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Estimation of insulin sensitivity from continuous glucose monitoring and insulin pump data in type 1 diabetes

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Abstract

Diabetes mellitus is a disease characterized by chronic hyperglycemia either due to a lack of secretion of the hormone insulin (Type 1 Diabetes Mellitus, T1DM) or due to impaired action of this hormone (Type 2 Diabetes Mellitus, T2DM). Due to its short- and long-term complications, it is currently one of the major health problems of the economically developed countries and at the same time, one of the first items of healthcare spending. Diabetic patients therefore need regular blood glucose monitoring associated with adequate insulin therapy whose goal is to keep glucose concentration within the normal safe range (70 ÷ 180 mg/dl), trying to limit excursions in hypoglycemic (20 ÷ 70 mg/dl), due to short-term complications, and hyperglycemic range (180 ÷ 600 mg/dl), due to long-term complications. In order to optimize insulin therapy, and then assess the correct amount of insulin to be administered to the patient, it is necessary to know its insulin sensitivity (SI), i.e. the ability of insulin to stimulate glucose utilization and inhibit its production, specific for each individual and changing during the day.

The aim of this thesis is to estimate an index of insulin sensitivity in patients with type 1 diabetes by using a recently proposed technique which exploits minimally invasive technologies used by diabetic patients for control therapy. This parameter will be estimated in correspondence of meals over the whole day and, in order to be able to estimate the index of insulin sensitivity even in the presence of meals close, a tool for the estimation of carbohydrates absorbed during the meal (Carbohydrates On Board, COB) will be proposed.

In Chapter 1 the glucose-insulin regulatory system, diabetes and its complications, conventional therapy for its control and indices of insulin sensitivity in literature are introduced.

In Chapter 2 the experimental protocols applied to the patients and the data available data are presented.

In Chapter 3 the recently proposed method for the estimation of SI using minimally invasive technologies and the COB function for the estimation of carbohydrates absorbed during the meal are presented.
In Chapter 4 the estimates of insulin sensitivity in different datasets with and without the COB function, which in turn was developed using simulated and real data, are presented.
# Table of Contents

1. Introduction .................................................................................................................. 3
   1.1. The glucose-insulin regulatory system ................................................................. 3
   1.2. Diabetes .................................................................................................................. 5
       1.2.1. Type 1 diabetes ............................................................................................. 5
       1.2.2. Type 2 diabetes ............................................................................................. 6
       1.2.3. Complications ............................................................................................... 6
   1.3 Control of Diabetes ................................................................................................. 7
       1.3.1. Continuous Glucose Monitoring Systems ..................................................... 8
       1.3.2. Subcutaneous Insulin Infusion Pumps .......................................................... 10
   1.4. Insulin sensitivity indices: state of the art ........................................................... 11
       1.4.1. Hyperinsulinemic-euglycemic clamp ............................................................. 12
       1.4.2. Intravenous glucose tolerance test minimal model ....................................... 13
       1.4.3. Other methods ............................................................................................. 15
   1.5. Objective ............................................................................................................... 17

2. Data base and protocols ............................................................................................. 19
   2.1. Data base 1 ........................................................................................................... 19
       2.1.1. Subjects ......................................................................................................... 19
       2.1.2. Protocol ......................................................................................................... 20
   2.2 Data base 2 ........................................................................................................... 22
       2.2.1. Subjects ......................................................................................................... 22
       2.2.2. Protocol ......................................................................................................... 23

3. Methods ..................................................................................................................... 27
   3.1. A new index of insulin sensitivity from minimally invasive technologies .......... 27
   3.2. Estimation of carbohydrates on board: COB ....................................................... 28
   3.3. Incorporation of COB in insulin sensitivity estimates .......................................... 30

4. Results ....................................................................................................................... 33
   4.1. COB from simulated data ..................................................................................... 33
   4.2. COB from real data .............................................................................................. 36
   4.3. Insulin sensitivity ................................................................................................. 41
       4.3.1. Data base 1 .................................................................................................... 43
       4.3.2. Data base 2 .................................................................................................... 44
4.4. Insulin sensitivity with COB ................................................................. 45
  4.4.1. Data base 1 .................................................................................... 47
  4.4.2. Data base 2 .................................................................................... 47
4.5. Comparison .......................................................................................... 49
4.6 Assessment of insulin sensitivity daily variability .................................. 53
5. Conclusions ............................................................................................. 55
References .................................................................................................. 57
To my parents

Ai miei genitori
1. Introduction

1.1. The glucose-insulin regulatory system

The study of glucose metabolism is fundamental both from a physiological, because glucose is the main source of energy for the whole body cells, and from a pathological point of view, because a malfunction of this system would lead to phenomena of glucose intolerance or, in the worst case, to diabetes.

The concentration of glucose in healthy subjects is tightly regulated by a complex neuro-hormonal control system. Insulin, which is secreted by the β-cells of the pancreas, is the primary regulator of glucose homeostasis, by promoting its use by tissues and inhibiting its endogenous production. On the other side, hormones such as glucagon, epinephrine, cortisol, and growth hormone play the role, on different time scales, to prevent hypoglycemia.

Glucose is generally absorbed by the gastro-intestinal tract through food digestion after a meal or, in fasting condition, it is provided primarily by the liver. It is distributed and used in the whole body and, based on the specific needs and roles in its regulation, we can classify tissues and organs (Fig. 1.1):

![Figure 1.1 The glucose-insulin control system, [1].](image-url)
- **Insulin-independent**: central nervous system and erythrocytes. Glucose is the substrate of choice and its extraction takes place at a constant speed, regardless of insulin concentration.

- **Insulin-dependent**: muscle, adipose tissue and liver. The utilization of glucose by these tissues is phasic; in fact it is modulated by the amount of circulating insulin.

- **Gluco-sensors**: pancreas β-cells, liver and hypothalamus. They are sensitive to glucose concentration and they could provide a proper secretory response.

In Fig. 1.1 is showed a schematic representation of the glucose-insulin control system. In the upper part we can find the production of glucose, mainly provided by the liver and its utilization, mediated and not by insulin action. In the lower part we can find the secretion of insulin from of the β-cells and its degradation by tissues. Dashed arrows show the mutual control between glucose and insulin, where insulin promotes glucose utilization and inhibits its production, while glucose stimulates insulin secretion. It is therefore evident that a well-regulated control system is in closed loop form (Fig. 1.2): glucose stimulates insulin secretion and this, in turn, acts on glucose production and utilization.

![Figure 1.2: Glucose-insulin control system in closed loop, [2].](image)

An imbalance of this feedback control system can lead to diseases such as diabetes.
1.2. Diabetes

Diabetes is a chronic disease characterized by either an autoimmune destruction of pancreas β-cells, leading to insulin deficiency (Type 1 Diabetes Mellitus, T1DM), or by insulin resistance (Type 2 Diabetes Mellitus, T2DM) which may be combined with impaired insulin secretion. As a result, in diabetic subjects the plasma glycaemic level exceeds the normal range, with several long- and short-term complications. It is expected that by the year 2030 there may be close to 400 million people with diabetes. It is important to note the fact that at least 50% of the entire diabetic population is unaware of its condition and in many countries this data reaches 80%. Every year 3.8 million deaths are caused by complications due to diabetes and, in fact, it is considered currently the fourth leading cause of death worldwide.

1.2.1. Type 1 diabetes

Type 1 diabetes is the form of diabetes which results from autoimmune destruction of insulin-producing β-cells of the pancreas (Fig. 1.3). The insulin deficiency results in the inability of cells (in particular fat and muscle) to utilize and store glucose, with immediate consequences:

- Accumulation of glucose in plasma which leads to strong hyperglycemia, to exceed the threshold of renal reabsorption causing glycosuria, polyuria and polydipsia.
- Use of alternative sources of energy such as the lipid reserves, bringing the loss of body fat and protein reserves with loss of lean body mass.

Type 1 diabetes is less than 10% of cases of diabetes and it is a disease of childhood thus affecting mostly children and adolescents, more rarely young adults (90% <20 years).
1.2.2. Type 2 diabetes

Type 2 diabetes is characterized by three physiological abnormalities: impaired insulin secretion, insulin resistance and overproduction of endogenous glucose.

