Tesi magistrale in bioingegneria
Modello della farmacodinamica del Pramlintide e sua inclusione nel simulatore del diabete di tipo 1

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Abstract

This thesis investigates the functions and prospective of Pramlintide, a drug used in combination with insulin for diabetes type 1 patient. This drug has been approved by FDA in late 2005 and it's being commercialized nowadays only in USA.The aim of this thesis is to develop a mathematical model which allows the quantitation of the effect of Pramlintide and to incorporate it into the meal simulation model of the glucose-insulin system. So starting with the introduction of what this new medicinal is, I go through a description of its dynamics, than I go through the model description and its inclusion in a Glucose-Insulin simulation model. These latest in silico *experiments should finally prove the benefits of Pramlintide action* vs *a usual insulin treatment.*

Due the fact that all therapy are focused on insulin therapies, the addiction of Pramlintide makes the blood glucose control problem less "insulincentric" giving the opportunity to achieve a better results in either diabetes mellitus type 1 and 2.

Sommario

Questa Tesi e' proposta in inglese poiche' sviluppata negli Stati Uniti d'America presso il centro di tecnologie per il diabete dell'University of Virginia nel 2012. In questa tesi ci si propone di investigare le possibilita' di un farmaco che si chiama Pramlintide, in commercio solo negli USA, che e' usato per la cura del diabete mellito sia di tipo 1 che 2. Partendo da cosa sia e quali sono i suoi effetti fisiologici, si passa ad una descrizione modellistica della sua dinamica. Una volta identificato l'opportuno modello di azione, sia nel caso di soggetto medio che individuale, con le relative ipotesi statistiche, lo inserisco per prove in silico in un simulatore del sistema Glucosio-Insulina, per dimostrare cosi', l'effettiva efficacia di questo nuovo medicinale. Quello che mi aspetto da questo farmaco e' un migliore controllo della concentrazione del glucosio, specialmente dopo un pasto. Il trattamento con Pramlintide non sostituisce quello regolare insulinico, ma lo integra.

Contents

Chapter 1

Introduction

1.1 Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1].

1.1.1 Diabetes mellitus type 1

Diabetes mellitus type 1 (Type 1 diabetes, T1DM, IDDM, or, formerly, juvenile diabetes) is a form of diabetes mellitus that results from autoimmune destruction of beta-cells of the pancreas. The subsequent lack of insulin leads to increased blood and urine glucose. The classical symptoms are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss. All these causes are due to the fact that the type 1 body is not able to use glucose as energetic source but uses other nutrients like fats. Type 1 is induced by genetically factors and usually appears in young age. It requires external insulin to make the subject live. Less than 10% of diabetes suffers of type1.

1.1.2 Diabetes mellitus type 2

Diabetes mellitus type 2 (formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency. The development of type 2 diabetes is caused by a combination of lifestyle and genetic factors. While some are under

personal control such as diet and obesity others such as increasing age, female gender, and genetics are not. It presents the same symptoms of type 1 and more compliances.The 90% of diabetes suffer of type 2[1] .

This paper regards mostly the case of diabetes type 1 and some references to the type 2. The other sort of non-mellitus diabetes, e.g. diabetes insipidus[2], is not topic of interest.

1.1.3 Epidemiology

Standing to United Nations publications the diabetes population is 6% of world population, it's about 240 millions of people. Time ago diabetes was mostly in the industrialized countries where the life style helped the disease. Nowadays diabetes is growing in the developing country, e.g. Central America and Arabia (Fig. 1.1, 1.2 and 1.3) due the new *junk* food available to low-class people. Diabetes hits both men and women in a range between 20 and 79 years old (unfortunately its growing in younger generation). WHO (World Health Organization) defines diabetes as epidemic, in fact the statistic says that diabetes has an exponential increase and in the 2025 the diabetics will be 380 millions, in which 80% from medium/low income country. WHO projects that diabetes deaths double from 2005 to 2030. This important disease is also expensive to cure. Its estimated that USA and Europe use 15/20% of health resources for diabetes, of which the 38% are used for the terrible diabetes compliances like ischemia, heart diseases, renal insufficiency and so on. For example the disease was estimated to cause $10.5 billion in annual medical costs ($875 per month per diabetic) and an additional $4.4 billion in indirect costs ($366 per month per diabetic)[2].

