC κάτω από το όριο	2,5	1,2	0,0	0,0	1,2	0,8	0,6	0,8

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	2:55	24%	1:55	8%	20:25	85%	14:05	59%	9:15	39%	9:05	38%	13:15	55%	70:55	45%
Εντός (70 - 140)	6:50	56%	18:30	77%	3:35	15%	9:55	41%	12:55	53%	12:15	51%	7:40	32%	71:40	46%
Κάτω από 70	2:25	20%	3:35	15%	0:00	0%	0:00	0%	1:50	8%	2:40	11%	3:05	13%	13:35	9%

Figure 5-10 Subject 05 Second Week Daily Overlay Report

During the second week of the program, Subject 05 shows an improvement in his/her glycemic behavior. Although the Mean presents a similar pattern, the duration allocation shows that 46% of the values lie within the range limits and 45% of the values are above the highest limit of 140 mg/dl.



	Τετ 24 Φεβ	Πεμ 25 Φεβ	Παρ 26 Φεβ	Σαβ 27 Φεβ	Κυρ 28 Φεβ	Δευ 29 Φεβ	Τρι 1 Μαρ	Μέση / Συνολική
# τιμών αισθητήρα	152	288	288	288	288	288	277	1.869
Ανώτατο	177	173	232	209	205	274	232	274
Κατώτατο	80	63	89	97	93	98	91	63
Μέσος όρος	135	121	154	140	146	154	138	142
Τυπική απόκλιση	26	26	49	30	29	41	31	36
MAD %	7,9	17,1	6,5	6,0	3,3	7,8	2,3	7,5
Συσχέτιση	Δ/Ι	Δ/Ι	0,99	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	0,91
# έγκυρων βαθμονομήσεων	5	4	4	4	4	4	3	28
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

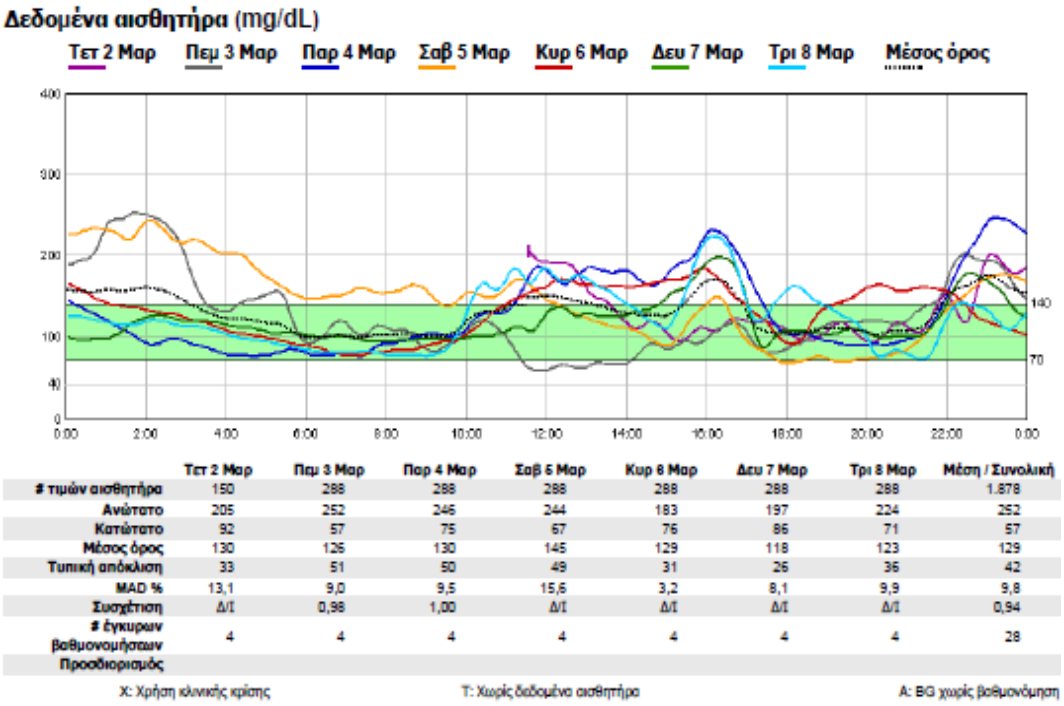
	Τετ 24 Φεβ	Πεμ 25 Φεβ	Παρ 26 Φεβ	Σαβ 27 Φεβ	Κυρ 28 Φεβ	Δευ 29 Φεβ	Τρι 1 Μαρ	Μέση / Συνολική
# υπερβάσεων	3	5	1	4	4	5	3	25
# υψηλών υπερβάσεων	3	4	1	4	4	5	3	24
# χαμηλών υπερβάσεων	0	1	0	0	0	0	0	1
AUC πάνω από το όριο	8,5	3,6	30,1	12,7	15,4	21,9	11,6	15,3
AUC κάτω από το όριο	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	5:00	39%	5:10	22%	12:30	52%	11:40	49%	12:10	51%	12:05	50%	11:05	48%	69:40	45%
Εντός (70 - 140)	7:40	61%	17:50	74%	11:30	48%	12:20	51%	11:50	49%	11:55	50%	12:00	52%	85:05	54%
Κάτω από 70	0:00	0%	1:00	4%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	1:00	1%

Figure 5-11 Subject 06 First Week Daily Overlay Report

Subject 06 was the sixth individual who followed the protocol. The report shows that the Mean fluctuates near the higher range limit. The number of 288 measurements was also performed on each day but only 152 on the first day. The duration allocation shows that the average percentage of the values was within the range limits as it is mirrored by the 54% of the pie. More hyperglycemic values than hypoglycemic were noted.



	Τετ 2 Μαρ	Πεμ 3 Μαρ	Παρ 4 Μαρ	Σαβ 5 Μαρ	Κυρ 6 Μαρ	Δευ 7 Μαρ	Τρι 8 Μαρ	Μέση / Συνολική
# τιμών αισθητήρα	150	288	288	288	288	288	288	1.878
Ανώτατο	205	252	246	244	183	197	224	252
Κατώτατο	92	57	75	67	76	86	71	57
Μέσος όρος	130	126	130	145	129	118	123	129
Τυπική απόκλιση	33	51	50	49	31	26	36	42
MAD %	13,1	9,0	9,5	15,6	3,2	8,1	9,9	9,8
Συσχέτιση	Δ/Ι	0,98	1,00	Δ/Ι	Δ/Ι	Δ/Ι	Δ/Ι	0,94
# έγκυρων βαθμονομήσεων	4	4	4	4	4	4	4	28
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

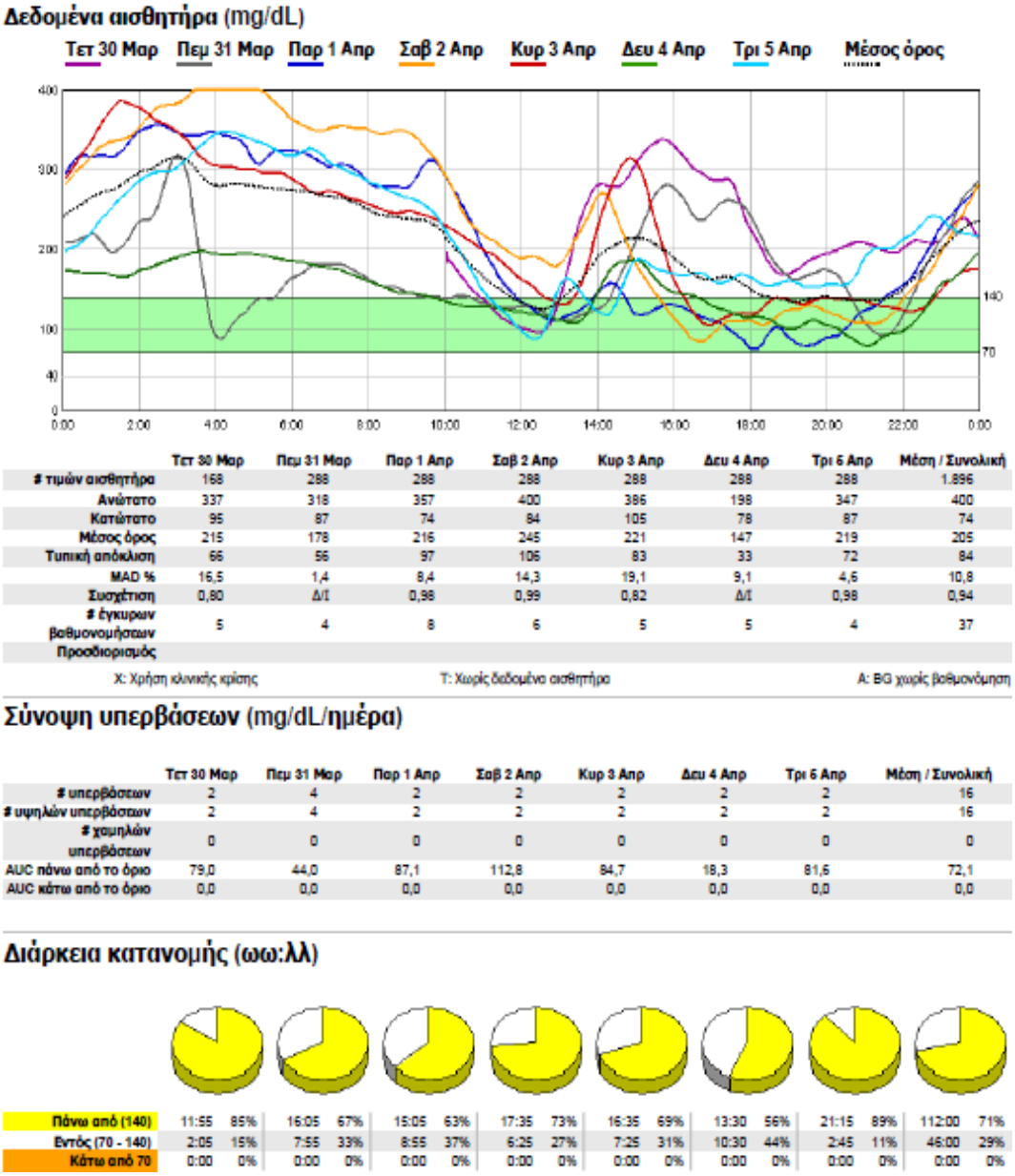
	Τετ 2 Μαρ	Πεμ 3 Μαρ	Παρ 4 Μαρ	Σαβ 5 Μαρ	Κυρ 6 Μαρ	Δευ 7 Μαρ	Τρι 8 Μαρ	Μέση / Συνολική
# υπερβάσεων	3	4	2	4	2	2	4	21
# υψηλών υπερβάσεων	3	3	2	2	2	2	4	18
# χαμηλών υπερβάσεων	0	1	0	2	0	0	0	3
AUC πάνω από το όριο	11,0	15,6	18,0	22,5	8,4	4,6	8,6	12,8
AUC κάτω από το όριο	0,0	0,8	0,0	0,1	0,0	0,0	0,0	0,1

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	3:45	30%	6:55	29%	8:30	35%	14:10	59%	10:05	42%	3:40	15%	6:50	28%	53:55	34%
Εντός (70 - 140)	8:45	70%	14:20	60%	15:30	65%	8:50	37%	13:55	58%	20:20	85%	17:10	72%	98:50	64%
Κάτω από 70	0:00	0%	2:45	11%	0:00	0%	1:00	4%	0:00	0%	0:00	0%	0:00	0%	3:45	2%

Figure 5-12 Subject 06 Second Week Daily Overlay Report

During the second week of the program Subject 06 presents an improvement in behavior. With an exception of the fourth day, the Mean ranges within the specified limits in each day. The duration allocation shows that the 64% lies within the range limits, 10% more than in first week's average percentage.



	Τετ 30 Μαρ	Πεμ 31 Μαρ	Παρ 1 Απρ	Σαβ 2 Απρ	Κυρ 3 Απρ	Δευ 4 Απρ	Τρι 5 Απρ	Μέση / Συνολική
# τιμών αισθητήρα	168	288	288	288	288	288	288	1.896
Ανώτατο	337	318	357	400	386	198	347	400
Κατώτατο	95	87	74	84	105	78	87	74
Μέσος όρος	215	178	216	245	221	147	219	205
Τυπική απόκλιση	66	56	97	106	83	33	72	84
MAD %	16,5	1,4	8,4	14,3	19,1	9,1	4,6	10,8
Συσχέτιση	0,80	Δ/Ι	0,98	0,99	0,82	Δ/Ι	0,98	0,94
# έγκυρων βαθμονομήσεων	5	4	8	6	5	5	4	37
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

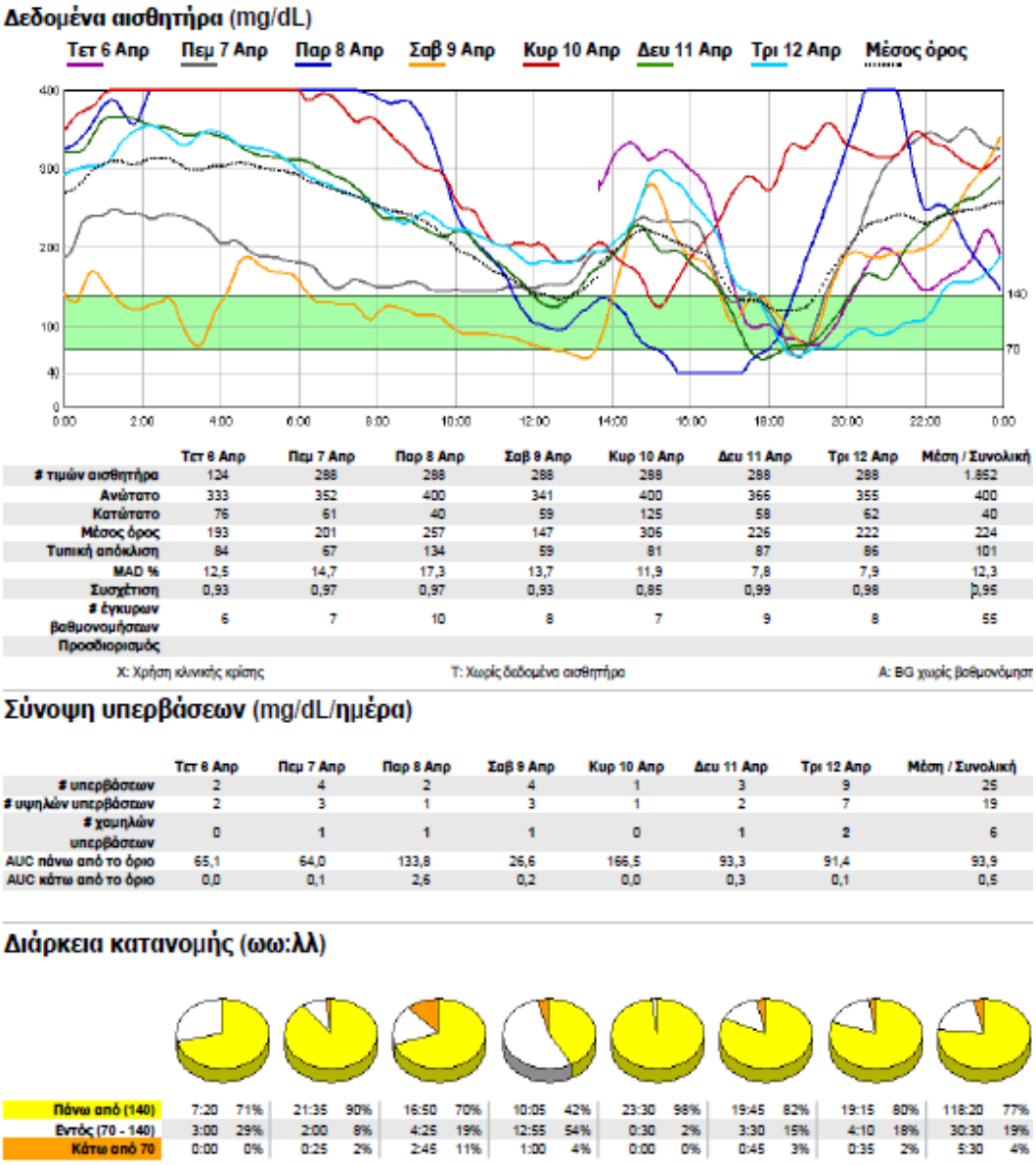
	Τετ 30 Μαρ	Πεμ 31 Μαρ	Παρ 1 Απρ	Σαβ 2 Απρ	Κυρ 3 Απρ	Δευ 4 Απρ	Τρι 5 Απρ	Μέση / Συνολική
# υπερβάσεων	2	4	2	2	2	2	2	16
# υψηλών υπερβάσεων	2	4	2	2	2	2	2	16
# χαμηλών υπερβάσεων	0	0	0	0	0	0	0	0
AUC πάνω από το όριο	79,0	44,0	87,1	112,8	84,7	18,3	81,6	72,1
AUC κάτω από το όριο	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	11:55	85%	16:05	67%	15:05	63%	17:35	73%	16:35	69%	13:30	56%	21:15	89%	112:00	71%
Εντός (70 - 140)	2:05	15%	7:55	33%	8:55	37%	6:25	27%	7:25	31%	10:30	44%	2:45	11%	46:00	29%
Κάτω από 70	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%	0:00	0%

Figure 5-13 Subject 07 First Week Daily Overlay Report

Subject 07 constitute the seventh individual of the program. As reflected from the iPro2 report, Subject 07 presents a hyperglycemic character. The Mean in each day shows values over the limit of 140 mg/dl. The hyperglycemic behavior of Subject 07 can be observed by the duration allocation pie, too. Only 29% of the values lie within the specified range limits in addition to 71% of the values that fluctuate above the higher limit.



	Τετ 6 Απρ	Πεμ 7 Απρ	Παρ 8 Απρ	Σαβ 8 Απρ	Κυρ 10 Απρ	Δευ 11 Απρ	Τρι 12 Απρ	Μέση / Συνολική
# τιμών αισθητήρα	124	288	288	288	288	288	288	1.852
Ανώτατο	333	352	400	341	400	366	355	400
Κατώτατο	76	61	40	59	125	58	62	40
Μέσος όρος	193	201	257	147	306	226	222	224
Τυπική απόκλιση	84	67	134	59	81	87	86	101
MAD %	12,5	14,7	17,3	13,7	11,9	7,8	7,9	12,3
Συσχέτιση	0,93	0,97	0,97	0,93	0,85	0,99	0,98	0,95
# έγκυρων βαθμονομήσεων	6	7	10	8	7	9	8	55
Προσδιορισμός								

X: Χρήση κλινικής κρίσης T: Χωρίς δεδομένα αισθητήρα A: BG χωρίς βαθμονόμηση

Σύνοψη υπερβάσεων (mg/dL/ημέρα)

	Τετ 6 Απρ	Πεμ 7 Απρ	Παρ 8 Απρ	Σαβ 8 Απρ	Κυρ 10 Απρ	Δευ 11 Απρ	Τρι 12 Απρ	Μέση / Συνολική
# υπερβάσεων	2	4	2	4	1	3	9	25
# υψηλών υπερβάσεων	2	3	1	3	1	2	7	19
# χαμηλών υπερβάσεων	0	1	1	1	0	1	2	6
AUC πάνω από το όριο	65,1	64,0	133,8	26,6	166,5	93,3	91,4	93,9
AUC κάτω από το όριο	0,0	0,1	2,6	0,2	0,0	0,3	0,1	0,5

Διάρκεια κατανομής (ωω:λλ)

Πάνω από (140)	7:20	71%	21:35	90%	16:50	70%	10:05	42%	23:30	98%	19:45	82%	19:15	80%	118:20	77%
Εντός (70 - 140)	3:00	29%	2:00	8%	4:25	19%	12:55	54%	0:30	2%	3:30	15%	4:10	18%	30:30	19%
Κάτω από 70	0:00	0%	0:25	2%	2:45	11%	1:00	4%	0:00	0%	0:45	3%	0:35	2%	5:30	4%

Figure 5-14 Subject 07 Second Week Daily Overlay Report

During the second week of the protocol, Subject 07 presents no improvement. The duration allocation pie shows that 77% of the values fluctuate above the higher limit and only the 19% lies within the specified limits. The Mean also presents values over the higher limit in each day. At the same time, hypoglycemic values were observed in the five of the seven days of the second week's program.