It is the most common type of diabetes (more than 90% of cases) and it is a typically disease of mature age (> 40 years), even if it starts to affect patients getting younger. The pathogenesis of type 2 diabetes is caused by a combination of lifestyle (obesity, lack of physical activity, etc.) and genetic factors. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages it is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels would be expected to result in even higher insulin values it they had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate glucose levels due to insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal [3].

1.2.3. Complications

All forms of diabetes increase the risk of long-term complications, which are mainly related to damage to blood vessels: in fact diabetes doubles the risk of cardiovascular disease [4, 5]. The main "macrovascular" diseases (related to atherosclerosis of larger arteries) are ischemic heart disease (angina and myocardial infarction), stroke and peripheral vascular disease; while the main "microvascular" complications (damage to the small blood vessels) are:

- **Diabetic retinopathy**: (70% T1DM, 40% T2DM) it affects blood vessel formation in the retina of the eye, leading to visual symptoms, reduced vision, and potentially blindness.

- **Diabetic nephropathy**: (20-30% of diabetic patients) the impact of diabetes on the kidneys can lead to scarring changes in the kidney tissue, loss of small or
progressively larger amounts of protein in the urine, and eventually chronic kidney
disease requiring dialysis.

- **Diabetic neuropathy:** (20-40% of diabetic patients) it is the impact of diabetes on
the nervous system, most commonly causing numbness, tingling and pain in the
feet and also increasing the risk of skin damage due to altered sensation. Together
with vascular disease in the legs, neuropathy contributes to the risk of diabetes-
related foot problems (such as diabetic foot ulcers) that can be difficult to treat and
occasionally require amputation.

Equally important are the short-term complications, conditions caused by either
hypoglycemia or hyperglycemia. The first case occurs in people with diabetes treated with
insulin or hypoglycemic agents and is more common in people who miss or delay meals
after an insulin bolus or do physical activity unexpectedly, causing an increase in glucose
utilization by tissues. In the second case [6] occurs diabetic ketoacidosis, usually in young
people with type 1 diabetes, and the main cause is the absolute or relative deficiency of
insulin. Among the risks are cerebral edema, hyperchloremic acidosis, lactic acidosis,
infections, gastric dilation, erosion and thromboembolism. Another complication that
affects, however, subjects with type 2 diabetes is the hyperglycemic-hyperosmolar
syndrome (mortality 10-60%) characterized by hyperosmolarity (plasma osmolality > 320
mosm / kg), severe hyperglycemia (blood glucose > 600 mg / dl), marked dehydration, the
absence of acidosis [7].

### 1.3 Control of Diabetes

For decades, the evaluation of the patient's glycemic control was based solely on
glycosuria [8] then, with the introduction of self-home capillary blood glucose, , a
fundamental level of quality was reached [9]. At the end of the 70s integrated indexes such
as HbA1c [10] and glycated proteins [11] have been joined, but, till now, the self-blood
glucose measurement (Self-Monitoring Blood Glucose, SMBG) remained an indispensable
element, enabling enormous progress, both in clinical terms, making possible to pass
towards a real self-care [12, 13], and in terms of knowledge, documenting a number of
aspects of the physiology and pathophysiology of glucose homeostasis [14, 15] that were
previously only intuited.
However, because of the wide and rapid variations in blood glucose due to physical activity, diet, and pharmacological therapy, SMBG values are not sufficient to identify episodes of post-prandial hyperglycemia and especially those of hypoglycemia caused by an overdose of insulin.

Since 2000 it has been possible to use techniques for continuous monitoring of blood glucose throughout the day, trying to limit the invasiveness (minimally invasive or non-invasive). In particular, systems have been proposed for continuous glucose monitoring (Continuous Glucose Monitoring, CGM), which have the advantage of being able to provide almost continuous glucose measurement, essential to recognize critical events in real time.

The standard treatment for patients with diabetes, especially for T1DM, is therefore based on multiple daily injections of insulin (bolus and basal doses), diet and exercise, tuned according to self-monitoring of blood glucose (SMBG) levels 3 to 4 times a day, but thanks to the availability of CGM sensors and insulin delivery systems has been possible to improve the management of diabetes. The SMBG however is still remained fundamental for control therapy due to possible systematic and random errors of CGM sensors, becoming of considerable importance the calibration procedure enabled by SMBG values.

### 1.3.1. Continuous Glucose Monitoring Systems

The difference between SMBG and CGM is evident: the amount of additional information that can be obtained from a tool that performs frequent measurements, without requiring the active intervention of the patient, even in times of the day which cannot be analyzed in detail with the traditional systems.

This difference is exemplified in glycemic profile shown in Fig. 1.3: a trend apparently satisfactory, if judged by isolated points detected with SMBG, reveals significant glucose excursions when the observation is made in a "continuous" way. So SMBG provides a limited and isolated number of accurate measurements, thus only roughly indicative of the overall picture, instead CGM, if correctly calibrated, gives a more detailed and representative picture of the real clinical situation.
Figure 1.3: Comparison between a glycemc profile obtained with SMBG (filled circles) and CGM (continuous line).

In the first continuous glucose monitoring system was offered only “offline” interpretation of the glucose profiles after disconnecting the sensor and uploading the results. In the past years, “online” or “real-time” continuous glucose monitoring systems have become available, allowing direct feedback of glucose levels.

CGM devices produced by Abbott, DexCom, and Medtronic have been approved by the U.S. Food and Drug Administration (FDA) and are available by prescription: these provide real-time measurements of glucose levels, with glucose levels displayed at 5-minute or 1-minute intervals. Users can set alarms to alert them when glucose levels are too low or too high. Special software is available to download data from the devices to a computer for tracking and analysis of patterns and trends, and the systems can display trend graphs on the monitor screen [16].

As other biological parameters continuously monitored, glucose monitoring is based on the use of biosensors, i.e. of analytical devices equipped with a detection system, associated to a system of translation of signals. This mechanism allows to translate variations induced by chemical reactions or by physiological changes in digital electronic signals, the intensity of which is proportionate to the concentration of the analyte in the biological material under examination.

- Conventionally, it is usual to distinguish continuous glucose monitoring devices in:
  - totally implantable glucose sensors
    - intravascular
    - subcutaneous
  - minimally invasive sensors
need loom
with systems of micro dialysis
based on ionophoresis

- non-invasive sensors
  - optical
  - based on spectroscopy
  - based on light scattering

In this thesis we will focus on minimally invasive instrumentation and in the particular the DexCom Seven Plus® (Fig. 1.6) used in our experimental protocols.

Figure 1.4: DexCom Seven Plus® composed of a small electrochemical sensor placed just under the skin, a one-use injector, a transmitter connected to the sensor and monitor which receives sensor signal and provides real-time results, [17].

1.3.2. Subcutaneous Insulin Infusion Pumps

Currently, people with type 1 diabetes have two treatment options for insulin delivery: multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) by pump or integrated systems such as pump-continuous glucose monitoring (Sensor Augmented Pump, SAP).

The insulin pump therapy or continuous subcutaneous insulin infusion was introduced over
30 years ago with the aim of improving glycemic control in patients with type 1 diabetes, trying to mimic insulin administration of a healthy patient. In fact, it can reduce the glycemic variability within-day and between-day instead occurs with multiple daily injections of insulin [18, 19]. This effect could be related to smaller deposit subcutaneous insulin during treatment with the pump (about 1 unit) and low coefficient of variation in absorption during the basal rate infusion [20]. The reduction of blood glucose fluctuations in patients allows to reduce the levels of glycated hemoglobin without increasing the risk of hypoglycemia [18].