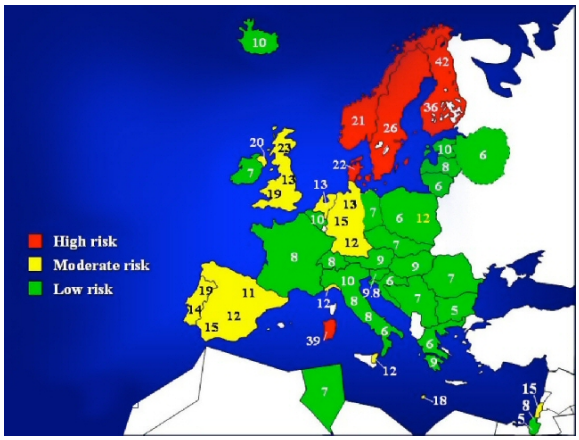

Figure 1.1: European Estimation Risk by IDF, 2007.

[1] In the 10% of not type 2 there aren't only the type 1, also the gestational diabetes (2% of diabetes population): this type of diabetes occurs in women who never had diabetes before but in pregnancy they present high glucose level.

[2] A rare disease due vasopressin dysfunction caused by a brain damage.

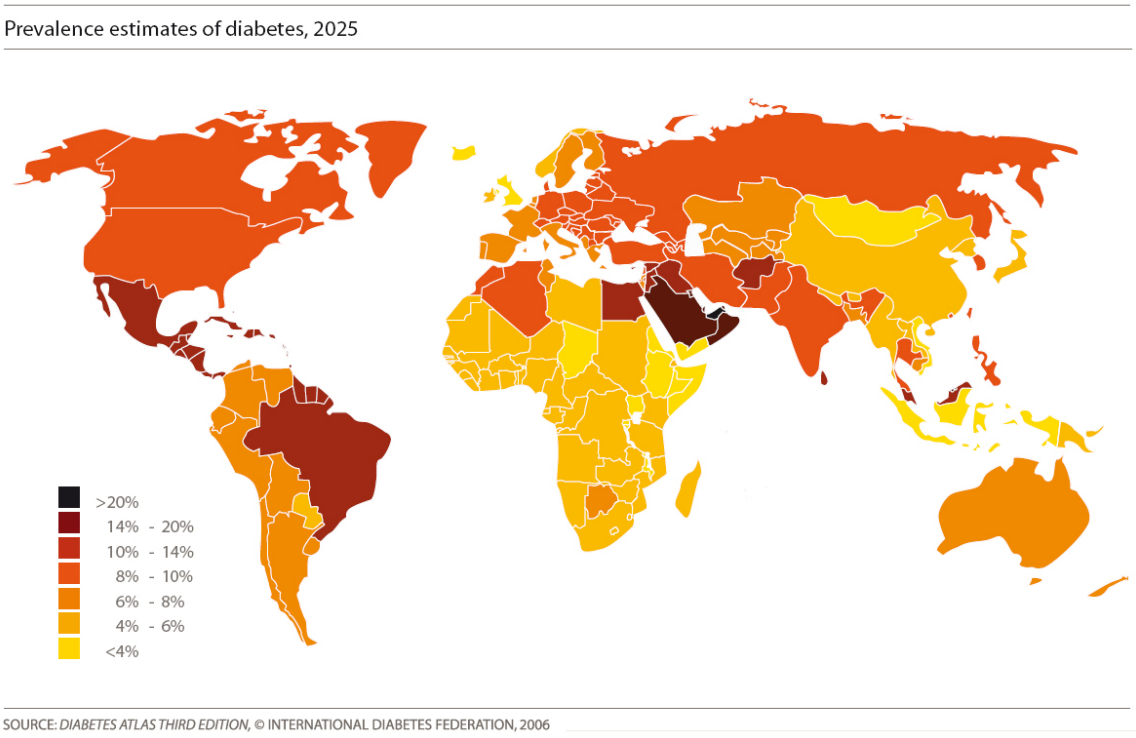

Figure 1.2: See how the middle income countries (e.g. Mexico, Brazil or Saudi Arabia) are in an alarming condition about their diabetes prevision.