5.4 ACTIVITY REPORTS FROM SENSEWEAR ARMBAND

BodyMedia SenseWear Armband constitutes a physiological monitoring device. More specifically, it is a useful device for tracking activity and other related metrics.

Studies and researches have shown that activity affects people's health and quality of life. During the last decade, wearable devices, with the ability to monitor physiological activity, have shown a broad public acceptance and the corresponding industry has shown a remarkable growth.

During this survey, SenseWear Armband was used to monitor individual's daily activity. As mentioned in Chapter 3, during the first week of the protocol the individual must refrain from any form of physical exercise. During the second week, the individual must participate in daily physical exercise. Therefore, SenseWear Armband gave the upper hand in order to monitor activity and, along with the other related metrics, to help us understand the correlation of glucose fluctuation with the presence of physical exercise.

Furthermore, Armband reports present metrics related to fitness with a friendly user way. More specifically, this device presents metrics like physical activity duration, total energy expenditure, active energy expenditure, lying down (noon to noon), sleep duration (noon to noon), number of steps, duration on body, average metabolic equivalent of task (METs), sleep efficiency, physical activity level (PAL). Last but not least, Armband reports include metrics related to energy expenditure at several levels of METs, like sedentary (up to 1,5 METs), light (1,5–3 METs), moderate (3–6 METs), vigorous (6–9 METs) and finally very vigorous (9 METs and higher). It is also worth noting that the individual's clinical characteristics are presented in the Armband report.

In conclusion, SenseWear Armband proved to be a useful tool in this survey, in monitoring the individual's physiological activity and helping us understand how physical exercise affects the glucose fluctuation. In order to have a clear understanding of the usage of Armband, the next figures depict the reports of all individuals who participated during the two week protocol.

Figure 5-15 Subject 01 SenseWear Activity Report

Subject 01 presents significant activity energy expenditure during the second week of the program. This is due to the fact that during the second week of the

protocol, physiological activity is allowed. This is reflected in the metrics of moderate physiological activity where in the second week of the program the time of exercise presents an increase. In addition, an excursion appears on Sunday, according to the table, referring to vigorous exercise.

Figure 5-16 Subject 02 SenseWear Activity Report

During the second week of the program, where physical exercise is allowed, Subject 02 presents excursions in activity energy expenditure. Subject 02 also presents significant excursions during the same week, in the tables of moderate and vigorous physiological activity due to physical exercise, which is presented in these tables.

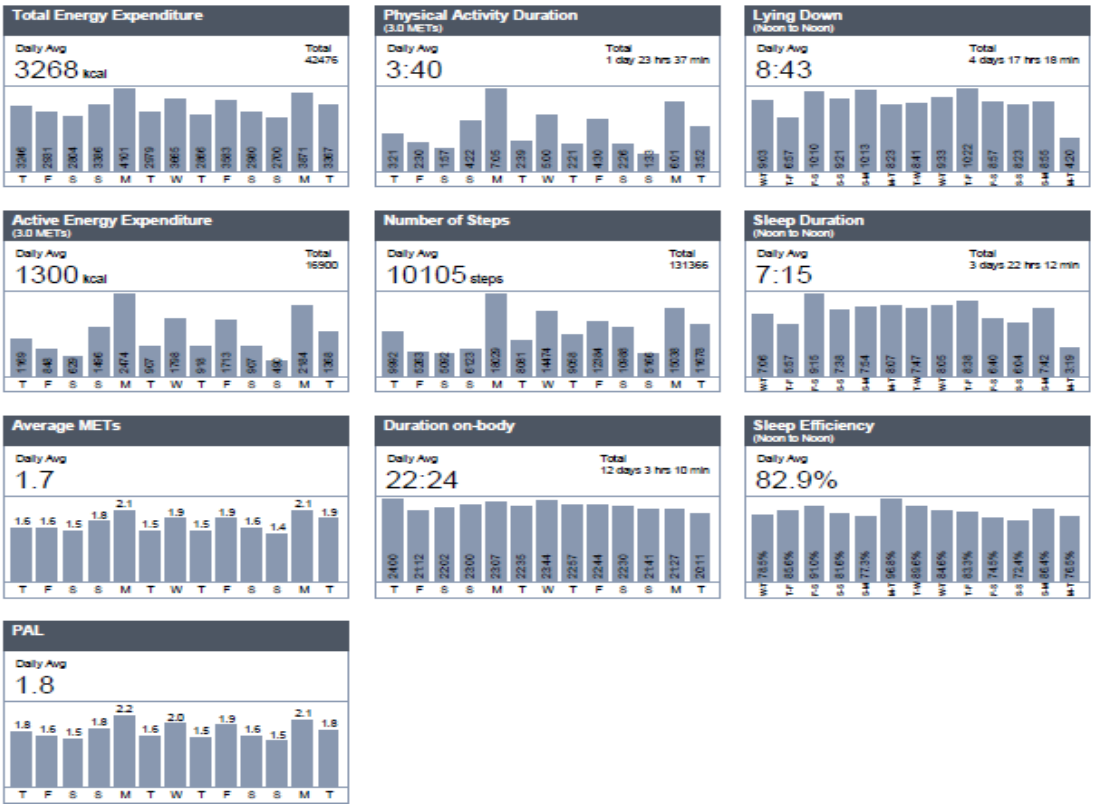


Figure 5-17 Subject 03 SenseWear Activity Report

During the second week of the program, Subject 03 presents excursions in active energy expenditure. Moreover, total energy expenditure presents small excursions while the number of steps during the second week of the protocol showed increased measurements. Physical exercise during the same week is obvious in the associated tables.

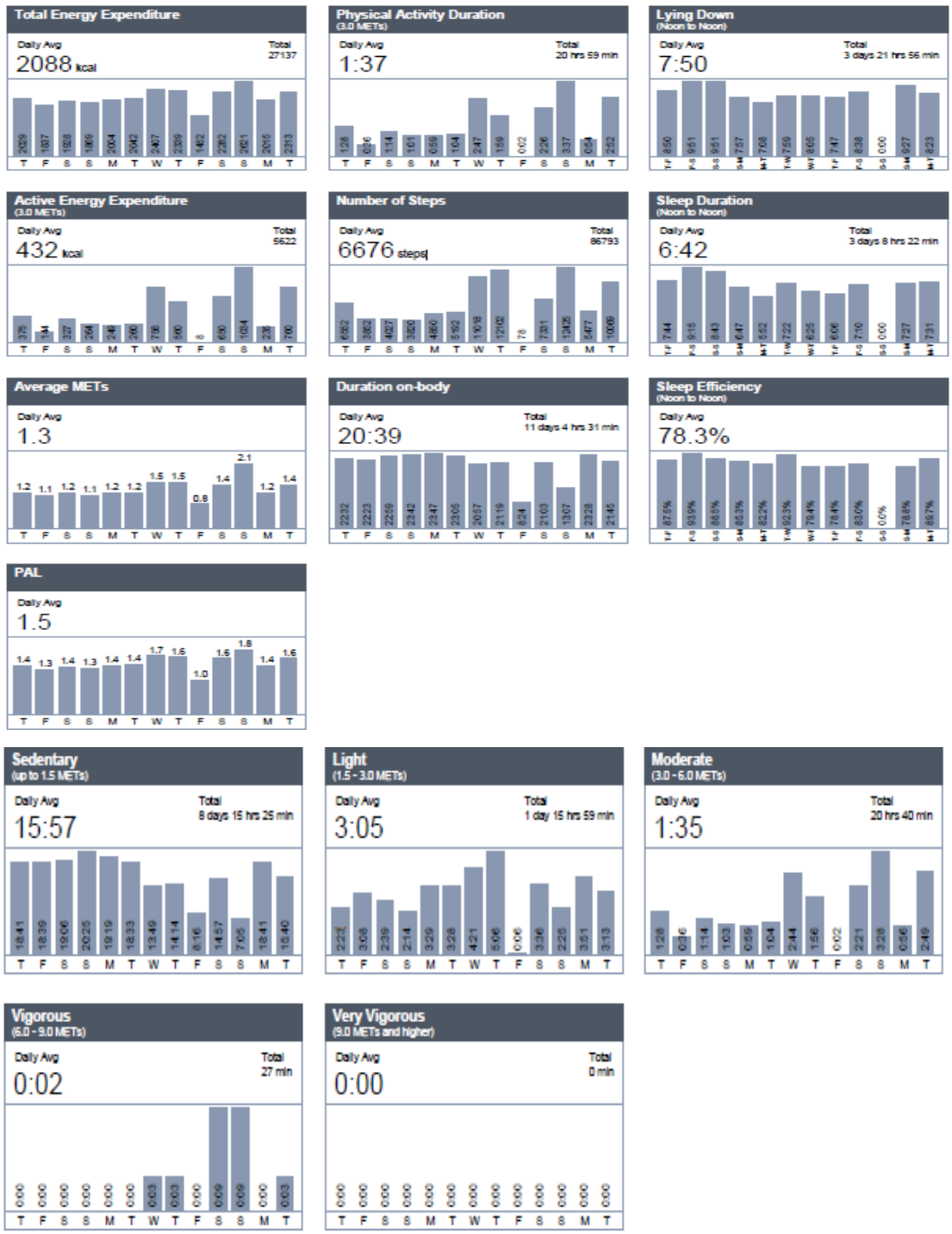


Figure 5-18 Subject 04 SenseWear Activity Report

During the second week of the program Subject 04 presents significant changes in activity energy expenditure. The tables showing moderate and vigorous exercise also present significant excursions due to the fact that during this week physical exercise was allowed.

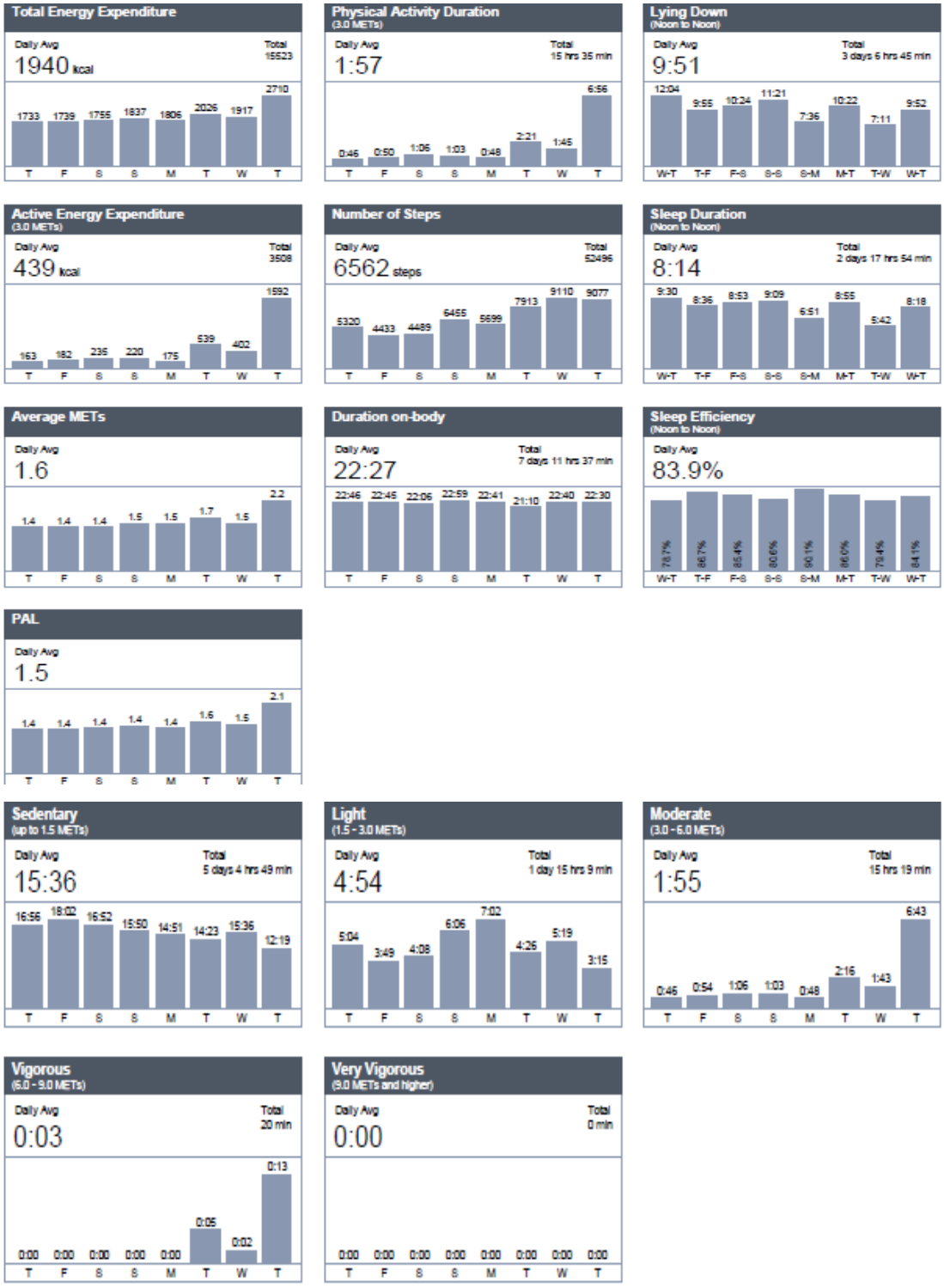


Figure 5-19 Subject 05 SenseWear Activity Report

Subject 05, presents an increase in activity energy expenditure during the second week of the program. This is reflected in the tables of moderate and vigorous physiological activity, too. During the second week of the program, physical exercise was performed by subject 05.

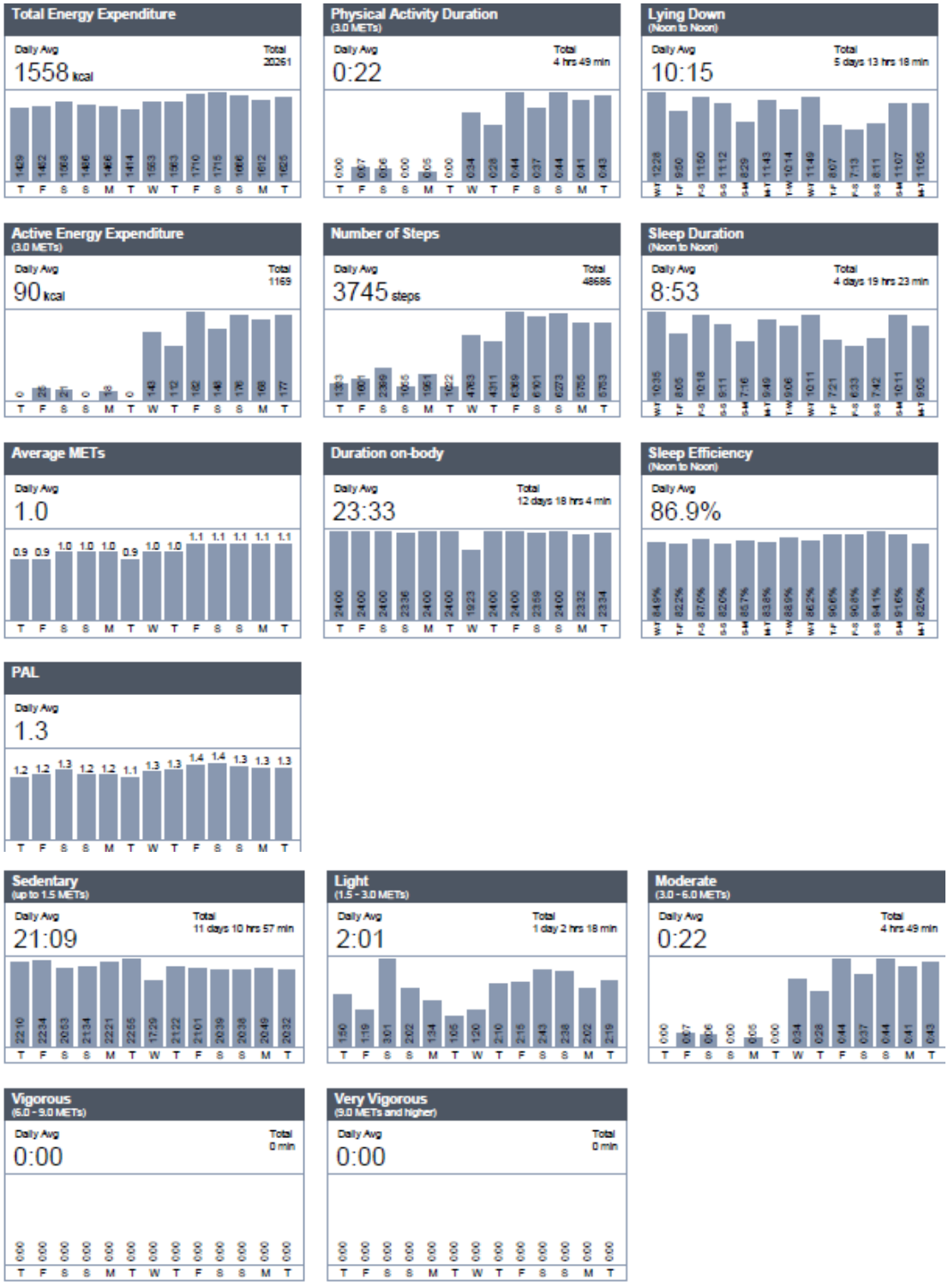


Figure 5-20 Subject 06 SenseWear activity report

Subject 06, presents excursions in regards to active energy expenditure, during the second week of the program. This can be seen in the table of the moderate physiological activity too, where, during the second week of the program, excursions

are presented. This is obvious due to the fact that physical exercise is performed during the second week of the protocol.