Insulin pumps therefore represent the technical basis on which the new generation of therapeutic tools for the administration of insulin is based. Since their appearance in the 80s they have seen a rapid technical evolution that has led them to be now able to manage insulin therapy in safety. Nowadays the devices in the market offer the possibility to adjust the basal infusion with different speeds depending on the time of the day and the same flexibility is guaranteed in the administration of boluses with meals. In recent years, the technology of the pump system has seen a sharp acceleration with the introduction of functionality to support the bolus calculation, on the basis of the amount of carbohydrates introduced and integration with systems of self-monitoring and continuous monitoring of blood glucose. Most modern systems also contain functions of alarms and alerts that allow you to:

- Inform in advance the person on the risk of occurrence of hypoglycemia and hyperglycemia;
- Operate in a feedback loop in the event of severe hypoglycemia by blocking the delivery of insulin.

### 1.4. Insulin sensitivity indices: state of the art

In order to correctly evaluate the amount of insulin which should be present in the administered bolus, it would be fundamental to know the value of insulin sensitivity (SI), which corresponds to the ability of insulin to stimulate glucose utilization and inhibit
glucose production. In fact, knowing the specific insulin sensitivity of the patient and its variation during the day will help in determining the optimal insulin treatment.

Several indices have been published, but the two most important have been favored in the past three decades: the clamp insulin sensitivity, $SI_{DF}$, defined by DeFronzo [21] as the ratio of glucose injection and insulin concentration during the hyperinsulinemic-euglycemic clamp (Fig. 1.8) and the insulin sensitivity, $SI_{BC}$, defined by Bergman and Cobelli [22] which uses minimal model of glucose regulation during an intravenous glucose tolerance test (Fig. 1.9).

The hyperinsulinemic-euglycemic glucose clamp technique is considered as the “gold standard” for quantifying insulin sensitivity in vivo because it directly measures the effects of insulin to promote glucose utilization under steady state conditions [21]. However, the glucose clamp is not easily applied in large scale investigations because i.v. infusion of insulin, frequent blood samples over a 3-h period and continuous adjustment of a glucose infusion are required for each subject studied. The second method is less invasive than clamp methods, although it involves frequent sampling of peripheral plasma after an intravenous glucose injection (IVGTT).

### 1.4.1. Hyperinsulinemic-euglycemic clamp

The subject is placed in closed loop and connected to two infusion pumps: a pump infuses glucose and a pump infuses insulin. The technique of "glucose clamp" (hyperinsulinemic-euglycemic clamp) is used to assess insulin sensitivity of the subject, and it represents the “gold standard” for the quantification of insulin sensitivity.

A prime-continuous insulin infusion (Fig. 1.5) is administered to the patient in order to raise and maintain insulin concentration at high level steady-state ($I_{ss}$). In order to keep constant glucose concentration at basal level, a variable glucose infusion is necessary. When the steady-state condition is achieved, the glucose infusion rate ($M$) equals glucose uptake by all the tissues in the body and is therefore a measure of tissue sensitivity to exogenous insulin (SI).
1.4.2. Intravenous glucose tolerance test minimal model

This method involves frequent sampling of peripheral plasma after an intravenous glucose tolerance test (IVGTT): the measured time course of plasma insulin concentration is considered as the “input” and the plasma glucose concentration as the “output” of the system. Model parameters can be estimated from a single IVGTT and provide an explicit, quantitative estimate of the insulin sensitivity of the tissues of given subject.

Glucose effectiveness (E) is defined as the quantitative enhancement of glucose disappearance due to an increase in the plasma glucose concentration

$$E = - \frac{\partial G}{\partial G}$$  \hspace{1cm} (1)

where $\dot{G}$ is the time rate of change of the plasma glucose concentration (G). Insulin sensitivity is then defined, in steady-state (SS), as the quantitative influence of insulin to increase the enhancement of glucose of its own disappearance

$$SI = \frac{\partial E_{ss}}{\partial I_{ss}}$$  \hspace{1cm} (2)
Figure 1.6: IVGTT Glucose Minimal Model, [23].

\[
\frac{dG}{dt} = (p_1 - x) G + p_4
\]  

\[
\frac{dx}{dt} = p_2 x + p_3 I(t)
\]  

where

\[
x = (k_4 + k_6) I^\prime
\]  

\[
p_2 = -k_3
\]  

\[
p_3 = k_2 (k_4 + k_5)
\]  

\[
p_4 = B_0
\]  

\[
p_5 = G(0)
\]  

Applying the definitions to the model:

\[
\dot{G} = (p_1 - x) G + p_4
\]  

Then

\[
E = x - p_1
\]  

Also at steady state

\[
X_{ss} = -\frac{p_3}{p_2} I_{ss}
\]  

So that
\[ E_{ss} = -\frac{P_2}{P_2} I_{ss} - P_1 \]  

(8)

Therefore, we define the insulin sensitivity index

\[ SI = -\frac{P_3}{P_2} \]

(9)

In this model, the rate of change of glucose is given by the difference between the net hepatic glucose balance, \( B \) (which may take on positive, production, or negative, uptake, values), and the disappearance of glucose into peripheral tissues only (\( U_p \)). Hepatic glucose balance varies according to a relation of the form:

\[ B = B_0 - (k_3 + k_6 I ) G \]  

(10)

where \( B \) is net glucose balance, and \( B_0 \) is the net balance expected when plasma glucose concentration is extrapolated to 0. It is assumed that the insulin acts from a remote compartment and the disappearance of glucose in peripheral tissues can be expressed as:

\[ U_p = (k_4 + k_6 I ) G \]  

(11)

where “remote” insulin is envisioned to increase the mobility of glucose across the cell membrane and this motility potentiates glucose disappearance.

However, this technique (as well as the glucose clamp) realizes experimentally a “no physiological milieu” since neither the elevated insulin-basal glucose condition of the clamp technique nor the rapid glucose and insulin perturbations of an IVGTT reflect the conditions of daily living. Therefore, it is highly desirable to have a method able to quantify insulin sensitivity in a normal life “physiological milieu,” e.g., during a meal.

### 1.4.3. Other methods

Recently, several methods for determining insulin sensitivity from oral glucose tolerance test (OGTT) or meal test (MTT) have been proposed, but the difficulty with oral tests is that the input of the system (rate of glucose appearance) is unknown. An approach to simultaneously identifying parameters describing glucose absorption and insulin sensitivity using seven or more blood samples from MTT or OGTT has been developed by Dalla Man et al. [24] and was validated against multiple tracer methods in non-diabetic subjects and
results were well correlated with results from hyperinsulinemic clamps. However, this method requires at least seven blood samples to measure plasma glucose and insulin concentrations and the identification of a model with sophisticated modeling software. Caumo et al. [25] derived an index of insulin sensitivity with an integral approach, but it also requires frequent measurements of plasma glucose and insulin concentrations after the meal; moreover, the method requires that both glucose and insulin concentrations have returned to basal values at the end of the experiment. This is a big limitation, since, in type 1 diabetic subjects, it is not unusual that glucose does not return to pretest glycemic basal value due to errors in insulin administration.

Other more empiric methods for determining insulin sensitivity from OGTT have also been proposed. Stumvoll et al. [26] empirically obtained an insulin sensitivity index based on glucose and insulin measurements during an OGTT that was correlated with the glucose infusion rate during an hyperinsulinemic clamp. Matsuda et al. [27] developed a composite insulin sensitivity index based on both fasting and mean values of glucose and insulin and showed that this measure was correlated with results from an hyperinsulinemic clamp. Hansen et al. [28] empirically determined measures of insulin sensitivity from OGTT that were correlated with SI measured by IVGTT. However, all of them use plasma measurements. A new empiric approach to evaluate insulin sensitivity has been proposed by Breton and Kovatchev [29]. It employs routine self-monitoring blood glucose (SMBG) data, collected over a period of 2-6 weeks and it is based on the theory of risk analysis of blood glucose data, combined with basic patient measurements. This method has the advantage to be easy to implement and uses simple data collected in normal daily life conditions, but, due to the long-time collected data, this not takes into account the intraday variability of this index which can be present in person’s natural environment.