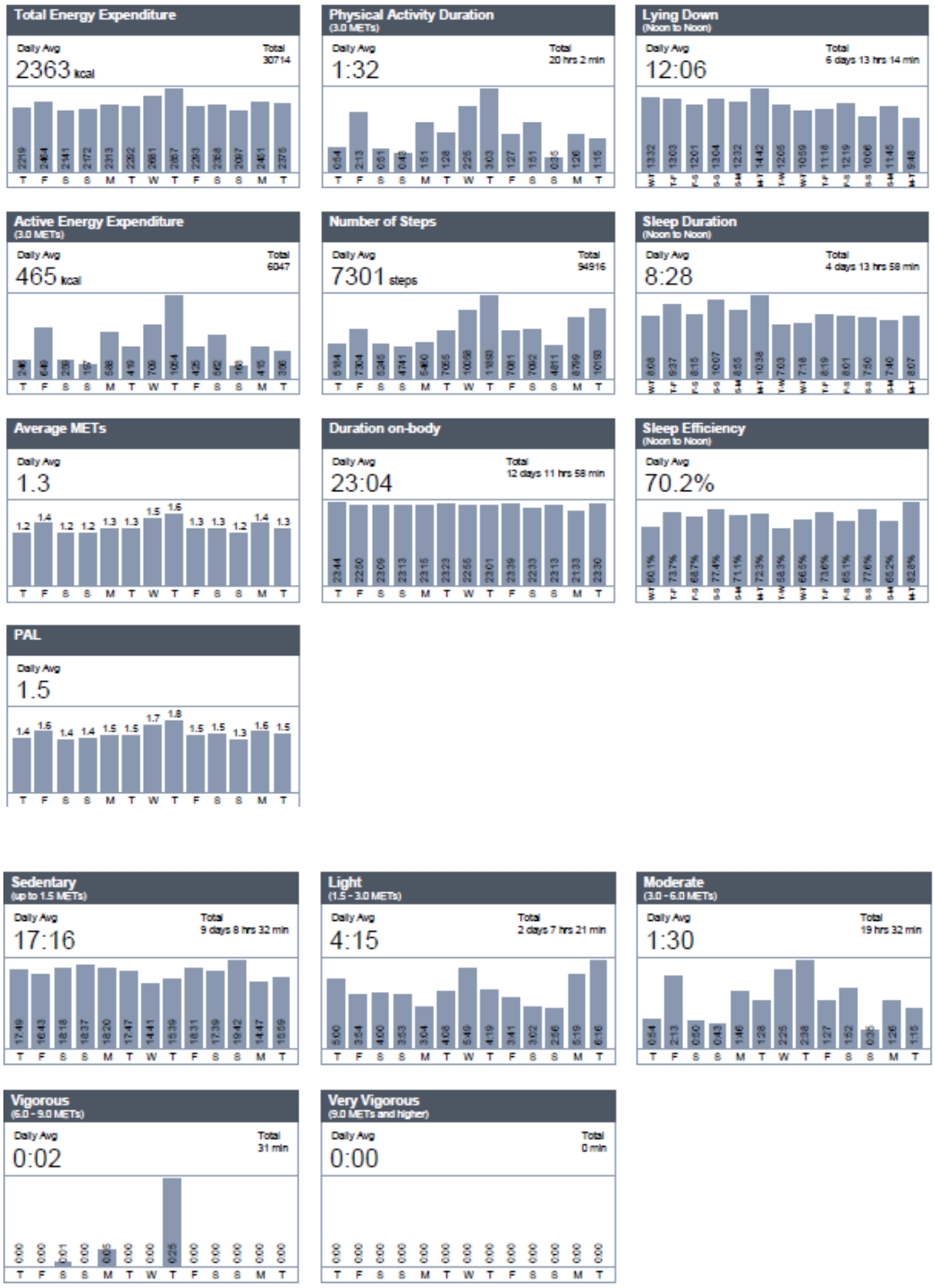


Figure 5-21 Subject 07 SenseWear activity report

Subject 07 presents some significant excursions in the active energy expenditure table, during the second week of the program. In addition, the tables of moderate and vigorous physiological activity present excursions due to physical exercise that was allowed during the second week of the program.

5.5 ACTIVITY REPORTS FROM FITBIT FLEX

Fitbit Flex constitutes an activity tracker that found use in this survey in order to continuously monitor the physiological activity of the individuals. Its reports have a friendly user character and along with the practicality in everyday use (like wearing a watch) was an ideal selection for research requirements.

More specifically, the report of this wearable device presents metrics related to the walking distance (km), steps taken, burned calories and active minutes. These metrics are calculated automatically by the device with the help of a 3-axis accelerometer. Fitbit Flex also has the ability to present metrics related to food intake, water intake, sleep duration, calories intake and weight levels. The difference with these metrics is that the device requires the manual entry of data, like the type and the quantity of the food. It basically includes a diary with the individual's lifestyle and daily routine.

In conclusion, Fitbit Flex was a valuable tool in order to monitor patient's physiological activity especially in the second week of the program where the patient exercises daily. The following figures present all the Fitbit reports.

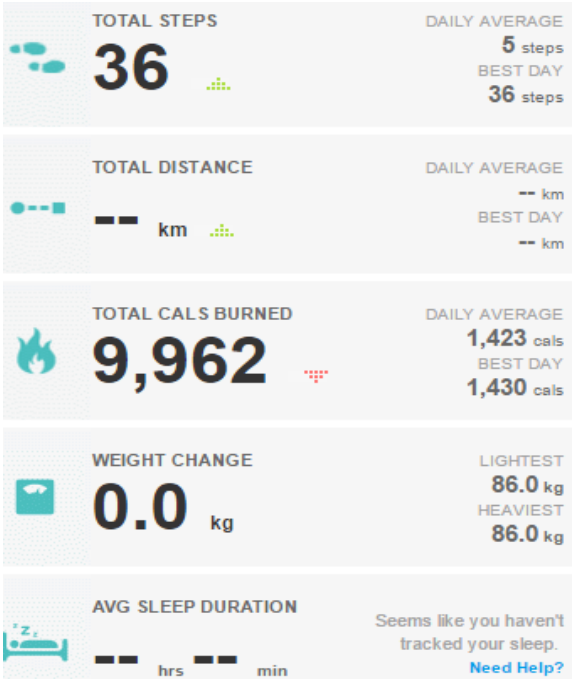


Figure 5-22 Subject 01 Fitbit First Week Report

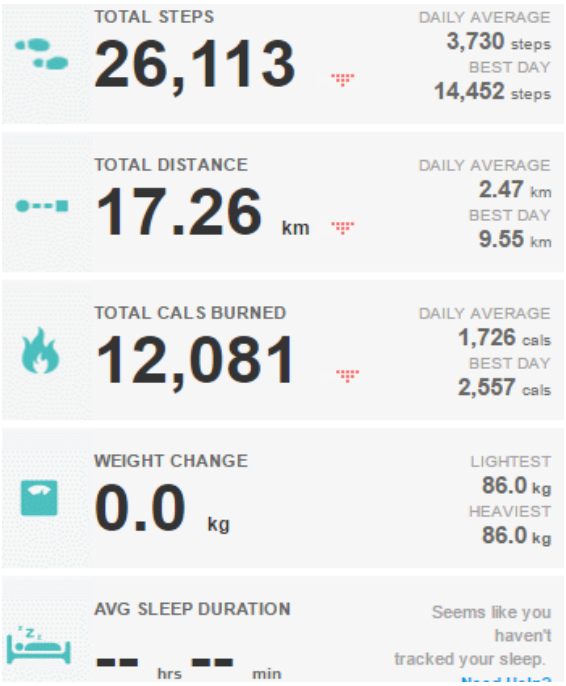


Figure 5-23 Subject 01 Fitbit Second Week Report

Observing subject 01, during the second week of the program where physical exercise is performed, the number of total steps present an increase in contrast with the total steps of week 1. The total calories burned also show a significant increase during the second week of the protocol due to physical exercise.

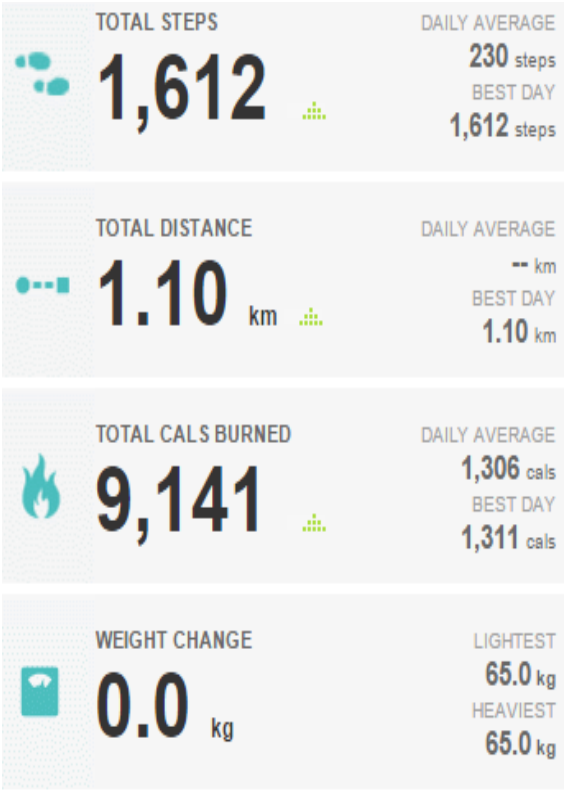


Figure 5-24 Subject 02 Fitbit First Week Report

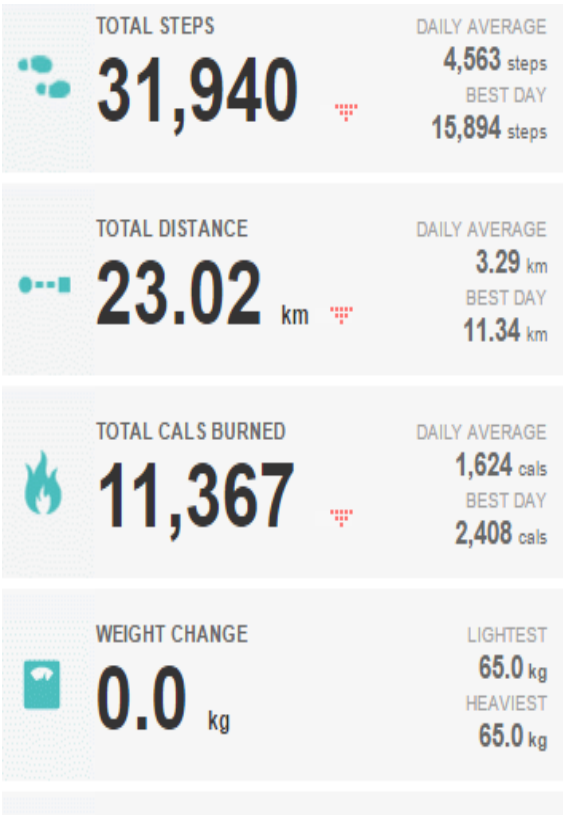


Figure 5-25 Subject 02 Fitbit Second Week Report

Subject 02 presents an increased number of total steps during the second week of the program due to physical exercise in this week. This is reflected in the metrics of distance and calories burned which both present a significant increase during the same week of the protocol.

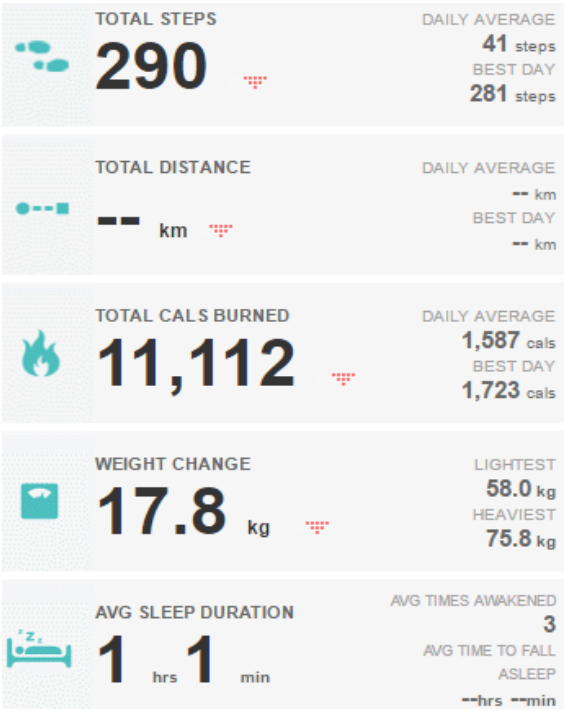


Figure 5-26 Subject 03 Fitbit First Week Report

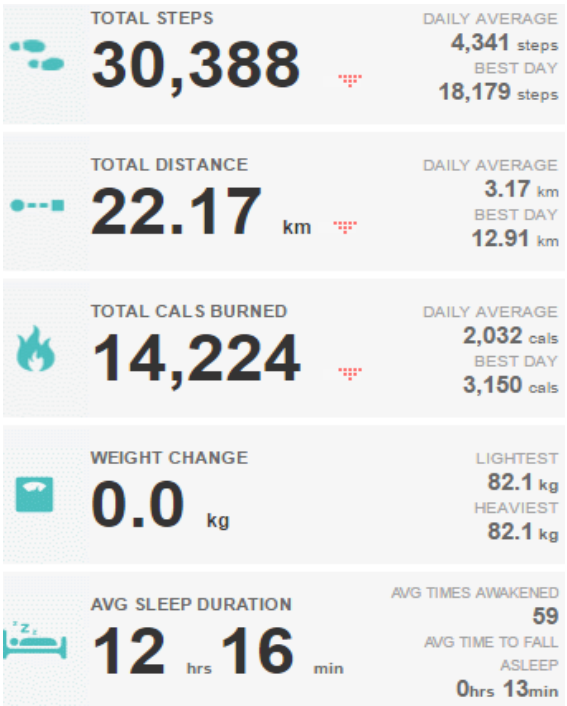


Figure 5-27 Subject 03 Fitbit Second Week Report

Subject 03, presents an increase in total steps metrics, during the second week of the program due to the physical exercise allowed in that week. This is also obvious in the metric tables regarding distance and calories burned during this week of the protocol.

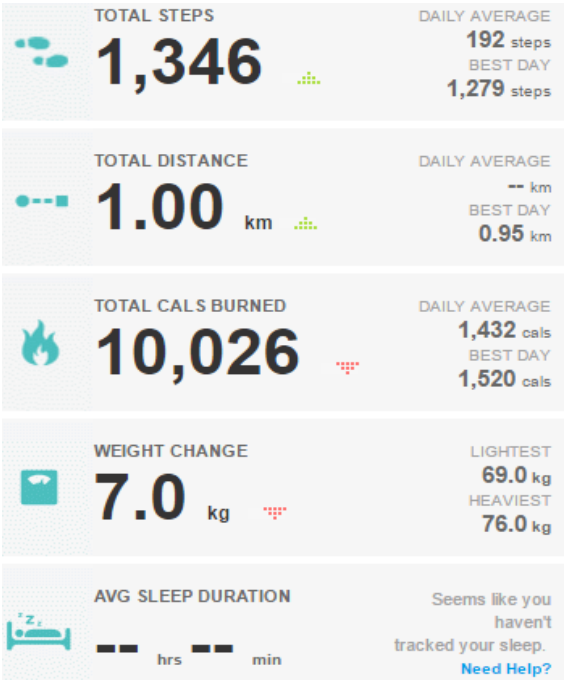


Figure 5-28 Subject 04 Fitbit First Week Report

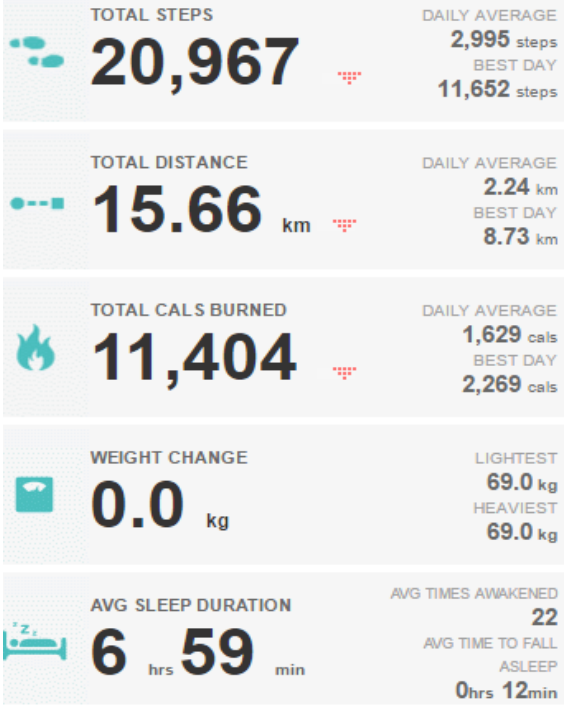


Figure 5-29 Subject 04 Fitbit Second Week Report

Subject 04, during the second week of the program, scores 20,967 total steps which is a significant number in contrast with the 1346 steps of the first week. This is also reflected in the tables of calories burned and total distance due to physical exercise in this week of the program.

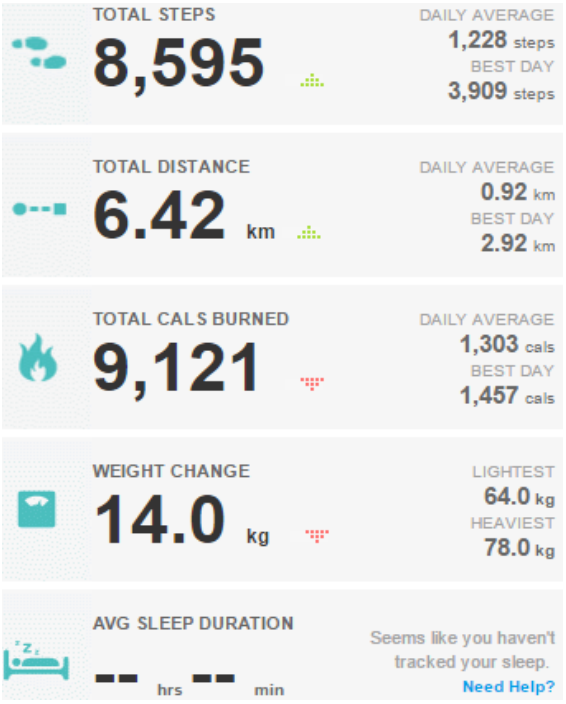


Figure 5-30 Subject 06 Fitbit First Week Report

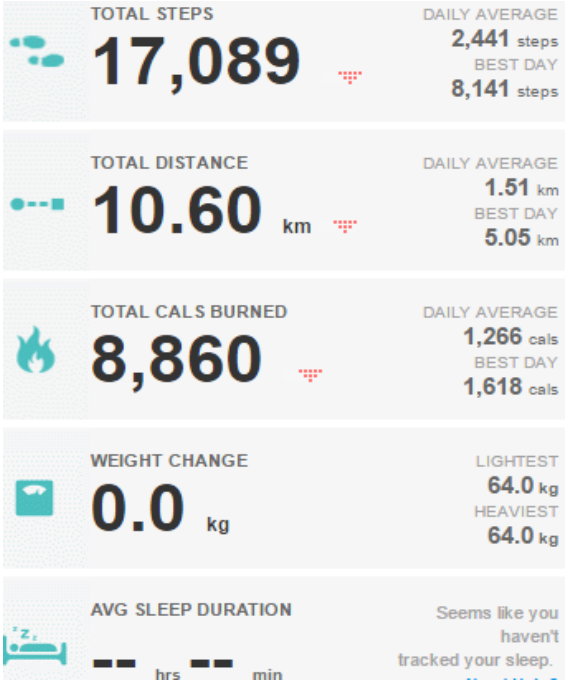


Figure 5-31 Subject 06 Fitbit Second Week Report

During the second week of the program, Subject 06 presents an increase in the number of total steps taken, due to physical exercise in this week. This is also reflected in the metrics of total distance in the second week of the protocol.