To best of our knowledge, until now, there is no method to estimate insulin sensitivity by using new technologies such as continuous glucose monitoring and subcutaneous insulin infusion devices which provide much more information about patient conditions respect to other devices.
1.5. Objective

The aim of the thesis is firstly to apply a new method for the estimation of insulin sensitivity (SI), in correspondence of meals, in patients with type 1 diabetes based only on CGM sensor and subcutaneous insulin pump. This technique is then applied on both data bases to evaluate, in the first one, the sensitivity to CGM errors, and, in the second one, SI daily variation.

Moreover, to improve the estimation of insulin sensitivity with meals close, a function which calculates, at each time, the amount of carbohydrates absorbed during the meal (Carbohydrates on Board, COB) is developed. At the end, results of insulin sensitivity estimation with and without the implementation of this function will be compared to allow to evaluate the improvement on insulin sensitivity estimation with meals close.
2. Data base and protocols

In this thesis, we used two databases. For the first study, the clinical trials began at the University of Virginia (UVA), followed by studies in Padova and Montpellier. These became the first clinical trials to receive regulatory approvals solely on in silico experiments. The second was a multicenter study in which each center followed the same protocol. Both databases are made up of patients with type 1 diabetes who use subcutaneous insulin pump and continuous glucose monitoring systems.

2.1. Data base 1

2.1.1. Subjects

Data base is made up of 11 patients aged between 21 and 64 years of any racial/ethnic group.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender [M/F]</th>
<th>Age [years]</th>
<th>Weight [Kg]</th>
<th>Height [Cm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6002</td>
<td>M</td>
<td>39</td>
<td>84</td>
<td>180</td>
</tr>
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</tr>
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<td>M</td>
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<td>F</td>
<td>49</td>
<td>61.9</td>
<td>165</td>
</tr>
<tr>
<td>6042</td>
<td>F</td>
<td>29</td>
<td>66</td>
<td>173</td>
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<td>F</td>
<td>37</td>
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<tr>
<td>6044</td>
<td>F</td>
<td>42</td>
<td>68.5</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2.1: Anthropometric characteristics of data base 1.

For each subject we have:
• CGM data of two sensors sampled every 5 minutes (CGM1 e CGM2).
• SMBG data.
• Insulin infusion data: basal insulin infusion [U/h] and pre-meal insulin blouses [U].
• Glucose loads: meals and hypotreatment [g].

Mealtimes are usually kept constant in all subjects:
• snack at 22:00.
• breakfast at 08:00.
• lunch at 14:00.

The start time data acquisition, for each subject varies between 18:00 and 21:00 as the end time data acquisition varies between 13:00 and 15:00 of the next day.

2.1.2. Protocol

a) Day of admission in the clinic and preparation

After the first visit (control) occurred a few days before, the subjects may be admitted to the clinic for the second visit; subjects can use their own insulin pump containing lispro (Humalog) insulin and the physician inserts two DexCom continuous glucose monitors (CGMs) sensors into their abdomen. Subjects are very familiar with this equipment as they had worn equipment CGM for about 6-8 weeks during a previous study, which was a requirement to participate in this phase.

Sensors measure the change in glucose levels and they send the information to a beeper-sized monitor, which stores the results. Subjects have to wear the CGMs until the completion of the second visit.

After wore the CGM, subjects have to perform all required calibrations with fingerstick glucose measurements (approximately two to four calibrations per day). All fingersticks are preceded by hand washing with warm water and a dry towel.

An i.v. catheter is placed to be used for frequent blood sampling during the admission. Heat may be applied to the arm with a hospital-supplied heating pad and two IV’s are placed in forearm or antecubital vein.
b) *Mixed meal study*

A standardized meal containing 0.9 grams carbohydrate per kilogram body weight is served for dinner on the evening of admission and insulin is bolused via the patient’s pump to cover the meal. The subject is offered a snack at 10:00 pm containing 20 grams carbohydrates and insulin may be bolused per the patient’s usual home regimen. The subject remains fasting except for sugar-free beverages until the following morning. Overnight, the subject’s glucose level is monitored via hourly BG measurement with the goal of avoiding hypoglycemia prior to the 8AM administration of the mixed meal. For glucose <80 mg/dl, 4 glucose tablets will be given (approximately 15-16 grams of carbohydrates).

In the morning (approximately 8 AM), the subject will undergo a mixed meal and insulin challenge as follows: an insulin bolus will be administered and a mixed meal nutrition drink will be consumed over 1-5 minutes. The mixed meal nutrition drink is selected for that individual to be most likely to raise the glucose levels by 100mg/dl and then return to baseline within a four-hour time period. The nutrition drink will be selected from the following types of product lines: Boost products (Nestle Nutrition), Ensure products (Abbott Nutrition), Carnation Instant Breakfast (Nestle Nutrition) or Glucerna (Abbott Nutrition). The pre-meal insulin bolus will be calculated to bring the subject to ~100mg/dl at 11AM.

If hypoglycemia of 50-69 mg/dl is not achieved by approximately 12 PM, a second insulin bolus will be administered with the goal to induce hypoglycemia of ~50-69 mg/dl, and again, if hypoglycemia is not occurred by approximately 1:00 PM or is not predicted to occur by 1:30 PM, (5-5.5 hours after a mixed meal), the study physician might decide to administer an additional insulin bolus at 1:00 PM.

Glucose is administered to resume euglycemia if blood glucose by YSI will be <60 mg/dl at any time during the protocol. The study physician should administer glucose prior to blood glucose reaching 60 mg/dl if there was a safety concern such as neuroglycopenic symptoms or rapid decline of glucose. Once the subject will reach a hypoglycemic target, the subject can have lunch and remain for observation.

For the mixed meal and insulin sensitivity measures, 8 AM (time 0) will be considered the start of the meal and blood samples will be collected at -120, -60, -30, -20, -10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 minutes for insulin, c-peptide, and glucose.
At approximately 12 PM (240 minutes after the meal), an insulin bolus will be given to target a blood glucose of 50-69 mg/dl for measurement of hypoglycemic counter regulatory responses.

2.2 Data base 2

2.2.1. Subjects

The data base available, that represents a subset of a larger one, is relative to the 8 subjects of the research center of Montpellier (MTP) and the 7 subjects in the center of Amsterdam (AMS) processed each with two closed-loop control algorithms (CAM, IAP), mentioned in the next paragraph, and in open-loop (OPEN).

<table>
<thead>
<tr>
<th>Center</th>
<th>Subject</th>
<th>Algorithm</th>
<th>Age [years]</th>
<th>Weight [Kg]</th>
<th>Height [Cm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMS</td>
<td>2</td>
<td>OPEN</td>
<td>8</td>
<td>62</td>
<td>162</td>
</tr>
<tr>
<td>AMS</td>
<td>3</td>
<td>OPEN IAP</td>
<td>33</td>
<td>107</td>
<td>207</td>
</tr>
<tr>
<td>AMS</td>
<td>4</td>
<td>OPEN CAM</td>
<td>62</td>
<td>93</td>
<td>193</td>
</tr>
<tr>
<td>AMS</td>
<td>6</td>
<td>CAM IAP</td>
<td>50</td>
<td>85</td>
<td>185</td>
</tr>
<tr>
<td>AMS</td>
<td>7</td>
<td>IAP OPEN</td>
<td>33</td>
<td>74</td>
<td>174</td>
</tr>
<tr>
<td>AMS</td>
<td>8</td>
<td>OPEN CAM</td>
<td>28</td>
<td>64</td>
<td>164</td>
</tr>
<tr>
<td>MTP</td>
<td>1</td>
<td>OPEN CAM</td>
<td>48</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>MTP</td>
<td>2</td>
<td>OPEN CAM</td>
<td>47</td>
<td>78</td>
<td>178</td>
</tr>
<tr>
<td>MTP</td>
<td>4</td>
<td>OPEN IAP</td>
<td>45</td>
<td>72</td>
<td>172</td>
</tr>
<tr>
<td>MTP</td>
<td>5</td>
<td>OPEN CAM</td>
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<td>68</td>
<td>168</td>
</tr>
<tr>
<td>MTP</td>
<td>11</td>
<td>OPEN CAM</td>
<td>40</td>
<td>74</td>
<td>174</td>
</tr>
<tr>
<td>MTP</td>
<td>20</td>
<td>OPEN CAM</td>
<td>47</td>
<td>63</td>
<td>163</td>
</tr>
</tbody>
</table>
Table 2.2: Anthropometric and protocol characteristics of data base 2.

For each subject and for each algorithm used, we have:

- CGM data sampled every 5 minutes.
- Plasma glucose concentration.
- Plasma insulin concentration.
- Insulin infusion data: basal insulin infusion [U/h] (sampled every minute) and pre-meal insulin boluses [U].
- Glucose loads: meals and hypotreatment [g].

2.2.2. Protocol

The protocol (called CAT protocol) described here was used for a multicenter study that aimed to compare two existing control algorithms MPC, namely the algorithms from Cambridge (CAM-A) and Padua-Pavia (PP-A), to open loop glycaemic control. Each patient will undergo 1 day of closed-loop glycaemic control with the Cambridge algorithm (CAM), 1 day of closed-loop control with the Padua-Pavia algorithm (IAP), and 1 day of open loop control with CSII treatment (control condition), all in randomized order to limit potential bias.

In total was planned to enroll 8 patients for each research center (only 7 in Montpellier), male or female, diagnosed with Type 1 Diabetes Mellitus, treated with CSII for a minimum of 3 months, aged 18 or above into the study.

Continuous Glucose Measurements will be provided by Seven®Plus CGM and Insulin Delivery will be collected from the insulin pump. This device can be linked to a computer so that recorded data can be easily exported to a file in xml, csv or text format. The study computer where the algorithms are installed will collect all the information in real-time.
a) *Day of admission in the clinic*

The day of the study, the patient should remain in the research center for 24 hours. Depending on the result of the randomization, this visit correspond either to an open-loop, either to a closed-loop with IAP algorithm or to a closed-loop with CAM algorithm.

- Subject arrives around 17:30 hours to the Centre.
- Two research clocks pre-set to the official time are placed in the room. The insulin pump, continuous glucose monitor, and all study procedures are referenced to the official time.
- Plasma glucose is measured using the YSI measurement device, which is calibrated and optimized until a 10 mmol/L glucose standard reads 9.8-10.2 mmol/L.
- The CGM device is calibrated with YSI glucose measurements.
- By 18:00 hours, two 20 Gauge indwelling catheters are placed in different antecubital veins for sampling of plasma glucose and insulin and infusion of glucose if required.
- Blood is sampled every 30 minutes from 19:00 the first day to 18:00 the second day except during the following events:
  - Night time: between 23:00 and 07:00 hours the sampling schedule is reduced to once every 60 minutes.
  - Meals: blood is sampled every 15 minute from the start of a meal until 2 hours afterwards.
  - Exercise: blood is sampled every 15 minute during exercise and until 2 hours after the start of the exercise.
- The study pump will be initiated and a new catheter inserted by 18:00 and the subjects own insulin pump are stopped.
- Initialization of the closed-loop system begins at 19:00 hours.
- Initialization of the controller occurs prior to closed loop initiation and includes configuration of nominal basal insulin pattern for the visit.
- Patient receives dinner at 19:00. This standardized meal consists of 80 grams of carbohydrates which should be fully ingested within 20 minutes. All meals will be identical on different study days. If the visit corresponds to a closed loop, the meal insulin bolus is calculated by the algorithm. Otherwise, patient calculates his need of insulin.
• Patients are allowed to sleep from 23:00 to 07:00 hours the following day.
• Patients receive breakfast at 08:00 hours with a carbohydrate content of 50 grams which should be fully ingested within 20 minutes.
• Patients receive lunch at 12:00 hours with a carbohydrate content of 60 grams which should be fully ingested within 20 minutes.
• Exercise follows at 15:00 hours.
• Automated closed-loop control ends at 18:00 and then patient is allowed to leave the Center when blood glucose is stable (>4.4 mmol/L).
3. Methods

3.1. A new index of insulin sensitivity from minimally invasive technologies

The estimation of this new index of insulin sensitivity ($S_{CGM&PUMP}$) [30] employs a simple integral approach with suitable approximations to simplify integral calculations, without the need to solve any differential equation. In particular the following ingredients are required:

- The area under the curve (AUC) of above basal CGM data during the meal.
- The area under the curve of subcutaneous insulin infusion (Inf) data during the meal and, possibly, an estimation of the delayed effect of insulin boluses administered before the meal by using an Insulin on Board algorithm (IOB) [31].
- The amount of glucose ingested during the meal ($D$).
- Patient specific parameters such as body weight (BW), age and height for the estimation, by using population models [32], of plasma insulin clearance (CL).
- Population values of glucose kinetics parameters [33] such as glucose effectiveness at zero insulin (GEZI), fraction of the ingested glucose which appears in the systemic circulation ($f$), and volume of glucose distribution ($V_G$).

The method used to calculate an estimation of insulin sensitivity is made up of four components (Fig. 3.1):

a) *Glucose module:* it considers continuous glucose monitoring data [mg/dl], from the start of the meal till six hours later (time at which the glucose absorption of the meal is assumed to be ended) and calculates the area under the curve (AUC) with the trapezoidal rule. If available, at least two SMBG references could be used for the calibration, with such an algorithm as [34], of CGM signal.

b) *Insulin module:* it considers the subcutaneous insulin infusion data [mU/min] from three hours before the start of the meal, to take into account, by using an Insulin on Board algorithm (IOB) [27], the delayed effect of insulin correction boluses
administered before the pre-meal bolus, till six hours later and calculates area under the curve.

c) **Patient module**: uses the specific data of the patient mentioned above and the dose of the meal.

d) **SI calculator**: is the core of the method and it employs a simple integral approach, without the need to solve any differential equation, to evaluate the insulin sensitivity by using simple algebra formula.

![Figure 3.1](image.png): Schematic diagram showing main modules used to calculate the SI_{CGM&PUMP}.

### 3.2. Estimation of carbohydrates on board: COB

This function is based on the model of gastro-intestinal tract (Fig. 3.2) which assumes two compartments for the stomach (one for the liquid and one for the solid phase) a gastric emptying rate \( (k_{empt}) \) dependent on the total amount of glucose in the stomach \( (q_{sto}) \), a single compartment for the intestine \( (q_{gut}) \) and a constant rate of intestinal absorption \( (k_{abs}) \). Many authors agree \([35-41]\) that the gastric emptying of liquids occurs exponentially and depends on the size of the meal, its energy density and the amount of nutrient in the stomach. On
the other hand, with increasing nutrient and caloric content of the liquid phase of the meal, there is a deceleration from the exponential model and closer approximation to linearity.

Starting from the knowledge of the amount of carbs ingested at time $t_m$, the COB function is able to evaluate, at each time $t > t_m$, the percentage of carbs not yet absorbed. For $t - t_m > 360\text{ min}$, it is assumed the percentage of carbs not yet absorbed is lower than 10% [42].

![Figure 3.2: Model of gastro-intestinal tract, [42].](image)

The model showed above is described by the following equations:

$$
\begin{align*}
\dot{q}_{sto1}(t) &= -k_{gri} \cdot q_{sto1}(t) + D \cdot \delta(t) \\
\dot{q}_{sto2}(t) &= -k_{empt} \cdot q_{sto1}(t) + k_{gri} q_{sto1}(t) \\
\dot{q}_{gut}(t) &= -k_{abs} \cdot q_{gut}(t) + k_{empt} \cdot q_{sto1}(t) \cdot q_{sto2}(t) \\
Ra(t) &= f_{\infty} \cdot k_{abs} \cdot q_{gut}(t)
\end{align*}
$$

(12)

where, if $D > 30\text{ g}$

$$
q_{sto}(t) = q_{sto1}(t) + q_{sto2}(t)
$$

(13)

$$
k_{empt}(q_{sto}) = k_{\min} + \frac{k_{\max} - k_{\min}}{2} \cdot \{\tanh[a \cdot (q_{sto} - b \cdot D)] - \tanh[c \cdot (q_{sto} - d \cdot D)] + 2\}
$$

else

$$
k_{empt} = k_{\max}
$$

(14)
With parameters $a$ and $c$ constrained by imposing that $k_{\text{empt}} = k_{\text{max}}$ for both $q_{\text{sto}} = D$ and $q_{\text{sto}} = 0$

$$a = \frac{5}{2 \cdot D \cdot (1 - b)}$$

$$c = \frac{5}{2 \cdot D \cdot d}$$  \hspace{1cm} (15)

We can calculate the value of $f$ by the ratio of AUCs between the rate of appearance of glucose after the meal, assuming that at the end of the meal the total fraction of the meal which appears into plasma is equal to $f_\infty = 0.9$.

$$f(t) = f_\infty \cdot \frac{\int_0^t R(t) dt}{\int_0^\infty R(t) dt} = \frac{\int_0^t R(t) dt}{D}$$  \hspace{1cm} (16)

Thus we can obtain the value of Carbs On Board, COB:

$$COB(t) = f_\infty - f(t)$$  \hspace{1cm} (17)

### 3.3. Incorporation of COB in insulin sensitivity estimates

This function can be used to estimate insulin sensitivity from CGM and insulin pump data [30] when meals are close each other, i.e. less than 360 minutes, and therefore the previous meal has not been fully absorbed.