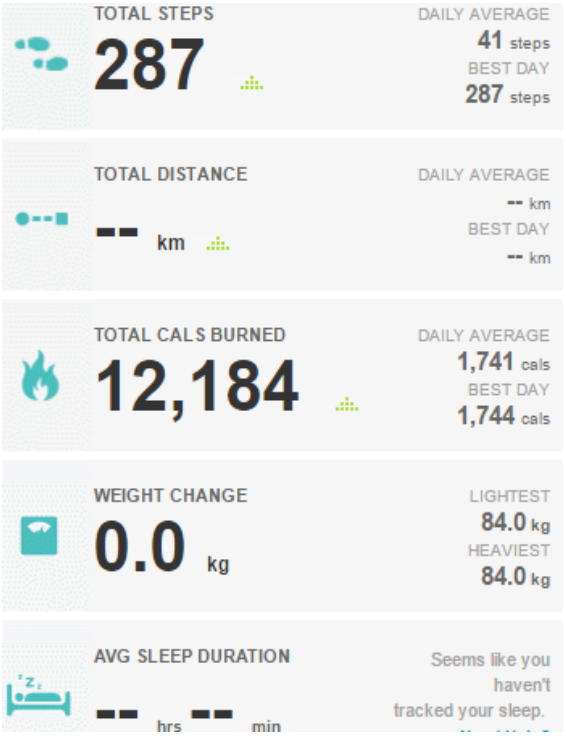


Figure 5-32 Subject 07 Fitbit First Week Report

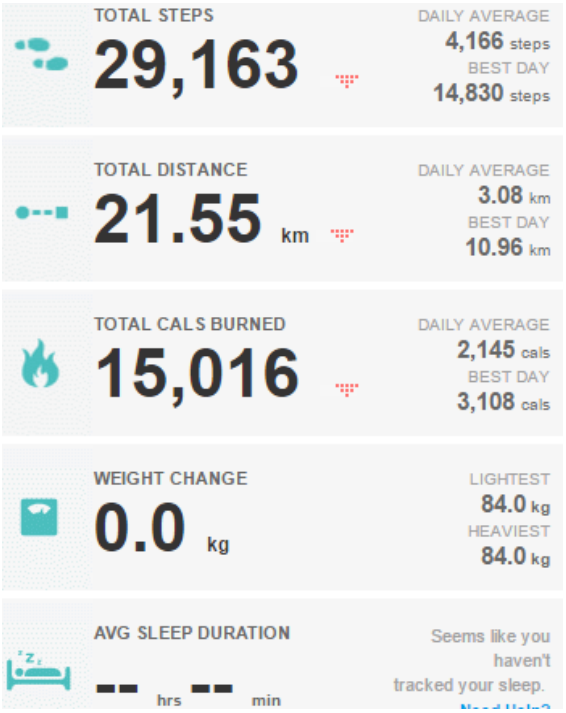


Figure 5-33 Subject 07 Fitbit Second Week Report

Subject 07, during the second week of the protocol, presents an increase in numbers in the metrics of total distance, calories burned and number of steps. This is likely due to the physical exercise that is performed during the second week of the protocol.

5.6 STATISTICAL ANALYSIS RESULTS

Statistical analysis, as it was proved, gave us valuable indicators about the goals of this thesis. As mentioned earlier, the target range was determined within the limits of [70 – 140] mg/dl. During the statistical analysis, credible measures were used concerning the most tangible results.

In order to have a clearer point of view and to generate more accurate results during the statistical analysis, the data was divided in nocturnal and diurnal measurements. This division led to interesting conclusions regarding glucose behavior and action either during the day or during the night. In turn, the whole data was analyzed in order to form a complete picture.

In the following tables, the statistical analysis results are presented and described in detail, beginning with the total statistical analysis results followed by the nocturnal and diurnal analysis results.

Table 5.2 Statistical analysis results (Mean, SD, % CV)

Patient	**Mean**	**SD**	**% CV**
Subject 01 1st Week	126,43	33,66	26,62
Subject 01 2nd Week	116,57	31,88	27,35
Subject 02 1st Week	118,03	33,03	27,98
Subject 02 2nd Week	109,16	32,24	29,54
Subject 03 1st Week	198,71	62,91	31,66
Subject 03 2nd Week	175,57	67,59	38,49
Subject 04 1st Week	145,18	56,44	38,88
Subject 04 2nd Week	145,84	53,11	36,41
Subject 05 1st Week	152,54	62,17	40,76
Subject 05 2nd Week	158,14	86,84	54,91
Subject 06 1st Week	141,95	36,97	26,05
Subject 06 2nd Week	130,69	42,74	32,71
Subject 07 1st Week	200,65	84,54	42,13
Subject 07 2nd Week	229,18	100,25	43,74

As it observed from table 5.2, during the second week of the protocol 4 patients reduce their Mean, 3 patients reduce their SD and only 1 individual reduce his/her

%CV. This is a first indicator that glycemic variability (GV) is affected with a negative way.

Table 5.3 Statistical analysis results (%< 70 mg/dl, % [70 – 140] mg/dl, % > 140 mg/dl)

Patient	**% < 70 mg/dl**	**% [70 – 140] mg/dl**	**% > 140 mg/dl**
Subject 01 1st Week	1,59	69,49	28,92
Subject 01 2nd Week	5,76	75,35	18,89
Subject 02 1st Week	6,21	68,68	25,11
Subject 02 2nd Week	9,11	75,90	14,99
Subject 03 1st Week	0,00	18,56	81,44
Subject 03 2nd Week	5,11	28,42	66,47
Subject 04 1st Week	3,16	50,46	46,38
Subject 04 2nd Week	4,84	48,04	47,12
Subject 05 1st Week	5,07	41,39	53,54
Subject 05 2nd Week	8,03	45,30	46,67
Subject 06 1st Week	0,69	53,74	45,57
Subject 06 2nd Week	2,50	58,92	38,57
Subject 07 1st Week	0,38	30,35	69,26
Subject 07 2nd Week	3,61	18,09	78,31

As it resulted from table 5.3, during the second week of the protocol 7 individuals increase their percentage under the 70 mg/dl, 5 individuals increase their percentage within the desired limits of 70 – 140 mg/dl and 5 individuals decrease their percentage over the limit of 140 mg/dl.

Table 5.4 Statistical analysis results (Average hours per day < 70 mg/dl, Average hours per day [70 -140] mg/dl, Average hours per day > 140 mg/dl)

Patient	**Average hours per day < 70 mg/dl**	**Average hours per day [70-140] mg/dl**	**Average hours per day > 140 mg/dl**
Subject 01 1st Week	0,35	15,14	6,30
Subject 01 2nd Week	1,24	16,20	4,06
Subject 02 1st Week	1,36	15,01	5,49
Subject 02 2nd Week	1,99	16,57	3,27
Subject 03 1st Week	0,00	4,02	17,67
Subject 03 2nd Week	1,12	6,23	14,56
Subject 04 1st Week	0,69	11,04	10,14
Subject 04 2nd Week	0,82	8,15	8,00
Subject 05 1st Week	1,12	9,05	11,70
Subject 05 2nd Week	1,75	9,88	10,17
Subject 06 1st Week	0,14	11,12	9,43
Subject 06 2nd Week	0,55	12,89	8,44

Patient	Average hours per day < 70 mg/dl	Average hours per day [70-140] mg/dl	Average hours per day > 140 mg/dl
Subject 07 1st Week	0,08	6,63	15,13
Subject 07 2nd Week	0,79	3,94	17,07

As it resulted from table 5.4, during the second week of the program 7 patients present an increasing rate in their time spending under the limit of 70 mg/dl, 5 individuals present increasing rate within the limits of 70 – 140 mg/dl and 6 individuals present decreasing rates over the limit of 140 mg/dl. This is another indicator that physical activity tends to reduce glucose values.

Table 5.5 Statistical analysis results (Minimum, Maximum, Range)

Patient	Minimum	Maximum	Range
Subject 01 1st Week	58,00	262,00	204,00
Subject 01 2nd Week	50,00	233,00	183,00
Subject 02 1st Week	58,00	201,00	143,00
Subject 02 2nd Week	53,00	235,00	182,00
Subject 03 1st Week	73,00	374,00	301,00
Subject 03 2nd Week	40,00	367,00	327,00
Subject 04 1st Week	40,00	330,00	290,00
Subject 04 2nd Week	55,00	317,00	262,00
Subject 05 1st Week	40,00	346,00	306,00
Subject 05 2nd Week	40,00	400,00	360,00
Subject 06 1st Week	63,00	274,00	211,00
Subject 06 2nd Week	57,00	252,00	195,00
Subject 07 1st Week	62,00	400,00	338,00
Subject 07 2nd Week	40,00	400,00	360,00

During the second week of the program as it observed from table 5.5, 5 individuals present more minimum values than in the first week of the program, 1 remains stable and the other one present higher value than in the first week of the protocol. Regarding the maximum values, 4 individuals present decreasing rates during the second week, 1 remains stable and the other one present's higher maximum value. Regarding the range 4 individuals present increasing rates during the second week of the protocol. These measures present a negatively affection of the glycemic variability when exercise is introduced during the second week of the protocol.

Table 5.6 Statistical analysis results (10^{th} percentile, 25^{th}percentile, 50^{th}percentile)

Patient	10^{th} percentile	25^{th} percentile	50^{th} percentile
Subject 01 1st Week	89,40	102,00	124,00
Subject 01 2nd Week	79,00	97,00	114,00
Subject 02 1st Week	73,00	93,00	115,00
Subject 02 2nd Week	71,00	84,00	107,00
Subject 03 1st Week	115,00	157,75	187,00
Subject 03 2nd Week	81,50	120,00	180,50
Subject 04 1st Week	82,00	101,00	134,00
Subject 04 2nd Week	83,00	108,00	136,00
Subject 05 1st Week	78,10	102,00	144,00
Subject 05 2nd Week	72,00	90,00	132,00
Subject 06 1st Week	99,00	114,00	135,00
Subject 06 2nd Week	80,00	97,00	122,00
Subject 07 1st Week	109,00	130,00	176,00
Subject 07 2nd Week	96,00	149,00	218,00

Table 5.7 Statistical analysis results (75^{th} percentile, 90^{th} percentile, IQR)

Patient	75^{th} percentile	90^{th} percentile	Interquartile Range (IQR)
Subject 01 1st Week	145,00	170,00	43,00
Subject 01 2nd Week	131,00	158,00	34,00
Subject 02 1st Week	140,00	169,90	47,00
Subject 02 2nd Week	129,00	148,00	45,00
Subject 03 1st Week	247,00	290,00	89,25
Subject 03 2nd Week	224,00	269,50	104,00
Subject 04 1st Week	180,25	231,00	79,25
Subject 04 2nd Week	178,00	220,00	70,00
Subject 05 1st Week	194,00	235,00	92,00
Subject 05 2nd Week	199,00	289,00	109,00
Subject 06 1st Week	165,00	194,00	51,00
Subject 06 2nd Week	161,00	187,70	64,00
Subject 07 1st Week	269,75	333,00	139,75
Subject 07 2nd Week	319,00	375,00	170,00

As regards to the whole picture of the statistical analysis results, it is clearly understood that five of the seven patients show lower values in Mean (mg/dl) during the second week of the program in contrast with the respective values during the first week of the program, one remains stable and one shows higher values in Mean (mg/dl). Target in range was improved in five of the seven patients, presenting more (%) values within the target range during the second week of the protocol while 2 patients show fewer values within the target in range during the same week. At the

same time, the percentage of values under the limit of 70% (mg/dl) presents an increase in all individuals while five individuals present a decrease in glucose values over the limit of 180 (mg/dl), one remained near the same level and the last one presents an increasing rate. In terms of average hour per day, the whole picture is similar. More specifically, 5 patients remain more time within the target range, while their remaining time under the lower limits presents an increasing rate. On the other hand, the remaining time over the higher limits in 6 patients presents a decreasing rate while the last one presents an increasing rate. All the above measures are indicators that all of the patients, except one, show a decrease in their hyperglycemic values during the second week of the program. Moreover, for four of them, the time within the range limits is larger during the same week.

Nevertheless, as regards to the three cardinal measures of glycemic variability (GV) the results are equivocal. Standard Deviation (SD) in four patients presents an increasing rate during the second week of the program, while in the other three it presents a decreasing rate. On the other hand, the coefficient of variation % (CV) in six patients presents an increasing rate during the second week of the program but a decrease in one patient. The third measure is Interquartile Range (IQR) which in three patients presents lower values in the second week of the protocol but in the same week the other four patients present higher values concerning the IQR. The above three measures of GV indicate that physical exercise may affect GV in a negative way. As mentioned in Chapter 4, high SD is an indicator of possible undesired problems, which may concern a lack of or surplus of insulin. Additionally, the % CV presents concrete results about GV but also equivocal during this thesis about the effect of physical exercise in GV. More specifically, high % CV is undesired and % CV beyond the limits of 36% may be an indicator of serious upcoming hypoglycemic events. So, as it was mentioned before, six patients present an increasing rate in their % CV during the second week of the protocol while one presents a decreasing rate. At the same time four patients present a percentage over the limit of 36% during the second week of the program while the other three have a percentage lower than 36%. Regarding the IQR which represents the 50 percent of the glucose values, the results are equivocal.

Furthermore, during the second week of the program five patients present minimum values lower than the first week, one remains stable and the last one

presents a maximize minimum glucose value in relation to the first week of the protocol. As regards to the maximum glucose values recorded, four of the seven patients present lower maximum values during the second week of the protocol, two patients present higher maximum glucose values and one patient still remains stable. Moreover, studying the range between maximum and minimum glucose values, four patients present a larger range during the second week of the protocol and the other three present a smaller range during the same week. The last five measures concern the 10^{th} percentile, 25^{th} percentile, 50^{th} percentile, 75^{th} percentile and 90^{th} percentile. More specifically, in the 10^{th} and the 50^{th} percentiles, the glucose values of six patients are decreased in contrast to the first week of the program. One patient presents an increase in his glucose values during the second week. In regard to the $25^{th,}$ 75^{th} and 90^{th} percentiles, during the second week of the protocol, five patients present reduced glucose values, while the other two show an increasing rate.

However, if we want to compare the results of the first week with the results of the second week for all patients, in terms of an average ensemble, then the picture starts to become clearer. More specifically, Mean ranges among lower levels during the second week while at the same time SD and %CV ranges among higher levels including the undesired 37,59% of %CV. From another point of view, glucose values under the limit of 70% mg/dl present an increasing rate during the second week of the protocol, as well as the glucose values within the range limits of 70 – 140 mg/dl. At the same time, glucose values over the limit of 170% mg/dl present reduce. In terms of average hours per day, the picture is similar. Average hours per day % < 70 mg/dl and Average hours per day % [70 – 140] mg/dl present an increasing rate during the second week of the protocol, while Average hours per day > 140 mg/dl presents reduce. Minimum glucose values recorded are reduced during the second week, but Maximums show a small increase. The Range between Maximum and Minimum during the second week of the program has increased too. With regard to the percentiles, the 10^{th} percentile, 25^{th} percentile and 50^{th} percentile have decreased in the second week, the 75^{th} percentile remains stable and the 90^{th} percentile shows a small increase. In terms of IQR, an increase occurs during the second week.

In conclusion, the results show that the GV and glucose fluctuation are affected by physiological exercise. As it seems, the glucose values present a decreasing rate

during the second week of the program, but GV seems to be affected in a negative way.

In order to have a better representation of the analysis results and to draw better conclusions, it was considered wise to separate and in turn analyse them into nocturnal and diurnal mesurements. The following tables represent the nocturnal analysis results.

Table 5.8 Nocturnal analysis results (Mean, SD, % CV).

Patient	**Mean**	**SD**	**% CV**
Subject 01 1st Week	111,26	27,75	24,94
Subject 01 2nd Week	119,98	34,05	28,38
Subject 02 1st Week	125,20	41,14	32,86
Subject 02 2nd Week	111,18	31,22	28,08
Subject 03 1st Week	196,22	51,80	26,40
Subject 03 2nd Week	193,47	75,07	38,80
Subject 04 1st Week	108,32	35,75	33,01
Subject 04 2nd Week	127,83	77,98	61,00
Subject 05 1st Week	142,63	54,58	38,26
Subject 05 2nd Week	149,56	99,68	66,65
Subject 06 1st Week	111,10	52,93	47,64
Subject 06 2nd Week	142,77	48,19	33,76
Subject 07 1st Week	265,37	87,13	32,83
Subject 07 2nd Week	301,24	88,94	29,53
Week 1			
Average	151,44	50,15	33,71
SD	58,95	19,09	6,20
Week 2			
Average	163,72	65,02	40,88
SD	66,33	27,16	16,19

As it resulted from table 5.8 during the second week of the nocturnal measurements, 5 individuals present higher Mean, also 5 individuals present higher SD during the same week. Reagrding the % CV 4 individuals present higher % CV during the second week of the protocol. As it observed from the above table nocturnal glucose measurements present higher glucose values in general than in the first week of the protocol.

Table 5.9 Nocturnal analysis results (% < 70 mg/dl, % [70 – 140] mg/dl, % > 140 mg/dl)

Patient	**% < 70 mg/dl**	**% [70 – 140] mg/dl**	**% > 140 mg/dl**
Subject 01 1st Week	4,17	78,97	16,87
Subject 01 2nd Week	8,73	67,66	23,61
Subject 02 1st Week	9,92	49,80	40,28
Subject 02 2nd Week	14,29	65,87	19,84
Subject 03 1st Week	0,00	14,09	85,91
Subject 03 2nd Week	9,72	18,06	72,22
Subject 04 1st Week	10,52	70,24	19,25
Subject 04 2nd Week	23,61	32,34	44,05
Subject 05 1st Week	10,32	36,31	53,37
Subject 05 2nd Week	7,54	59,52	32,94
Subject 06 1st Week	16,67	47,62	35,71
Subject 06 2nd Week	0,00	57,54	42,46
Subject 07 1st Week	0,00	8,73	91,27
Subject 07 2nd Week	0,00	7,74	92,26
Week 1			
Average	7,37	43,68	48,95
SD	6,20	26,32	29,83
Week 2			
Average	9,13	44,10	46,77
SD	8,23	24,45	26,45

During the second week of the protocol as it presented in table 5.9, 4 individuals present increase in their percentage under the limit of 70 mg/dl, 4 individuals present increasing rates within the limits of 70 – 140 mg/dl, also during the second week of the protocol 4 individuals present increasing rates in their values over the limit of 140 mg/dl.