When two meals are close one to each other, we can split the carbs content into two components:

1. **First component**: by using the function $f$, we can obtain the amount of the meal absorbed up to the desired instant by multiplying the meal dose for the value of the function at that time.

2. **Second component**: by using the function $COB$, we can obtain the amount of the meal which has not been yet absorbed by multiplying the previous meal dose for the value of the function at that time. This value can then be added to the next meal dose to obtain the total carbs amount which will be absorbed by the start of the second meal.
This procedure can be repeated for the following meals. Moreover, also a hypotreatment is administered close to a meal could affect the estimation of the insulin sensitivity because the relative increase in glucose concentration due to hypotreatment, which could reduce the estimation of insulin sensitivity, in this case it is compensated by an opportune addition of carbs to the meal dose.

It should be considered, therefore, that the development of the functions $f$ and $COB$ cannot be identical for each type of meal, but as we shall see later, varies depending on the composition and the amount of the meal assumed.
4. Results

4.1. COB from simulated data

In this first phase COB and f functions are extracted in a simulated environment thank to the availability of a type 1 diabetes mellitus simulation model of the glucose-insulin system during a meal accepted by the Food and Drugs Administration (FDA) as a substitute to animal trials for certain insulin treatments [43]. T1DM simulator is equipped with a cohort of in silico subjects which permit to simulate inter-individual variability of key metabolic parameters in the T1DM population. In silico subjects are generated from a distribution of the parameters which characterize the physiological model of the glucose-insulin system described in [42]. Parameters estimates are obtained by fitting the model to individuals data collected in clinical trial.

To extract COB and f functions, 100 in silico subjects were generated using the distribution of the parameters variations of the entire glucose-insulin model.

Starting from the gastro-intestinal model [42] and the parameters extracted from the 100 in silico subjects, COB and f functions for both hypotreatment and standard meal were defined.

For each subject, we calculated the glucose rate of appearance in plasma (Ra) using the relation shown in Section 3.2 both for hypotreatment and meal. The glucose rate of appearance in plasma after a hypotreatment (D = 20 g), due to its known rapid absorption, is obtained after linearization of the model of the gastro-intestinal tract (Fig. 4.1); while for a standard meal (D = 50 g), which is known to be slower than the previous, is obtained by the standard gastro-intestinal model (Fig. 4.2).
Figure 4.1: Median ± IQR range of glucose rate of appearance (Ra) after a hypotreatment (D = 20 g) of the 100 in silico subjects.

Figure 4.2: Median ± IQR range of glucose rate of appearance (Ra) after a standard meal (D = 50 g) of the 100 in silico subjects.
Then, as previously defined, the functions $f(t)$ and $COB(t)$ are obtained, for each time, by the area under the curve of glucose rate of appearance $Ra(t)$ and hypotreatment/meal dose.

Below are shown the median COB, obtained by the 100 in silico subjects, for a hypotreatment (Fig. 4.3) and for a standard meal (Fig. 4.4), respectively.

**Figure 4.3:** Median ± IQR range of COB function of the 100 in silico subjects after a hypotreatment.

**Figure 4.4:** Median ± IQR range of COB function of the 100 in silico subjects after a standard meal.
As known from literature the hypotreatment administered to the patient is absorbed more quickly than a standard meal.

4.2. COB from real data

Parameters of the gastro-intestinal tract, extracted with an identification study using the Bayesian estimation technique MAP (Maximum A Posteriori), where the a priori information of the glucose-insulin model [44] is available in [45], from plasma glucose and insulin concentration are available.

For every single subject and meal, because parameters are thought to be dependent on meal composition, the parameters of the gastro-intestinal tract (\(k_{abs}\), \(k_{max}\) and \(k_{min}\), while \(b\) and \(d\) are maintained constant for all meals) have been identified, obtaining a total of 258 groups. Among these groups, only parameters which presented a satisfactory fit of the model compared to the data are selected.

In the same way for simulated data, functions \(f\) and \(COB\) are obtained for every different meal (breakfast = 50 g, lunch = 60 g and dinner = 80 g).

Below are shown the average parameters (Table 4.1 and Fig. 4.5) and distributions (Fig. 4.6-7-8) of meal-varying parameters of the gastro-intestinal tract identified from all subjects.

<table>
<thead>
<tr>
<th>Mean(sd)</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{abs})</td>
<td>0.1853(0.0746)</td>
<td>0.1944(0.2071)</td>
<td>0.2117(0.1787)</td>
</tr>
<tr>
<td>(k_{max})</td>
<td>0.0638(0.2906)</td>
<td>0.0356(0.0251)</td>
<td>0.0824(0.0186)</td>
</tr>
<tr>
<td>(k_{min})</td>
<td>0.0175(0.0124)</td>
<td>0.0087(0.0069)</td>
<td>0.0095(0.0057)</td>
</tr>
</tbody>
</table>

**Table 4.1:** Average meal-varying parameters identified for all subjects with standard deviation subdivided by meal type.
Figure 4.5: Graphic display of average parameters with their standard error.

Figure 4.6: Distribution of the parameter $k_{abs}$ for breakfast (red), lunch (green) and dinner (blue).
Figure 4.7: Distribution of the parameter $k_{max}$ for breakfast (red), lunch (green) and dinner (blue).

Figure 4.8: Distribution of the parameter $k_{min}$ for breakfast (red), lunch (green) and dinner (blue).
As can be seen from the figures shown above, the distribution of the parameters is similar as regards lunch and dinner, while breakfast is different from the previous especially in the case of $k_{\text{min}}$ and $k_{\text{max}}$.

To demonstrate it, also from a statistical point of view, a two-tailed t-test for each combination of the same parameters for different meals (Breakfast vs Lunch, Breakfast vs Dinner and Lunch vs Dinner) was performed in Table 4.2.

<table>
<thead>
<tr>
<th></th>
<th>Breakfast vs Lunch</th>
<th>Breakfast vs Dinner</th>
<th>Lunch vs Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{abs}}$</td>
<td>0.819</td>
<td>0.4736</td>
<td>0.5702</td>
</tr>
<tr>
<td>$K_{\text{max}}$</td>
<td>0.0018</td>
<td>0.0002</td>
<td>0.3564</td>
</tr>
<tr>
<td>$K_{\text{min}}$</td>
<td>$4.7 \cdot 10^{-6}$</td>
<td>$7.97 \cdot 10^{-8}$</td>
<td>0.4606</td>
</tr>
</tbody>
</table>

**Table 4.2**: P-value of two-tailed t-test for each combination of the same parameters for the different meals.

As we can see in Table 4.2, $k_{\text{max}}$ and $k_{\text{min}}$ are different for breakfast respect to lunch and dinner while parameter $k_{\text{abs}}$ does not change. This feature, due to the different composition of the meal (percentage of fats, proteins and carbohydrates), is thus presented also on the COB functions extracted (shown below) and it will allow us, in the implementation phase, to distinguish the COB function depending on meal composition (Fig. 4.9-10-11).
**Figure 4.9:** Median ± IQR range of COB function of the real subjects during breakfast.

**Figure 4.10:** Median ± IQR range of COB function of the real subjects during lunch.
As expected, the difference can be seen especially between breakfast respect to lunch and dinner, which are similar each other, this meal is in fact absorbed about 100 minutes earlier than lunch or dinner while the latters have a remarkably similar pattern.

4.3. Insulin sensitivity

Firstly for the estimation of insulin sensitivity, it was necessary to extract the time window of analysis for the calculation of the area under the curve of the CGM signal and the area under the curve of basal insulin infusion with meal boluses. Moreover, for some subjects, was also necessary to apply linear interpolation to fill the areas in which CGM values were missing due to an incorrect sampling of the sensor.

In the time windows analyzed only one meal at a time is analyzed and they contain data (CGM and insulin infusion) of at least 3 hours prior to the meal, so as to extract the insulin
on board (IOB), and data about 6 hours after administration of the meal to allow the CGM signal to return to steady state (Fig. 4.12).

**Figure 4.12:** Up: two CGM signals (blue and black line) with the time window analyzed for SI estimation (red); middle: meals; bottom: basal insulin infusion and pre-meal boluses (blue and black line, respectively) with the pre-meal time window for IOB calculation (yellow).

In the Section 4.3 we estimate insulin sensitivity with the standard formulation, i.e. without the introduction of the COB function, to analyze:
• **Data base 1**: by protocol there are two CGM sensors, so this part is dedicated to the analysis of the sensitivity of the formula to the error of the sensor.

• **Database 2**: by protocol there are three different meals (dinner, breakfast and lunch) so this part is dedicated to the extraction of SI during a day in normal living conditions and to assess whether there are patterns of SI.

### 4.3.1. Data base 1

In this data base we have two CGM signals for each patient, so we estimate two values of insulin sensitivity, two for each patient, and then we calculate the average SI ($SI_{cgmM}$) for each patient (Tab. 4.3).

<table>
<thead>
<tr>
<th>Subject</th>
<th>$SI_{cgm1}$</th>
<th>$SI_{cgm2}$</th>
<th>$SI_{cgmM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6002</td>
<td></td>
<td>8.73</td>
<td></td>
</tr>
<tr>
<td>6009</td>
<td>16.23</td>
<td>10.81</td>
<td>13.32</td>
</tr>
<tr>
<td>6012</td>
<td>14.59</td>
<td>2.04</td>
<td>6.4</td>
</tr>
<tr>
<td>6014</td>
<td>20.9</td>
<td>16.58</td>
<td>18.87</td>
</tr>
<tr>
<td>6034</td>
<td>48.49</td>
<td>52.65</td>
<td>50.51</td>
</tr>
<tr>
<td>6035</td>
<td>20.7</td>
<td>24.95</td>
<td>23.28</td>
</tr>
<tr>
<td>6038</td>
<td>9.44</td>
<td>16.7</td>
<td>12.27</td>
</tr>
<tr>
<td>6041</td>
<td>21.82</td>
<td>25.99</td>
<td>25.33</td>
</tr>
<tr>
<td>6042</td>
<td>6.06</td>
<td>9.03</td>
<td>7.42</td>
</tr>
<tr>
<td>6043</td>
<td>17.13</td>
<td>27.44</td>
<td>21.58</td>
</tr>
<tr>
<td>6044</td>
<td>12.12</td>
<td>8.8</td>
<td>10.28</td>
</tr>
</tbody>
</table>

**Table 4.3**: SI estimates, two for each subjects, and the average SI.

SI estimates are similar for each subjects, depending on CGM signals, except for 3 of them (marked in red) where the percentage relative distance ($d$) from the respective average SI is in average of 50%.

\[
d = \frac{|SI_{cgm1} - SI_{cgmM}| + |SI_{cgm2} - SI_{cgmM}|}{2} \times \frac{100}{SI_{cgmM}} \tag{18}
\]
The absolute difference between SI estimates $|\text{SI}_{\text{cgm1}} - \text{SI}_{\text{cgm2}}|$ is significantly correlated ($\rho = 0.8236, p = 0.003$) to the difference between the areas under the curve (AUC) of the two CGM signals $|\text{AUC}_{\text{cgm1}} - \text{AUC}_{\text{cgm2}}|$, where, in this case, the calculated area under the basal glycemic value is added with positive sign.

Instead, the correlation between the SI index and the respective AUC is: $\text{RHO} = -0.66$ with $\text{PVAL} = 0.0016$.

In conclusion, the result of correlation emphasizes the importance of a good calibration of the sensor during the study to obtain an SI estimation as accurate as possible.

### 4.3.2. Data base 2

In this data base we have three different meals, thus we estimate insulin sensitivity for each meal. In the table below (Table 4.4) is shown the mean SI estimates for each meal.

<table>
<thead>
<tr>
<th></th>
<th>Dinner</th>
<th>Breakfast</th>
<th>Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>45.53</td>
<td>24.33</td>
<td>35.17</td>
</tr>
<tr>
<td>Median</td>
<td>42.44</td>
<td>17.80</td>
<td>33.02</td>
</tr>
<tr>
<td>SD</td>
<td>35.535</td>
<td>22.26622</td>
<td>25.011</td>
</tr>
<tr>
<td>SE</td>
<td>9.1053</td>
<td>4.86544</td>
<td>7.0344</td>
</tr>
</tbody>
</table>

**Table 4.4**: Mean SI estimates, for each meal, obtained by all the subjects of data base 2.

It may happen that a few SI estimates result negative: these values are associated to subjects who did not return to pretest glycemic basal value, but continued to rise even long time after the meal. This is probably caused by too small pre-meal insulin bolus which is not able to compensate the total amount of glucose entering the system after the meal.
As we can see in Fig. 4.13, the physiological change in insulin sensitivity during different times of the day is confirmed by the estimates obtained: during dinner and lunch mean SI results higher than breakfast, where, before awakening, greater quantities hormones that cause insulin resistance such as cortisol and growth hormone are produced [46, 47].

### 4.4. Insulin sensitivity with COB

In this Section, we carry out the same analysis of Section 4.3, but with the incorporation of COB function, to take into account the contribution of meals close, into the estimation of insulin sensitivity. In Fig. 4.14 is shown a schematic diagram of what has been described in Section 3.3 for calculation of the total dose at the instant of the SI estimation.
**Figure 4.14**: Graphical view of how COB and $f$ functions work. The term $B$ is the dose that corresponds to breakfast, $L$ lunch, and $H$ to a hypothetical hypotreatment. The temporal distances $d$, $d1$, $d2$, $d3$ are used to calculate the corresponding values of COB and $f$.

For the estimation of insulin sensitivity in correspondence of a meal it is necessary to know:

- Dose of the meal analyzed.
- Dose of meals, or hypotreatments, administered before the start of the time window analyzed, because they could not have been completely absorbed up to the start of the meal.
- Dose of meals administered before the end of the time window analyzed, because they could be partially absorbed.

Thus COB and $f$ functions are used for this purpose, i.e. to account for meals not completely absorbed before or after the start of the meal during the time window analyzed.
4.4.1. Data base 1

In this section has been implemented the functions $f$ and $COB$ for the estimation of insulin sensitivity. In this case the dose used will be reduced by the function $f$ because the calculation of SI is stopped before 360 minutes (time which is assumed for the complete absorption of the meal).