Table 5.10 Nocturnal analysis results (Average hours per day < 70 mg/dl, Average hours per day [70 – 140] mg/dl, Average hours per day > 140 mg/dl)

Patient	**Average hours per day < 70 mg/dl**	**Average hours per day [70-140] mg/dl**	**Average hours per day > 140 mg/dl**
Subject 01 1st Week	0,25	4,74	1,01
Subject 01 2nd Week	0,52	4,06	1,42
Subject 02 1st Week	0,60	2,99	2,42
Subject 02 2nd Week	0,86	3,95	1,19
Subject 03 1st Week	0,00	0,85	5,15
Subject 03 2nd Week	0,58	1,08	4,33
Subject 04 1st Week	0,63	4,21	1,15

Patient	Average hours per day < 70 mg/dl	Average hours per day [70-140] mg/dl	Average hours per day > 140 mg/dl
Subject 04 2nd Week	0,56	1,94	2,64
Subject 05 1st Week	0,62	2,18	3,20
Subject 05 2nd Week	0,45	3,57	1,98
Subject 06 1st Week	0,14	2,86	2,14
Subject 06 2nd Week	0,00	3,45	2,55
Subject 07 1st Week	0,00	0,52	5,48
Subject 07 2nd Week	0,00	0,46	5,54
Week 1			
Average	0,32	2,62	2,94
SD	0,29	1,58	1,79
Week 2			
Average	0,43	2,65	2,81
SD	0,32	1,47	1,59

During the nocturnal measuremnts of the second week of the protocol 3 individuals present their spending time under the limit of 70 mg/dl increased. Regarding their spending time within the desired limits of 70 – 140 mg/dl at night 4 patients increase their values. Also 4 individuals present increasing rates over the limit of 140 mg/dl during the second week of the protocol at night. As it observed glucose variability presend an increasing character.

Table 5.11 Nocturnal analysis results (Minimum, Maximum, Range)

Patient	Minimum	Maximum	Range
Subject 01 1st Week	64,00	213,00	149,00
Subject 01 2nd Week	50,00	229,00	179,00
Subject 02 1st Week	60,00	187,00	127,00
Subject 02 2nd Week	53,00	163,00	110,00
Subject 03 1st Week	93,00	308,00	215,00
Subject 03 2nd Week	40,00	292,00	252,00
Subject 04 1st Week	40,00	224,00	184,00
Subject 04 2nd Week	0,00	317,00	317,00
Subject 05 1st Week	40,00	240,00	200,00
Subject 05 2nd Week	57,00	400,00	343,00
Subject 06 1st Week	0,00	191,00	191,00
Subject 06 2nd Week	75,00	252,00	177,00
Subject 07 1st Week	87,00	400,00	313,00
Subject 07 2nd Week	74,00	400,00	326,00
Week 1			
Average	54,86	251,86	197,00
SD	31,75	76,85	59,44

Patient	Minimum	Maximum	Range
Week 2			
Average	49,86	293,29	243,43
SD	25,36	87,70	89,99

As it resulted from table 5.11, 5 individuals present more minimum values during the second week of the protocol, and 4 individuals present increasing maximum values. Regarding the range, 5 individuals present increasing rates in their values.

Table 5.12 Nocturnal analysis results (10^{th} percentile, 25^{th} percentile, 50^{th}percentile)

Patient	10^{th} percentile	25^{th} percentile	50^{th} percentile
Subject 01 1^{st} Week	79,00	91,00	106,00
Subject 01 2^{nd} Week	87,90	103,00	115,00
Subject 02 1^{st} Week	69,90	76,00	121,50
Subject 02 2^{nd} Week	67,00	82,00	120,00
Subject 03 1^{st} Week	115,00	175,00	188,00
Subject 03 2^{nd} Week	70,00	115,50	214,00
Subject 04 1^{st} Week	67,90	82,00	103,00
Subject 04 2^{nd} Week	0,00	73,50	131,00
Subject 05 1^{st} Week	68,00	95,00	146,50
Subject 05 2^{nd} Week	72,90	82,50	102,50
Subject 06 1^{st} Week	0,00	92,00	126,00
Subject 06 2^{nd} Week	90,90	104,00	127,00
Subject 07 1^{st} Week	145,90	190,50	296,00
Subject 07 2^{nd} Week	164,80	238,00	329,00
Week 1			
Average	77,96	114,50	155,29
SD	45,34	47,28	68,45
Week 2			
Average	79,07	114,07	162,64
SD	48,45	56,65	81,94

Table 5.13 Nocturnal Analysis Results (75^{th} percentile, 90^{th}percentile, IQR)

Patient	75^{th} percentile	90^{th} percentile	Interquartile Range (IQR)
Subject 01 1^{st} Week	130,00	148,00	39,00
Subject 01 2^{nd} Week	137,00	164,00	34,00
Subject 02 1^{st} Week	162,50	179,10	86,50

Patient	75th percentile	90th percentile	Interquartile Range (IQR)
Subject 02 2nd Week	136,00	149,00	54,00
Subject 03 1st Week	227,00	270,00	52,00
Subject 03 2nd Week	256,00	278,00	140,50
Subject 04 1st Week	131,00	162,10	49,00
Subject 04 2nd Week	174,00	227,10	100,50
Subject 05 1st Week	189,00	214,10	94,00
Subject 05 2nd Week	166,50	326,50	84,00
Subject 06 1st Week	146,00	163,00	54,00
Subject 06 2nd Week	176,00	223,20	72,00
Subject 07 1st Week	339,50	375,10	149,00
Subject 07 2nd Week	366,00	400,00	128,00
Week 1			
Average	189,29	215,91	74,79
SD	74,71	81,58	38,54
Week 2			
Average	201,64	252,54	87,57
SD	82,78	89,38	38,42

As mentioned earlier, the division of measurements into diurnal and nocturnal gave a helping hand in order to analyze the nocturnal measurements and in turn compare them with the corresponding results of diurnal measurements.

More specifically, five patients show their Mean increase during the second week of the protocol while the other two present decreasing rates. The same picture is depicted from the SD results. Five patients had increased SD and the other two had decreased nocturnal SD during the second week of the program. Four patients had %CV increased during the second week, while the other three present decreasing rates in %CV. In terms of percentage under the limit of 70% mg/dl, four individuals had an increase in their percentage in contrast with the first week of the program while two patients had a decrease in their percentage and the last one remains with no glucose values under the limit of 70% mg/dl. During the same week, four patients increase their percentage within the desirable limits while the other three present decreasing rates. Furthermore, in reference to the percentage over the higher limit, one patient practically remains stable, while three present decreasing rates and the other three present increasing rates. In terms of Average hours per day, one patient remains stable with no time (nocturnal) under the limit of 70% mg/dl, three individuals present decreasing rates while, at the same time, the other three patients present increasing rates. Four individuals increase their average hours per day within the desirable limits

while the other three present decreasing rates. Four individuals present increasing rates over the limits of 140 mg/dl while the other three present decreasing rates.

Furthermore, as regards to the Minimum and Maximum recorded glucose values, five patients reduce their minimum glucose values during the second week of the program while the other two increase their minimum glucose values during the same week. On the other hand, one patient remains stable regarding his maximum glucose value recorded, while six patients present decreasing rates and the other three present increasing rates. With regard to the percentiles, four patients present increase rates in the 10^{th} percentile and 25^{th} percentile while the other three present decreasing rates. Five patients present increasing rates in their glucose values in the 50^{th} percentile and 75^{th} percentile, while the remaining two present decreasing rates. Six patients in the 90^{th} percentile present increasing rates in their glucose values, while one individual shows a decreasing rate. In terms of IQR, four individuals present decreasing rates during the second week of the program while the other three increase their glucose values. Finally, the Range between their minimum and maximum recorded glucose values increased in five individuals, while the other two decrease their range.

However, in terms of average measurements, Mean, SD, %CV were increased during the second week of the program. In terms of the percentage under the limit of 70% mg/dl and the percentage within the desirable limits, both were increased, while on the other hand the percentage over the limit of 140 mg/dl decreased. Exactly the same picture was observed in the measurements of Average hours per day. Minimum value was decreased during the second week of the program, while maximum value was increased during the same week. Excluding the 25^{th} percentile, where a decreasing rate is presented, in all the other percentiles increasing rates are presented during the second week of the program. The range between minimum and maximum values has also increased during the second week of the program. In terms of IQR an increasing rate occurs during the second week.

In conclusion, in contrast with the diurnal analysis results, it seems that glucose values tend to increase during the nights of the second week of the protocol. GV seems to be affected in a negative way as well. These results were indicators of the effect that physiological exercise has on GV and glucose fluctuation. Of course, it is

necessary to examine the differences and similarities with the results of diurnal analysis. The following tables represent diurnal glucose analysis results.

Table 5.14 Diurnal Analysis Results (Mean, SD, % CV).

Patient	**Mean**	**SD**	**% CV**
Subject 01 1st Week	115,91	53,81	46,42
Subject 01 2nd Week	99,23	49,10	49,48
Subject 02 1st Week	101,59	46,19	45,46
Subject 02 2nd Week	95,34	46,69	48,97
Subject 03 1st Week	174,09	91,30	52,45
Subject 03 2nd Week	149,17	80,43	53,92
Subject 04 1st Week	140,28	73,93	52,70
Subject 04 2nd Week	94,94	78,99	83,21
Subject 05 1st Week	137,71	78,84	57,25
Subject 05 2nd Week	141,60	92,68	65,45
Subject 06 1st Week	126,14	61,47	48,73
Subject 06 2nd Week	111,28	55,09	49,50
Subject 07 1st Week	155,06	86,56	55,83
Subject 07 2nd Week	177,09	107,32	60,60
Week 1			
Average	135,83	70,30	51,26
SD	24,22	16,94	4,54
Week 2			
Average	124,09	72,90	58,73
SD	32,15	23,24	12,50

As it observed from table 5.14 during the second week of diurnal analysis, 5 individuals present increasing rates in their Mean, 4 individuals present increasing rates in their SD and all of them present increasing rates in their % CV.

Table 5.15 Diurnal Analysis Results (% < 70 mg/dl, % [70 -140] mg/dl, % > 140 mg/dl)

Patient	**% < 70 mg/dl**	**% [70 – 140] mg/dl**	**% > 140 mg/dl**
Subject 01 1st Week	12,83	57,80	29,37
Subject 01 2nd Week	17,86	67,46	14,68
Subject 02 1st Week	16,14	66,80	17,06
Subject 02 2nd Week	18,32	70,11	11,57
Subject 03 1st Week	12,83	17,66	69,51
Subject 03 2nd Week	14,62	28,57	56,81
Subject 04 1st Week	12,17	37,90	49,93
Subject 04 2nd Week	35,71	34,52	29,76

Patient	% < 70 mg/dl	% [70 – 140] mg/dl	% > 140 mg/dl
Subject 05 1st Week	14,62	38,16	47,22
Subject 05 2nd Week	19,44	35,05	45,50
Subject 06 1st Week	13,62	45,90	40,48
Subject 06 2nd Week	14,81	52,45	32,74
Subject 07 1st Week	12,43	33,93	53,64
Subject 07 2nd Week	16,60	19,31	64,09
Week 1			
Average	13,52	42,59	43,89
SD	1,42	16,16	17,04
Week 2			
Average	19,62	43,92	36,45
SD	7,32	19,67	20,05

As it resulted from table 5.15 during the second week of the protocol all of the patients present increasing rates in their percentage under the limit of 70 mg/dl, 4 of them present increasing rates in their percentage within the desired limits of 70 – 140 mg/dl and 6 of them present decreasing rates in their percentage over the limit of 140 mg/dl.

Table 5.16 Diurnal Analysis Results (Average hours per day < 70 mg/dl, Average hours per day [70 – 140] mg/dl, Average hours per day > 140 mg/dl)

Patient	Average hours per day < 70 mg/dl	Average hours per day [70-140] mg/dl	Average hours per day > 140 mg/dl
Subject 01 1st Week	0,10	10,40	5,29
Subject 01 2nd Week	0,71	12,14	2,64
Subject 02 1st Week	0,76	12,02	3,07
Subject 02 2nd Week	1,13	12,62	2,08
Subject 03 1st Week	0,00	3,18	12,51
Subject 03 2nd Week	0,54	5,14	10,23
Subject 04 1st Week	0,06	6,82	8,99
Subject 04 2nd Week	0,26	6,21	5,36
Subject 05 1st Week	0,50	6,87	8,50
Subject 05 2nd Week	1,30	6,31	8,19
Subject 06 1st Week	0,00	8,26	7,29
Subject 06 2nd Week	0,55	9,44	5,89
Subject 07 1st Week	0,08	6,11	9,65
Subject 07 2nd Week	0,79	3,48	11,54
Week 1			
Average	0,21	7,67	7,90
SD	0,30	2,91	3,07
Week 2			
Average	0,75	7,91	6,56

Patient	Average hours per day < 70 mg/dl	Average hours per day [70-140] mg/dl	Average hours per day > 140 mg/dl
SD	0,36	3,54	3,61

As it resulted from table 5.16 during the second week of the protocol, all of them present increasing rates in their spending time under the limit of 70 mg/dl, 4 of them present an increasing rate within the limits of 70 -140 mg/dl, 6 individuals present decreasing rates in their spending time over the limit of 140 mg/dl.

Table 5.17 Diurnal Analysis Results (Maximum, Range)

Patient	Maximum	Range
Subject 01 1st Week	262,00	262,00
Subject 01 2nd Week	233,00	233,00
Subject 02 1st Week	201,00	201,00
Subject 02 2nd Week	235,00	235,00
Subject 03 1st Week	374,00	374,00
Subject 03 2nd Week	367,00	367,00
Subject 04 1st Week	330,00	330,00
Subject 04 2nd Week	311,00	311,00
Subject 05 1st Week	346,00	346,00
Subject 05 2nd Week	400,00	400,00
Subject 06 1st Week	274,00	274,00
Subject 06 2nd Week	246,00	246,00
Subject 07 1st Week	361,00	361,00
Subject 07 2nd Week	400,00	400,00
Week 1		
Average	306,86	306,86
SD	62,99	62,99
Week 2		
Average	313,14	313,14
SD	76,41	76,41

Table 5.18 Diurnal Analysis Results (25th percentile, 50th percentile)

Patient	25th percentile	50th percentile
Subject 01 1st Week	100,00	124,00
Subject 01 2nd Week	83,00	109,00
Subject 02 1st Week	85,00	111,00
Subject 02 2nd Week	76,00	99,00
Subject 03 1st Week	123,00	180,00
Subject 03 2nd Week	101,00	152,00
Subject 04 1st Week	99,00	139,00
Subject 04 2nd Week	0,00	107,00

Patient	25th percentile	50th percentile
Subject 05 1st Week	90,00	135,00
Subject 05 2nd Week	82,00	127,00
Subject 06 1st Week	107,00	129,00
Subject 06 2nd Week	82,50	111,00
Subject 07 1st Week	110,50	145,50
Subject 07 2nd Week	101,50	180,00
Week 1		
Average	102,07	137,64
SD	12,81	21,74
Week 2		
Average	75,14	126,43
SD	34,58	29,43

Table 5.19 Diurnal Analysis Results (75th percentile, 90th percentile, IQR)

Patient	75th percentile	90th percentile	Interquartile Range (IQR)
Subject 01 1st Week	145,50	172,00	45,50
Subject 01 2nd Week	125,00	149,30	42,00
Subject 02 1st Week	130,00	150,00	45,00
Subject 02 2nd Week	122,00	144,00	46,00
Subject 03 1st Week	241,00	290,00	118,00
Subject 03 2nd Week	208,00	246,00	107,00
Subject 04 1st Week	194,00	236,00	95,00
Subject 04 2nd Week	150,50	202,00	150,50
Subject 05 1st Week	190,00	241,00	100,00
Subject 05 2nd Week	193,00	268,30	111,00
Subject 06 1st Week	166,00	198,00	59,00
Subject 06 2nd Week	154,00	175,00	71,50
Subject 07 1st Week	208,00	277,00	97,50
Subject 07 2nd Week	248,50	328,00	147,00
Week 1			
Average	182,07	223,43	80,00
SD	37,99	52,36	29,53
Week 2			
Average	171,57	216,09	96,43
SD	46,70	67,93	44,59

From a different point of view, diurnal analysis results led to significant observations. More specifically, Mean was reduced in five patients while in the other two it was increased. Of course, this increase in one of them wasn't very significant. Mean constitutes the first indication that glucose values are reduced in most of the patients during the second week of the program. On the other hand, Standard Deviation (SD) presents an equivocal behavior. In three patients it presents a

decrease in numbers during the second week, while on other three these numbers are increased in the same week. The remaining individual presents a very insignificant increase which means that it is practically stable. A much clearer picture is produced by the results of Coefficient of Variance % (CV) which were increased in all individuals in the second week of the protocol.

Furthermore, all individuals present their percentage under the lower limit of 70% mg/dl increased. At the same time, their percentage within the target range has a different behavior. Three patients have reduced percentage of values within the target range while the other four increased their percentage of glucose values within the target range. Six patients present a reducing percentage of glucose values over the higher limit of 140 mg/dl while the seventh patient present an increase in his percentage over the high limit. In terms of Average hours per day, the picture is similar. All individuals present an increase in their time under the lower limit of 70% mg/dl during the second week. Four patients present increasing rates in their time within the target range while the other three present a decrease. Six individuals present decreasing rates in their time over the limit of 140 mg/dl while the last one presents increasing rates during the second week.

However, Maximum values were increased in three patients while the other four present reducing values. All individuals present reducing values in 25^{th} percentile, while the six of them guard their rate in 50^{th} percentile during the second week of the program. In 75^{th} percentile and 90^{th} percentile, five patients present reducing rates during the second week while the other two present increasing rates. In terms of Interquartile Range (IQR) two patients present decreasing rates while the other five present increasing rates during the second week. The above results constitute indicators that physical exercise affects GV while at the same time seems to have a behavior of reducing the glucose values.

In conclusion, according to average measurements in all individuals, Mean present a reducing rate during the second week of the program while at the same time during the same week SD and %CV present an increasing rate. The percentage under the lower limit of 70% mg/dl increased as well as the percentage of glucose values within the target range in contrast to the percentage of glucose values over the higher limit of 140 mg/dl, where a reducing rate appears. Exactly the same picture was presented in the measurements of Average hours per day. The average maximum

value was increased during the second week of the protocol. All percentiles that have been presented were reduced significantly. On the other hand IQR present higher values during the second week than in the first week of the program. These values indicate that physiological activity affects glucose fluctuation reducing its values to lower levels.

5.7 GLUCOSE DATA ANALYSIS REPORTS

In order to visualise the glucose data taken from the iPro2 devices, it was considered appropriate to use Matlab as the basic data management software. The subsequent reports present glucose concentration (mg/dl) and how it fluctuates as a function of time during the first week of the protocol, where there wasn't any physical exercise and the same is also presented in regards to the second week where the patient exercised daily.