<table>
<thead>
<tr>
<th>Subject</th>
<th>$SI_{cgm1}$</th>
<th>$SI_{cgm2}$</th>
<th>$SI_{cgmM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6009</td>
<td>23.03</td>
<td>17.08</td>
<td>19.98</td>
</tr>
<tr>
<td>6012</td>
<td>14.57</td>
<td>2.04</td>
<td>6.39</td>
</tr>
<tr>
<td>6014</td>
<td>20.87</td>
<td>16.56</td>
<td>18.84</td>
</tr>
<tr>
<td>6034</td>
<td>33.81</td>
<td>36.41</td>
<td>35.07</td>
</tr>
<tr>
<td>6035</td>
<td>24.26</td>
<td>30.67</td>
<td>27.25</td>
</tr>
<tr>
<td>6038</td>
<td>9.29</td>
<td>16.68</td>
<td>12.16</td>
</tr>
<tr>
<td>6041</td>
<td>21.66</td>
<td>25.96</td>
<td>25.30</td>
</tr>
<tr>
<td>6042</td>
<td>6.31</td>
<td>11.54</td>
<td>8.75</td>
</tr>
<tr>
<td>6043</td>
<td>11.59</td>
<td>21.46</td>
<td>15.26</td>
</tr>
<tr>
<td>6044</td>
<td>12.04</td>
<td>8.74</td>
<td>10.21</td>
</tr>
</tbody>
</table>

Table 4.5: SI estimates, two for each subject, and the average SI with the function COB implemented.

The absolute difference between SI estimates $| (SI_{cgm1} - SI_{cgm2}) |$ is correlated ($\rho = 0.8415, p = 0.002$) with the difference between the areas under the curve (AUC) of the two CGM signals $| (AUC_{cgm1} - AUC_{cgm2}) |$, where, in this case, the calculated area under the basal glycemic value is added with positive sign.

4.4.2. Data base 2

The use of the $COB$ and $f$ functions influence the estimation of the insulin sensitivity of each meal:

- Dinner: if there is a hypotreatment in the time window of analysis.
- Breakfast: in general, the dose is not completely absorbed because of the proximity to the lunch (separated by less than 360 minutes) and hypotreatment could be administered.

- Lunch: in general the dose of breakfast is not completely absorbed before the start of the lunch, thus we have to take into account its contribution to the meal dose, and any hypotreatment administered. Moreover, the dose may be reduced if the time window after the meal is less than 360 minutes.

<table>
<thead>
<tr>
<th></th>
<th>Dinner</th>
<th>Breakfast</th>
<th>Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>47.33</td>
<td>25.23</td>
<td>41.79</td>
</tr>
<tr>
<td>Median</td>
<td>42.71</td>
<td>17.41</td>
<td>37.65</td>
</tr>
<tr>
<td>SD</td>
<td>35.31</td>
<td>23.71</td>
<td>24.36</td>
</tr>
<tr>
<td>SE</td>
<td>7.06</td>
<td>4.74</td>
<td>4.87</td>
</tr>
</tbody>
</table>

Table 4.6: Mean SI estimates, for each meal, obtained by all the subjects of data base 2.

![Figure 4.15: Average and standard error of the SI for each meal using the COB.](image)
4.5. Comparison

The comparison between the estimates obtained with and without the implementation of the function COB emphasizes how the proximity of two meals may affect the calculation of insulin sensitivity and the importance of assessing the amount of carbohydrates not yet absorbed.

In addition a glucose load administered between meals should be taken into account during the estimation of insulin sensitivity, in fact, it implies a glycemic excursion and thus it modifies its area under the curve which is used for the calculation of the SI.

In Fig. 4.16 is shown the comparison between the mean SI estimates obtained in data base 1 with and without the COB function.

![Figure 4.16](image)

**Figure 4.16**: Comparison between mean SI estimates with and without COB for data base 1.

As we can see the mean SI estimates for breakfast with the COB function are slightly lower respect to the ones without the function, thus confirming the hypothesis. The function lowers the dose administered due to time window is less than 360 minutes, even if the effect on mean SI estimates is small due to the fast absorption of meal dose.
In Fig. 4.17 is shown the comparison between the mean SI estimates obtained in data base 2 with and without COB function for dinner.

![Bar graph showing comparison of mean SI estimates for dinner with and without COB.]

**Figure 4.17:** Comparison of mean SI estimates for dinner with and without COB.

As hypothesized, SI estimates with and without the COB function is, on average, very similar: in fact there is no information about previous meals. The slight increase of SI with COB is probably due to the presence of hypotreatments which are not accounted without the COB function.

In Fig. 4.18 is shown the comparison between the mean SI estimates obtained in data base 2 with and without COB function for breakfast.
Fig. 4.18 shows an increase of SI with the COB function. This initially appears rather unexpected, because a decrease of insulin sensitivity due to the presence of the next close meal, which does not allow the complete absorption of the dose, is expected. The slight increase of SI with COB is probably due to the compensation on the total dose, in some subjects, between the administration of hypotreatments, which are accounted to be rapidly absorbed by the COB function, and the reduction of the breakfast meal dose due to the presence of the next meal close.

In Fig. 4.19 is shown the comparison between the mean SI estimates obtained in data base 2 with and without COB function for lunch.
**Figure 4.19**: Comparison of mean SI estimates for breakfast with and without COB.

In this case there is a pronounced increase of SI estimates with the COB function, compared to the previous cases, probably due to both the portion of breakfast meal dose which is not yet absorbed and the presence of hypotreatments, which are not taken into account without the COB function.
4.6 Assessment of insulin sensitivity daily variability

In this section, the Fig. 4.19 is shown to demonstrate insulin sensitivity daily-variability that, even after using COB function, it maintained a certain pattern although the average values vary.

![Figure 4.19: Mean SI with and without COB for each meal with their standard error.](image)

It is evident that the average value of insulin sensitivity for breakfast is significantly lower than the SI for lunch and dinner and this is concordant with what we know from the literature.
5. Conclusions

The purpose of this work is to estimate an index of insulin sensitivity in patients with type 1 diabetes through a new method which exploits minimally-invasive technologies such as subcutaneous glucose (continuous glucose monitoring, CGM) sensing and insulin delivery in everyday life condition. This method allows the estimation of an index of insulin sensitivity, in correspondence of meals, by integration of CGM and insulin infusion data and using subject-specific parameters approximated using field-measurable characteristics of the patient. From data base 1, thanks to the presence of two CGM signals, a study of the sensitivity of the formula to the error of the sensor is performed. It was obtain that the sensitivity of the SI estimates is strongly correlated ($\rho = 0.8236$ and $p = 0.003$) with the area under the curve of the CGM sensor, as one might expect, and this allowed us to confirm how important is a good calibration of the CGM sensor to correctly evaluate patient condition. From data base 2 an analysis of SI daily variability, thanks to the availability of three contiguous meals (dinner, breakfast and lunch), is performed. These preliminary results showed a pattern of SI during the day, in fact, on average, insulin sensitivity at breakfast is lower than lunch and dinner, as known from the literature.

However, the method used for the estimation of insulin sensitivity, to be properly applied, assumes the distance between contiguous meals to be at least six hours because, at that time, the carbs amount ingested should be completely absorbed. In fact, in presence of meals close, the dose of a meal that is not completely absorbed should contribute to glucose excursions of the next meal, thus it is necessary to take into account of this contribution. To overcome this limitation, a function which evaluates the percentage of carbs not yet absorbed by the gastro-intestinal tract is developed. This function, called Carbs on Board (COB), is firstly estimated in a simulated environment thanks to the availability of 100 in silico subjects generated using the distribution of the parameters variation of the type 1 diabetes mellitus simulation model of the glucose-insulin system during a meal accepted by the Food and Drugs Administration (FDA) [48]. This function is then estimated using real data thanks to the availability of parameters of the gastro-intestinal tract obtained by an identification study on different meals from plasma glucose and insulin concentration [45]. Through a statistical analysis conducted on parameter
estimates, it was observed that some parameters differ among meals, especially for breakfast \(p << 0.05\) respect to lunch and dinner, which is in agreement with meals composition, i.e. lunch and dinner are very similar respect to breakfast. By using this information, in order to make more reliable the estimation of carbs not yet absorbed between meals close, we developed different COB functions depending on the type of meal administered. Therefore the COB function is used to improve the estimation of insulin sensitivity, described before, also in condition of meals close. The comparison of results showed, also in this case, the previously observed pattern of SI during the day, i.e., on average, insulin sensitivity at breakfast is lower than lunch and dinner, even though mean values of SI are different from the previous one thanks to the contribution of meals close in the insulin calculation process.
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