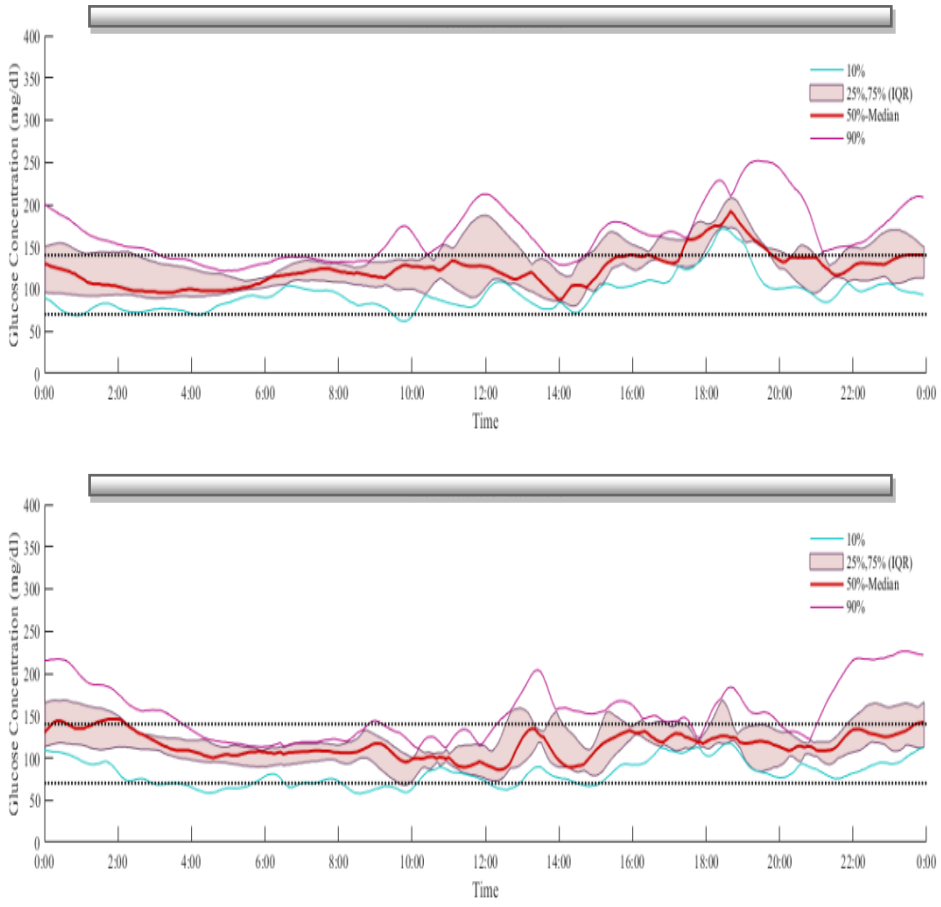


Figure 5-34 Subject 01 Analysis Report

Subject 01 presents a well controlled glycemic profile during the first week of the program. During the second week of the protocol, as seen in the above report, the glucose values present reduction in general. This is likely due to the effect physical exercise. This is feflected from all the above lines especially from the median and IQR. The line of 90% presents high glucose values between 20:00 – 0:00 p.m. The nocturnal analysis results show the same picture during this time space.

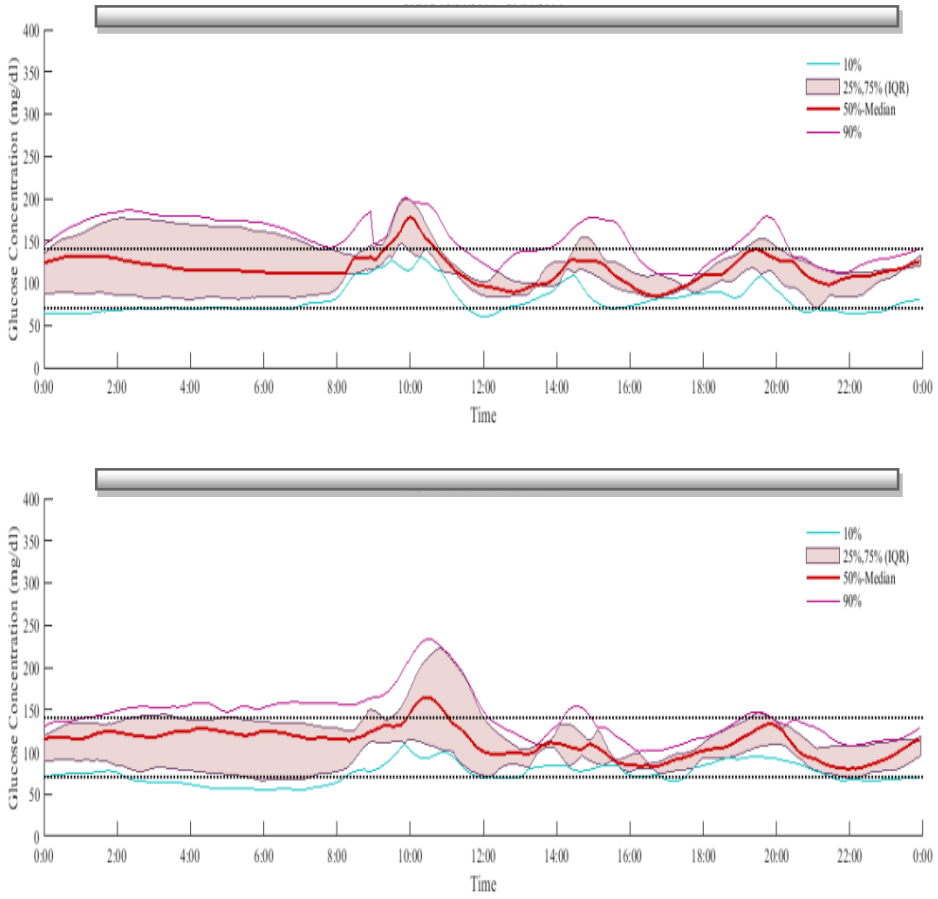


Figure 5-35 Subject 02 Analysis Report

Subject 02 during the first week of the program presents a well control glycemic profile. The glucose values present a few high peaks during 08:00 – 11:00 a.m. As in the case of subject 01 the glucose values of subject 02 show decreasing rates during the second week of the protocol excluding the time space between 10:00 – 11:30 a.m. The most important thing is that the most of the glucose values are limited within the desired limits of [70 – 140] mg/dl. Also the median shows a well normalized curve,

confirming that the physiological activity has an effect on glucose fluctuation, reducing glucose values.

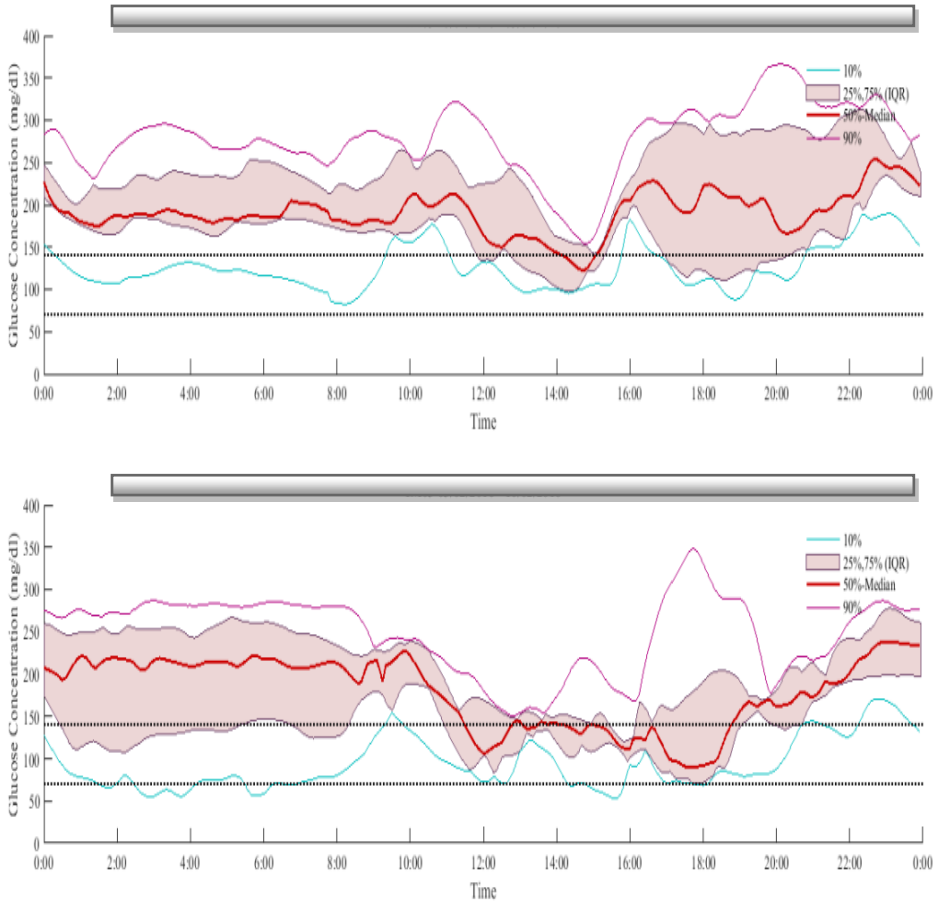


Figure 5-36 Subject 03 Analysis Report

Subject 03 during the first week of the protocol presents a bad glycemic profile with very high glucose values. Subject 03 lies permanently in the hyperglycemic area. Only the 10% percent of the glucose values present a normal picture. During the second week of the protocol physiological exercise's effects decrease the glucose values leading more values within the desired limits of [70 -140] mg/dl. Especially in the space time of 12:00 – 20:00 p.m. the glucose values lie within the target range. Also, as it concluded from the statistical analysis results, during the second week of the protocol the glycemic profile presents better picture than during the first week of the program.

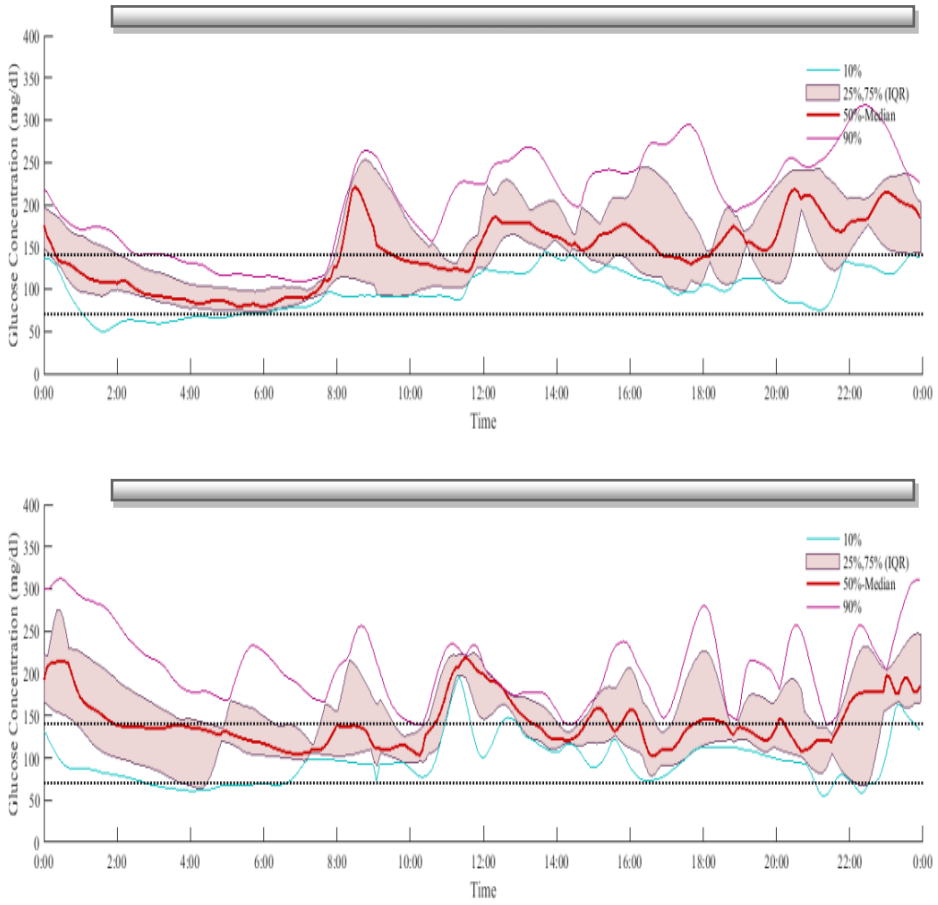


Figure 5-37 Subject 04 Analysis Report

Subject 04 during the first week of the protocol presents a bad glycemic profile with glucose values higher than normal. From 0:00 a.m to 08:00 a.m the glucose values show a normal picture but after 08:00 a.m the glucose values show an increasing rate. During the second week of the protocol the glucose values present a decreasing rate but the high GV still remains. It is worth noting that more glucose values are within the range limits as it resulted from the statistical analysis results too. This is a significant indicator that physical exercise has a relationship with glucose values.

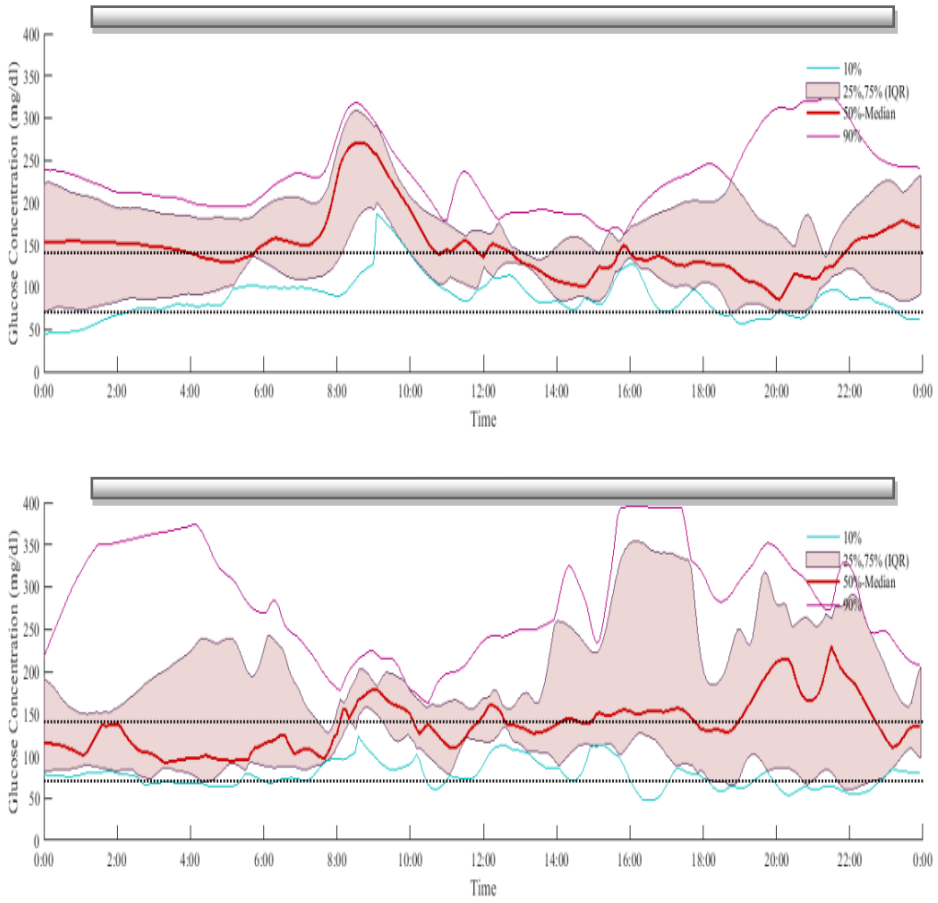


Figure 5-38 Subject 05 Analysis Report

Subject 05 during the first week of the protocol presents a bad glycemic profile in which the majority of glucose values are in the hyperglycemic area. During the second week of the program the bad glycemic profile still remains but the difference is that more glucose values are within the desired limits. The GV still remains high but the median follows a more normalize path in contrast with the first week of the protocol. This is an indicator that physical exercise has a relationship with glucose fluctuation and variability.

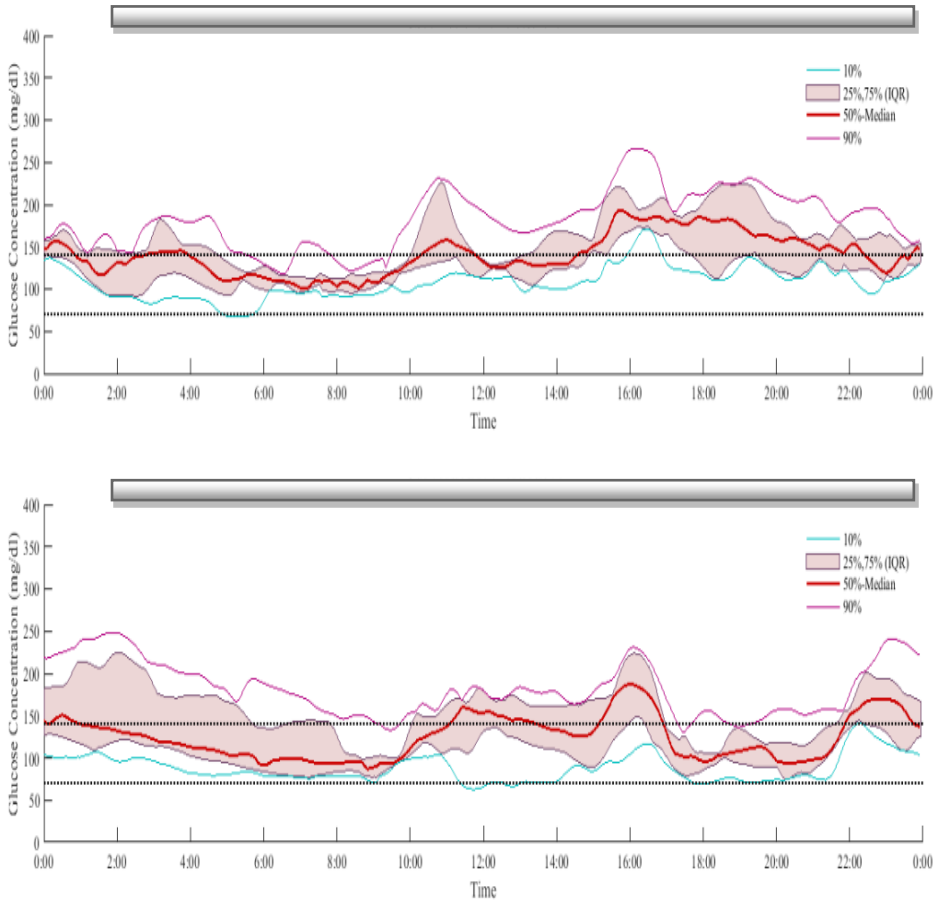


Figure 5-39 Subject 06 Analysis Report

Subject 06 during the first week of the protocol shows a controlled glycemic profile with high glucose values from 11:00 a.m to 12:00 p.m and some peaks during the space time between 16:00 p.m to 20:00 p.m. The median follows a well normalized path but many glucose values still remain in the hyperglycemic area. Subject 06 during the second week of the program still guards the controlled glycemic profile. Also during the second week more glucose values are within the desired range limits. The glucose values present a decreasing rate during the second week indicating that physical exercise has a relationship with the glucose fluctuation and variability.

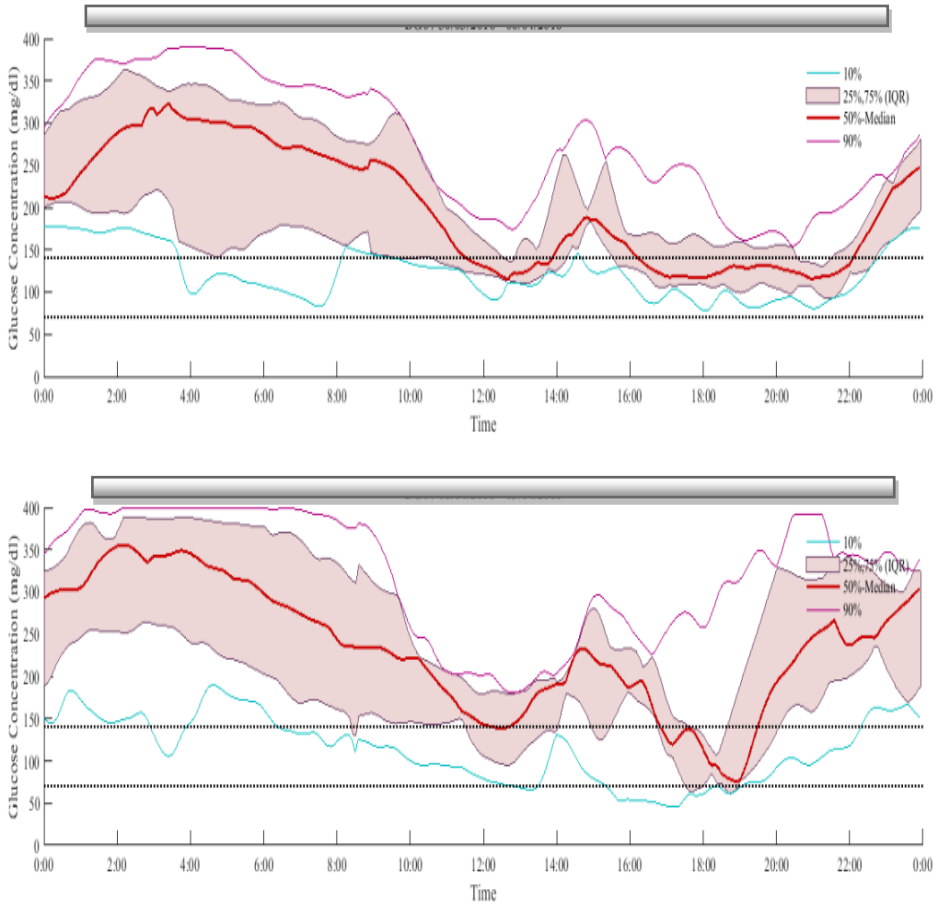


Figure 5-40 Subject 07 Analysis Report

Subject 07 during the first week of the protocol presents glucose values that are nearly permanently in the hyperglycemic area. The majority of the IQR values are high. Subject 07 presents a bad glycemic profile from the beginning of the protocol. During the second week of the program, subject 07 still guards the big percent of glucose values over the desired limits of 140 mg/dl but at the same time presents glucose values within the desired limits. More specifically from 12:00 p.m to 18:00 p.m more glucose values are within the limits of [70 - 140] mg/dl.

As referred to in Chapter 3, all patients are monitored by the iPro2 CGM system. They also refrain from any form of physical exercise, following a steady diet during the first week of the protocol.

During the second week of the protocol, the same diet still exists by adding physical exercise according to the protocol. It is worth noting that consistent adherence to the same diet as in the first week is very important in order to ensure, as

much as possible, a higher quality in the data. In order to draw better conclusions, regarding the association of physical exercise and glucose fluctuation, the following figures represent the total analysis report of all subjects for each week of the protocol.

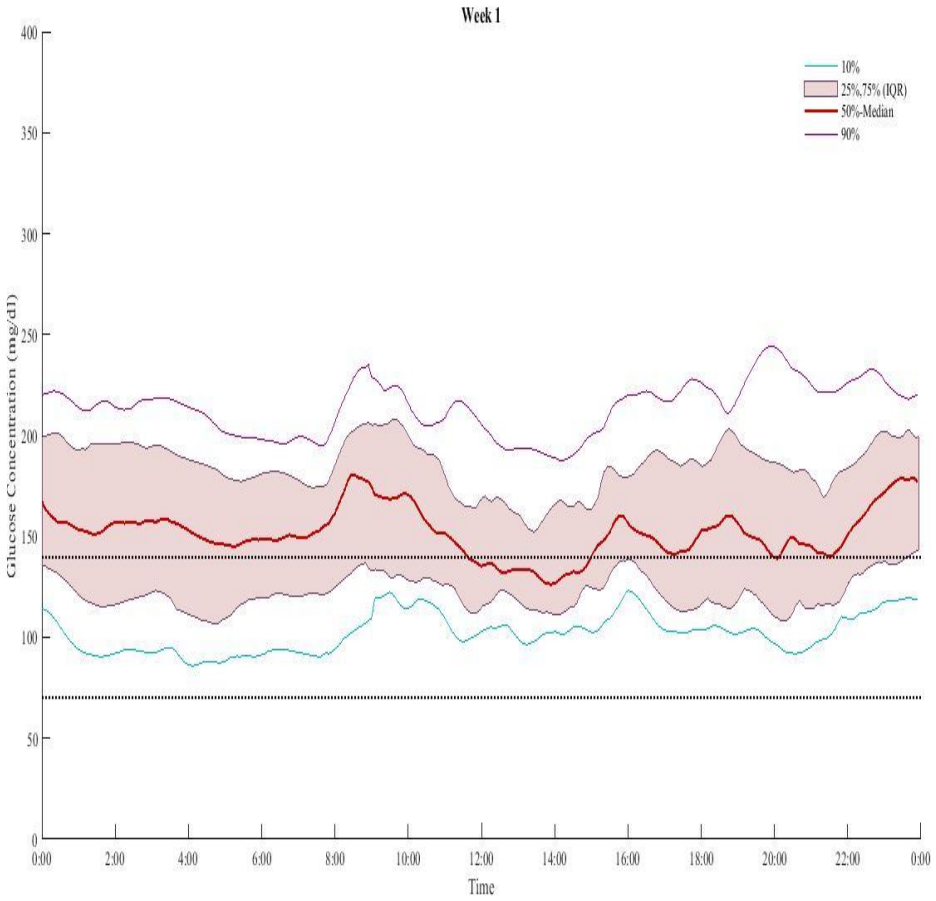


Figure 5-41 First Week Total Analysis Report

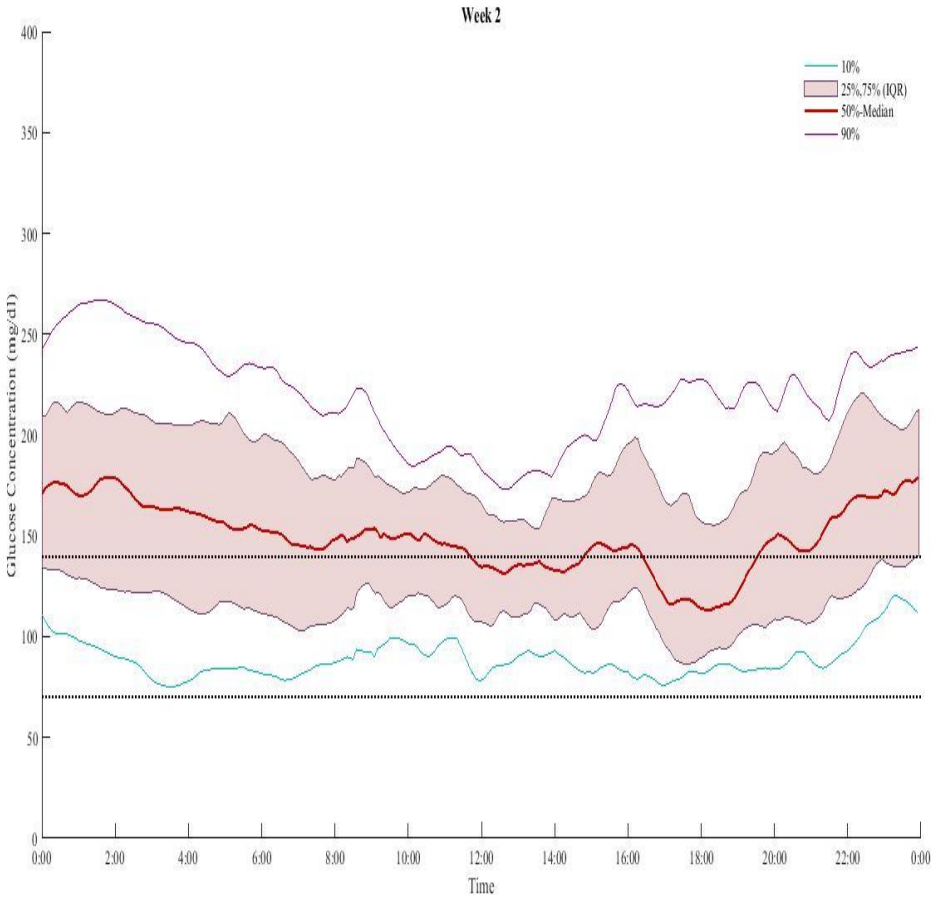


Figure 5-42 Second Week Total Analysis Report

In conclusion, as seen in the above analysis reports, the glucose values during the second week of the protocol present a downward rate in general. This is an indication that physical exercise affects the fluctuation of glucose. As it has been shown in the iPro2 daily overlay reports in the subsections before, patients with consistent hyperglycemic values, during the second week, present more values within the target range and in some cases hypoglycemic values appeared. This is another indication which shows that constant and intense physical exercise may lead to undesired results like more hypoglycemic values.

5.8 CORRELATION ANALYSIS RESULTS

As it referenced in Chapter 4, correlation analysis gave the upper hand in order to achieve the best results regarding the correlation between physical exercise and

glucose concentration. The next tables present both the results of Pearson's correlation coefficient and Spearman's correlation coefficient respectively.

5.8.1 Pearson's Correlation Coefficient Results

Table 5.20 Pearson's correlation analysis results between energy expenditure and glucose concentration.

Patient	**r**	**p-value**	**C**
Subject 01 1st Week	0,0067	0,7790	0,0002
Subject 01 2nd Week	-0,0756	0,0014	-0,0003
Subject 02 1st Week	0,0338	0,1443	0,0010
Subject 02 2nd Week	0,0479	0,0573	1,8334
Subject 03 1st Week	0,0079	0,7326	0,0035
Subject 03 2nd Week	-0,2180	5,6239e - 20	-0,1087
Subject 04 1st Week	0,1872	2,6306e – 16	0,0083
Subject 04 2nd Week	-0,0656	0,0207	-0,0040
Subject 05 1st Week	0,0458	0,0476	0,0019
Subject 05 2nd Week	-0,0398	0,0877	-0,0039
Subject 06 1st Week	0,0773	8,1675e - 14	0,0010
Subject 06 2nd Week	-0,1007	8,1369e – 06	-0,0026
Subject 07 1st Week	-0,2984	2,3116e – 41	-0,0239
Subject 07 2nd Week	-0,3850	6,1926e - 67	-0,0044

Pearson's correlation coefficient is a statistical measure which often finds use in statistical science in order to find the relationship between two variables and in turn how strong this relation is.

Furthermore, in order to find a relation between glucose fluctuation and physiological activity, Pearson's correlation coefficient proved a valuable method. More specifically, excluding subject 02 who presents positive values in both weeks, all the other individuals present negative values during the second week of the protocol. This practically means that the relation between physiological activity and glucose has an opposite behavior. Basically what this first indicator says is that when the physiological activity increases, then glucose values starts to decrease.

However, regarding how strong this relation is, firstly, it's worth noting that high correlation occurs when the values fluctuate from -0,5 to -1 or 0,5 to 1, medium correlation occurs when the values fluctuate from -0,3 to -0,5 or 0,3 to 0,5 and finally low correlation exists from -0,01 to -0,3 or 0,01 to 0,3. The correlation analysis

shows that six subjects fluctuate in the low correlation area while the seventh subject fluctuates in the medium correlation area.

In conclusion, the above results indicate that a relation between physical exercise and glucose fluctuation occurs, but in the majority of individuals the correlation between these two variables is low. Of course, Pearson's correlation coefficient depends strongly on the linearity between the two sets of data, but in the real world the linearity in glucose data might be a 'forbidden' word.

5.8.2 Spearman's correlation coefficient

Spearman's correlation coefficient presented as r_s and Table 5.21 presents the correlation analysis using Spearman's correlation coefficient.

Table 5.21 Spearman's correlation analysis results between energy expenditure and glucose concentration.

Patient	**r_s**	**p-value**	**C**
Subject 01 1st Week	0,0149	0,5310	0,0002
Subject 01 2nd Week	-0,1535	6,5467e - 11	-0,0003
Subject 02 1st Week	-0,0559	0,0157	0,0010
Subject 02 2nd Week	-0,0561	0,0258	1,8334
Subject 03 1st Week	0,0519	0,0240	0,0035
Subject 03 2nd Week	-0,3119	6,4275e - 43	-0,1087
Subject 04 1st Week	0,3518	6,2549e - 56	0,0083
Subject 04 2nd Week	-0,0072	0,7986	-0,0040
Subject 05 1st Week	0,0897	1,0457e - 04	0,0019
Subject 05 2nd Week	0,0650	0,0053	-0,0039
Subject 06 1st Week	0,0969	2,6620e - 05	0,0010
Subject 06 2nd Week	-0,0962	2,0528e - 05	-0,0026
Subject 07 1st Week	-0,3812	2,1486e - 68	-0,0239
Subject 07 2nd Week	-0,4652	9,2223e - 101	-0,0044

Spearman's correlation coefficient is a very useful statistical measure to verify and quantify the relationship between two sets of data. As referred to above, in contrast to Pearson's correlation coefficient which evaluates the linear relationship between two sets of data, Spearman's correlation coefficient evaluates the monotonic relationship between two sets of data. In terms of the degree of correlation, the whole idea is the same with Pearson's correlation coefficient.

Furthermore, in order to find a possible relationship between physiological exercise and glucose fluctuation, Spearman's correlation coefficient was proved to be very useful. More specifically, excluding subject 05, who presents positive values in both weeks, all the other individuals present negative values during the second week of the program. Subject 07 and subject 03, during the second week of the protocol, fluctuate in the medium correlation area between -0,3 to -0,5. All the other subjects fluctuate in the low correlation area, between -0,01 to -0,3. Only subject 05 presents positive values in the second week of the program, fluctuating in the low correlation area between 0,01 to 0,3.

In conclusion, as seen in the results, two subjects present medium correlation and five subjects present low correlation. The above results indicate that physical exercise has a correlation with glucose fluctuation. More specifically, when physiological activity tends to increase, glucose values tend to decrease.

6 CONCLUSIONS

The upper goal of this research is to extract conclusions regarding the relationship between glucose concentration and physical exercise. The effect of physical exercise in glucose fluctuation and glycemic variability (GV) plays a cardinal role in diabetes mellitus management and treatment.

During this thesis, diabetic patients undergo a measurement procedure in order to observe their glucose fluctuation and variability with or without the presence of physical exercise. In the first week of the protocol, all individuals must refrain from any form of physical exercise. During the second week of the program, the same individuals must perform moderate daily physical exercise. Furthermore, during the two weeks of the program, the patients must follow the same diet. The desired limits of target in range were determined in [70 – 140] mg/dl. All individuals suffered from type 1 diabetes mellitus. Advanced monitoring devices were used in order to accumulate valuable data for our purpose.

Furthermore, the received data were analyzed and visualized in tables and graphs, respectively. Firstly, the received data undergoes a statistical analysis for each individual in detail drawing conclusions about glucose fluctuation and GV for each week of the program. Secondly, the received data undergoes a correlation analysis in order to find a correlation between physical exercise and glucose fluctuation. Two types of correlation analysis were performed. The first type of correlation analysis concerns Pearson's correlation coefficient which depends on the linearity of the data set. The second type of correlation analysis concerns Spearman's correlation coefficient which doesn't depend on the linearity of the data set but evaluates the monotonic relationship between the data sets. Finally, the statistical and correlation analysis results can find use in prediction models.

Nevertheless, in order to draw more accurate conclusions, regarding glucose behavior, the data set was divided into nocturnal and diurnal measurements. Subsequently, analysis of the whole data set was performed and in turn commented on. It was considered appropriate, at the end of every analysis subsection, to comment on the analysis results, in order to find common or different points. In addition, concluding this thesis, it is worth commenting on all the results that were presented,

associated with the final conclusions about the relationship between physical exercise and glucose fluctuation.

More specifically, as mentioned above, the received data undergoes a statistical analysis, concerning measures of glycemic variability. Measures like Mean, SD, and % CV as it was proven, were significant indicators, concerning the effect of physical exercise with GV. In general, from the statistical analysis, it was concluded that during the second week of the program, the glucose values tend to decrease. From the GV point of view, the physiological activity seems to have an impact on GV, tending to increase the range between glucose values. The majority of individuals show more glucose values within the range limits of 70 – 140 mg/dl and more glucose values under the limits of 70 mg/dl. Furthermore, another significant indicator is that the average time per day within the range limits and under the lower limit of 70 mg/dl, has increased. During statistical analysis, the Mean presents a decrease in the majority of the patients during the second week of the program, while at the same time both %CV and SD presents an increase in most of the subjects. IQR also presents an increase in most individuals. These measures are indications that physical exercise has an effect on glycemic variability and glucose fluctuation.

From the above results, it is concluded that while glucose values tend to decrease, at the same time the GV tends to increase the range between glucose values. Some might say that this is a paradox, but it is wise to consider that the diabetic body undergoes a shock during the second week of the program due to physical exercise. The human body is made to be adjusted, depending on its surroundings and on every change in it. So, the individuals need more time for their bodies to adapt to the effects that physical exercise has on them. Additionally, the possible differences in the analysis results between individuals occur, due to the fact that each patient's body responds differently to physical exercise, therefore having a different effect on them.

Of course, beyond statistical analysis, the correlation analysis results present some significant indicators too, leading to conclusions regarding the relationship between physical exercise and glucose concentration.

Moreover, correlation analysis gave a helping hand in order to draw more accurate conclusions about the purpose of this thesis. Two types of correlation analysis were performed; the first concerns Pearson’s correlation coefficient and the second concerns Spearman’s correlation coefficient which gave significant indicators.

Energy expenditure and glucose concentration were the two variables that were considered throughout Spearman's and Pearson's correlation analysis.

As it presented in Chapter 5, excluding subject 02 who shows a positive sign, all the other individuals during Pearson's correlation analysis show a negative sign in the second week of the protocol. This is due to the fact that energy expenditure and glucose concentration are correlated in opposite directions. As mentioned before, energy expenditure is high correlated with physical exercise. More specifically, when physical exercise is more vigorous, the energy expenditure is greater. So when energy expenditure presents high values, then glucose concentration presents a decreasing rate. As it was presented before, most of the subjects in Pearson's correlation analysis, results in the low correlation are between -0,01 and -0,3 excluding one subject who lies in the medium correlation area between -0,3 and -0,5. Of course, it is worth noting that Pearson's correlation coefficient is a measure of linearity, so it would be wise to have a deeper look at the relationship between physical exercise and glucose concentration using Spearman's correlation coefficient.

Furthermore, it is worth noting that Spearman's correlation coefficient doesn't depend on the linearity of the data set like in the case of Pearson's correlation coefficient. Moreover, in the case of Spearman's correlation coefficient the two variables that were considered appropriate to use were energy expenditure and glucose concentration. Excluding subject 05, who presents a positive sign, all the other subjects present a negative sign during the second week of the protocol. Although, as concluded from the analysis results, two subjects were in the medium correlation area, but in general the subjects fluctuate in the low correlation area.

However, diabetes mellitus consists of many mysteries which require discovering and in turn solving. As concluded, physical exercise affects glucose fluctuation, tending to decrease glucose values when physical exercise increases. Of course, it is worth noting that the level of exercise was moderate and the effects of intense and vigorous exercise are parameters which should be studied further. Further study and research, including more individuals, will give more results about the relationship between glucose fluctuation and physical exercise.

In conclusion, diabetes mellitus is a chronic metabolic disorder which affects millions of people globally. For many years now, studies and researches have been trying to find solutions in order to manage and treat this chronic disease. These

results can also be used in prediction models. Of course, it is worth noting that several factors can potentially affect the final results including the individual's commitment to diet, glycemic control and statistical error of the glucose monitoring devices. Finally, this thesis can potentially be the kick–off for a deeper and more complete look at diabetes mellitus behavior with or without the presence of physical exercise.

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APPENDIX

In order to accomplish the present thesis and obtain permission to carry out the measurement procedure of the clinical protocol which was presented in chapter 3, the Ethics Committee of the University Hospital of Ioannina had to read and validate the licence application associated with the clinical protocol. The Ethics Committee in turn gave us the green light to start the measurements procedure on diabetic patients. This clinical protocol was written from Evangelos Rizos, Evangelia Ntzani and Dimitrios Fotiadis. The original form of the clinical protocol is presented below:

PHYSICAL ACTIVITY AND HEART RATE MONITORING ASSOCIATIONS WITH GLUCOSE VARIABILITY IN TYPE 1 DIABETIC ADULTS

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Introduction

Continuous glucose monitoring (CGM) is a useful tool for assessing glucose variability in diabetic patients. Its major use is in patients with type 1 diabetes mellitus (DM), often combined with insulin infusion pump [1]. CGM provides in-depth information on glucose excursions and helps to calibrate the rate and correction of insulin infusion through the pump by the patient or the physician. CGM is also an essential part of some insulin pumps, guided by the real-time data of glucose levels in cases of hypoglycemia (sensor augmented pumps). Beyond type 1 DM, CGM is continuously used for women with diabetes during pregnancy, and also in type 2 diabetics with poor glucose control, profound glucose excursions, and frequent or unexplained hypoglycemia. The road to the eagerly anticipated closed-loop devices is necessarily through the incorporation of CGM application. Based on the information provided by CGM, dietary habits and lifestyle of the diabetic patients, physicians usually modify insulin treatment and counsel for certain lifestyle and dietary modifications.

One of the major determinants for glucose variability beyond the dietary habits is physical activity. The intensity and type of physical activity are crucial factors affecting blood glucose regulation. Normally in healthy people the control of glucose homeostasis during exercise is dictated by a complex interaction between numerous factors including hormones, the nervous system, and various molecular regulators within the skeletal muscles and the liver. In contrast, the control of glucose homeostasis during exercise is extremely challenging for insulin-treated patients as insulin levels cannot change rapidly and appropriately in response to exercise [2-4]. During prolonged moderate-intensity aerobic exercise the failure of insulin levels to decrease at the onset of exercise causes a reduction in glucose concentration [5], whereas during anaerobic exercise there could be an increase in blood glucose levels due to catecholamine elevation which are not offset by the increase in insulin availability [4,6]. This exercise-induced hyperglycemia may last for minutes to hours after the end of the activity and can compromise the overall glycemic control and the subsequent exercise performance. In reality, many sporting activities are a combination of both aerobic and anaerobic patterns [7]. Following physical activity and independently of the exercise pattern, the insulin-treated diabetics are prone to late-onset post-exercise hypoglycemia appearing or lasting for several hours

following the end of activity [2, 8, 9]. Exercise also masks some of the symptoms of hypoglycemia such as sweating, dizziness and tiredness. As a result, diabetic subjects are frequently unable to sense hypoglycemia during the exercise or post-exercise period.

Existing tools assessing physical activity are questionnaires as well as objective monitoring devices. Questionnaires are cheap and easy to administer, but suffer from recall bias, floor effects (the lowest response category is too high for many respondents) and are unreliable for walking, one of the most predominant activities in adults [10-12]. Motion sensors (pedometers and accelerometers) are the most popular and well validated devices that provide objective physical activity measurements. Pedometers are cheap and measure step count but not intensity; therefore they cannot distinguish between walking speeds or types of exercise when the legs are not practiced [2,13]. Accelerometers measure the body's acceleration in one or more directions continuously for long periods and some of them can also record step counts. The magnitude and the intensity of the acceleration provide an output, the activity counts per unit of time, which is used to distinguish between different walking speeds and intensity levels. Another way to estimate total energy expenditure is heart rate monitoring. In general, there is no difference when heart rate monitoring was compared with doubly labelled water (the gold standard for the assessment of total energy expenditure), although the individual differences were large as shown by the standard deviation (SD) of the mean, ranging from -17% [14,15] to 52% [14,16]. Heart rate monitoring is also applied as an indicator of activity intensity [17] and in combination with body movement as a measure of physical fitness [18, 19]. Heart rate monitoring is an objective method affected by more factors than physical activity. Thus it could be a useful indicator for the activity of sympathetic nervous system, which also regulates glucose levels [14].

Insulin degludec is currently the basal insulin with the longest half-life and has reported to decrease the glucose variability throughout all day compared to insulin glargine, the most widely used basal insulin. Adult patients with type 2 DM on basal insulin have attained a certain way of life including more stable dietary habits, as well as restricted and specific physical activity patterns. They represent an ideal group to see the effect of physical activity on glucose variability depending on the basal insulin they use, as well to test the performance of the various types for physical activity measurement.

Currently, there are few reports on the effect of physical activity on glucose excursions assessed by CGM with various results depending on the type of exercise, the age of the population studied and the type of diabetes [20, 21]. Although the existing evidence suggests that CGM could reduce the glycated haemoglobin (HbA1c) in type 1 diabetic adults [22, 23] and may lower the number and time spending during hypoglycemia in type 1 diabetic children, there is a gap of knowledge regarding the effect of physical activity and the type of monitoring devices, as well as what parameters of these devices correlate best with glucose variability. This kind of information would be precious for the creation of algorithms applied to technically supported insulin infusion systems for type 1 diabetics in order to attain better glucose control and avoid hypoglycemia. Thus, the step to the closed-loop system would come even closer.

We aim to objectively measure the effect of physical activity on glucose excursions assessed by CGM and to examine what data correlate best with glucose variability in type 1 diabetic adults. Moreover, we tried to find the role of heart rate measurement not only as an indicator of physical activity [24], but also as a surrogate marker of sympathetic nervous system activation, which is a key-player for glucose homeostasis in these patients.

Patients and Methods

Study design and participants

The study was performed in Caucasian Greek adults with type 1 DM aged 18-65 years. All patients did not suffer from a concomitant medical illness that would limit in any way their physical activity. Eligible subjects were counselled to avoid swimming (due to technical failure) and record the time and duration of specific activities (e.g. road biking, elliptical, stair climbing, incline walking). Eligible subjects were instructed to wear the CGM system and simultaneously the devices for physical activity and heart rate monitoring for 7 consecutive days. During that period all subjects were obliged to abstain from any structured exercise program (only walking with their usual pace was permitted). Then all subjects had to continue the CGM and their physical activity - heart rate monitoring for 7 consecutive days but to perform their usual type of exercise once daily. If any of the individuals did not already perform a certain exercise in his everyday life, he/she was instructed to walk

for more than 30 minutes with a walking speed of more than 5 km/hour once daily. The follow up for each participant will stop 14 days later.

Pregnant women, patients with previous stroke, pulmonary disease, peripheral arterial disease, any condition related to movement disability, renal (serum creatinine levels greater than 1.5 mg/dl) or liver disease (transaminase levels greater than threefold the upper normal values) were excluded from the study. Diabetics on exclusively anaerobic exercise programs were also excluded from the study.

All male subjects were counselled to eat 1600 calories daily and all women 1400 calories daily of a predefined choice of foods for the first 7 days. Then they had to repeat the same dietary pattern for the next 7days. The participants had to record their food intake, the total calories and the carbohydrate content (in grams) of each meal. Information on insulin regime (i.e. type of insulin, injection dosage and time) was also recorded on a daily basis using a specially designed paper diary.

Monitoring devices

We used the Medtronic iPro2, a CGM system approved both for the US and the European market. It allows collection of data in a blinded way in order to better evaluate the patient treatment [25]. This system does not require calibration during its use; instead the calibration data are entered retrospectively and thus allowing elimination of the bias from patient interaction. The results can be stored and reviewed later by the health care professional. The patient provides the list of glucose measurements, the meals and exercise time. This system claims better accuracy with 11% mean absolute relative difference compared to 16% from the other CGM device by the same Company, the Guardian RT [25-28]. The CGM sensor is calibrated when blood (and interstitial) glucose are in a steady state (glucose levels changing less than 2 mg/dl/min) [29].

The enhanced BodyMedia SenseWear System was used to assess the physical activity of the participants, which includes the SenseWear Armband, the SenseWear Software and, optionally, the SenseWear Display device [30]. The SenseWear Armband collects physiological data at a rate of 32 times/second from the following sensors: (i) a 3-axis accelerometer which measures motion and steps taken, (ii) a galvanic skin response (GSR) sensor which measures the electrical conductivity of the skin (iv) a sensitive electronic thermometer which measures the surface temperature

of the body, and (v) a heat flux sensor which measures the rate at which heat is dissipating from the body. The SenseWear Software records and analyses physiological parameters, and uses algorithms to report daily movement, calories burned degree of physical activity, and steps taken. Participants were instructed to wear the device on the back of the upper arm on their non-dominant hand, while awake, and to remove it for water activities such as swimming. The system's output was initialized and downloaded using the SenseWear Software, including raw data from sensors as well as comprehensive reports on energy expenditure, MET levels, number of steps and total distance, physical activity levels and duration (including sedentary, light, moderate, vigorous, and very vigorous levels), sleep duration and efficiency, lying down time and On/Off body time. Data were included if the subject had accumulated a minimum of 10 hours of valid activity recordings per day for the whole study duration (14 days) [31,32].

Participants wore also either the Fitbit Flex or the Withings Pulse wearable activity trackers [33, 34]. An integrated 3-axis accelerometer allows both devices to track movement 24 hours a day with a sampling rate of 1 minute. The recorded data consists of steps taken, calories burned, distance travelled and active minutes. Withings Pulse has also an altimeter which allows it to determine the daily active elevation gain, whereas it uses an optoelectronic sensor and a LED to measure the instant heart rate and blood oxygen level (SpO2). Fitbit flex is typically worn on the wrist like a hand watch, whereas Withings Pulse is usually attached to the belt or apparel using a clip. Both devices sync automatically and wirelessly to leading smartphones and computers using low consumption and short range communication Bluetooth wireless technology.

Heart rate monitoring was performed with the Empatica E4 Wristband, a wearable wireless device designed for continuous, real-time data acquisition in daily life [35]. The E4 Wristband has four embedded sensors: (i) a photoplethysmography sensor which measures blood volume pulse (BVP) from which heart rate (HR), heart rate variability and other cardiovascular features may be derived, (ii) a 3-axis accelerometer which captures motion-based activity, (iii) an electrodermal activity (EDA) sensor (GSR sensor) which measures sympathetic nervous system arousal to derive features related to stress, engagement and excitement, and (iv) an infrared thermopile which reads peripheral skin temperature. The E3's custom PPG sensor illuminates the skin and measures the light reflected, where each cardiac cycle

appears as a peak of light absorption. Moreover, through a proprietary artifact removal technique, which is based on a combination of multiple wavelengths, E3 attenuates noise even when there are no repetitive movements that affect the sensor. The data output consists of four time series i.e. EDA (also known as GSR), BVP, acceleration, HR, and temperature. The data recorded are seamlessly uploaded in Empatica's secure cloud platform.

Physical Activity Logs were completed with the participants recorded their activities at the end of each day. A list of activities was provided for the participant on the daily log sheet and the broad categories included: household activities, lawn/garden activities, volunteer/occupational, care of others, transportation, walking, dancing, sports, conditioning, and inactivity. Participants had to record how long each activity was performed. MET values were assigned to each activity [36] and multiplied by the number of minutes each activity performed resulting in MET/min. All physical activity logs were checked by the researcher and reviewed with each participant to ensure completeness. When there are obvious discrepancies between the three objective monitoring devices of physical activity, then we have to check the accordance of their outcome with the report of the questionnaires.

Outcomes

The primary outcome will be the correlation of physical activity data with glucose variability recorded by the CGM. A co-primary outcome will be the difference of glucose variability between the first 7 days when structured physical activity is prohibited compared to the last 7 days when structured physical activity is performed. Secondary outcomes will be the associations of various parameters assessing glycemic variability with different variables of physical activity/heart rate monitoring.

Glycemic variability will be assessed by the following metrics: (i) the coefficient of variation (CV, which represents the SD divided by the mean glucose from the CGM expressed as a percentage), (ii) the mean amplitude glucose excursion (MAGE), (iii) the continuous overlapping net glycemic action (CONGA) between a time difference of 4 hours (CONGA4), and (iv) the area under the curve (AUC) for the time spending in hypo- and hyper- glycemia states [37-39]. MAGE, probably the most popular index for CGM applications, has been designed to take into account the

glycemic peaks and nadirs encountered during a day, beyond average glucose values. MAGE is estimated by the formula:

$$MAGE = \sum \frac{\lambda}{x} \text{ if } \lambda > y,$$

where λ is the difference from peak to nadir, x is the number of valid observations, and y is 1 SD of mean glucose in a 24-h period. MAGE generally considers more heavily the major variations of glucose levels compared to minor ones. Only variations exceeding 1 SD of the average glycemic value during the observation period are considered [37-39]. CONGA has been designed as a tool for the analysis of CGM data and is widely considered as the most appropriate index to assess glycemic variability from CGM data. Contrary to the other indexes targeting the interday glucose variability, CONGA illustrates the intraday variability. For each observation after the first n hours of observations, the difference between the current observation and the observation n hours prior is calculated. CONGAn is defined as the SD of the differences. Mathematically, CONGAn can be described by the formula:

$$CONGAn = \sqrt{\sum_{t=t_1}^{t_k} \frac{\left(D_t - \bar{D}\right)^2}{k-1}},$$

where $D_t = GR_t - GR_{t-m}$, $\bar{D} = \sum_{t=t_1}^{t_k} D_t \Big/ k$, k is the number of observations in which there is an observation $n \times 60$ min ago, GR_t is glucose reading at time t in minutes after start of observations, t_i is time in minutes after start of ith observation [37-39]. The time difference of 4 hours was chosen as a relevant duration for assessing glucose variability during resting conditions as well as following exercise. For the AUC, we defined hhypoglycemia as glucose levels below 70 mg/dL and hyperglycemia as glucose levels above 180 mg/dL. AUC is defined as the absolute distance from the described limits, multiplied by the time spent outside those limits.

The physical activity levels assessed by the accelerometer recordings are presented as 1) mean axis acceleration units (called counts) per minute (cpm), 2) number of minutes spent in intensity-specific categories, 3) physical activity energy expenditure (MET-minutes), 4) number of steps registered per day [40]. Mean cpm is

a measure of overall physical activity and is expressed as the total number of registered counts for all valid days divided by wearing time in that day across all valid days. Count thresholds corresponding to the level of physical activity were as follows: sedentary activity (e.g. sitting, reclining, lying down) is defined as all activity below 100 cpm, low-intensity physical activity as 100 – 759 cpm, lifestyle activity (e.g. slow walking, grocery shopping, laundry, vacuuming, sweeping, child care) as 760 – 2019 cpm, and moderate to vigorous physical activity (MVPA) [equivalent to an energy expenditure of ≥ 3 METs such as gardening, walking at speeds of ≥ 78 m/min (4.7 km/h)] is defined as all activity ≥ 2020 cpm [5998 cpm (≥ 6 METs) discriminates moderate vs. vigorous physical activity]. The numbers of minutes per day at different intensities were determined by summing all minutes where the count met the criterion for that intensity, divided by the number of valid days. The number of steps per day was registered as number of cycles of the signal, which is claimed to be representative of the number of steps taken [41].The number of steps taken will be also and more accurately assessed from the activity trackers. We will use the number of steps recorded daily as well as during the exercise periods.

Heart rate (HR) is not a very good predictor of low to lifestyle physical activity (LLPA), but correlates well with MVPA. A typical method of individual calibration is the ‘‘flex method’’ whereby the flex HR is identified as HR obtained during sitting or standing. Under free-living conditions, if an individual’s HR falls below the flex HR, they are credited with 1.0 MET. Above the flex HR, the energy expenditure is estimated from a linear HR-to-energy expenditure relationship [18]. We will use the percentage of HR increase above the flex HR daily as well as during the exercise periods.

Statistical analysis

Power calculations could not be performed due to the lack of information from previous studies on the potential correlation estimates. We thus chose to include 30 patients in order to capture successfully physical activity and glycemic control variations. The primary analysis will be the correlation of AUC for hypoglycemia (AUC_{70}), hyperglycemia (AUC_{180}), and normoglycemia ($AUC_{70\text{-}180}$) with physical activity energy expenditure (MET-min) during the days when physical activity is performed. Any correlation will be tested during the exercise period, as well as during

a 6-hour post-exercise period. A co-primary end point will be the difference of 24-hour AUC for hypo-, hyper-, or normo- glycemia between the days when structured exercise is encouraged compared to the days when structured exercise is forbidden.

For secondary analyses we will assess any correlation during the exercise period, as well as during a 6-hour post-exercise period, from the CGM output: 1) AUC for hypo- (AUC_{70}), hyper- (AUC_{180}), or normo- glycemia (AUC_{70-180}), 2) CV, 3) MAGE and 4) CONGA4 with 1) cpm, 2) number of minutes spent in LLPA or MVPA, 3) physical activity energy expenditure (MET-minutes), or 4) number of steps; or with 1) the percentage of increase HR above the flex HR (average daily living HR during rest) measured by the heart rate monitor.

Moreover, we will evaluate if there is any difference between any associations from the CGM output with physical activity/heart rate monitoring when the structured physical activity period will be compared to the non-structured physical activity period.

The comparison for AUC will be mean (± SD). AUCs were calculated with the trapezoidal rule for the glucose levels on a minute-to-minute basis for total AUC. AUCs were compared between the groups with related samples Wilcoxon signed rank test. We will examine the associations of physical activity with blood glucose variability using linear regression models (Pearson correlation). Descriptive statistics will be reported as means and standard deviations. Normality of distribution will be tested by calculating skew and kurtosis values. Continuous variables were compared using Student's t test or the Wilcoxon test or analysis of variance, as appropriate. Correlations will be analysed in order to investigate the relationship between physical activity parameters and the glucose variability indicators. Pearson's correlation coefficient will be computed and a univariate analysis will be conducted using linear regression to assess independent predictors among demographic and clinical characteristics. On the basis of the outcome of the univariate analysis, a multivariate regression analysis will be performed to assess independent predictors of glucose variability and physical activity indicators. Models will be initially adjusted for the potential confounders of age (years), sex, height (cm), body mass index (BMI), waist circumference (cm). Statistical significance will be assumed for $p<0.05$. The statistical data analysis will be conducted with Stata/SE 11.0 software for Windows (StataCorp LP, Texas, USA).

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