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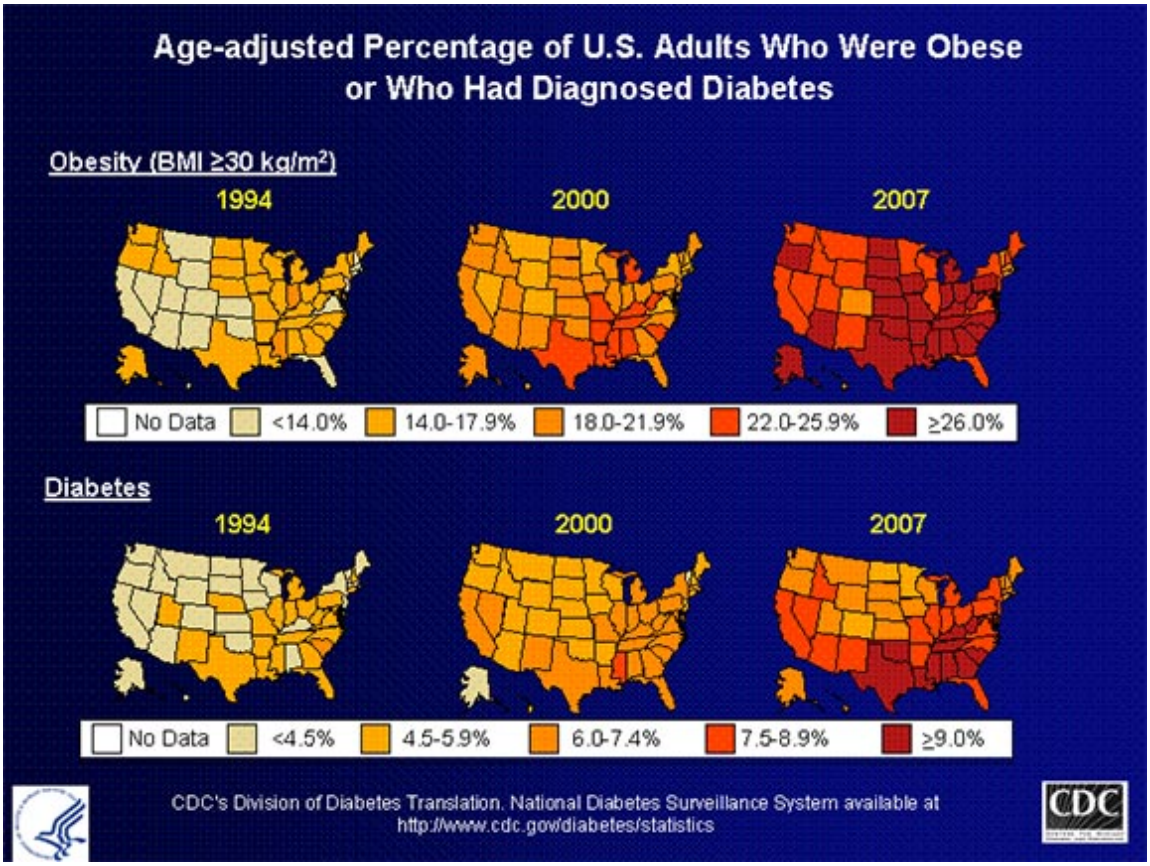

Figure 1.3: American Map: alarming data are estimated from US population (by US Health Center). Nowadays one third is overweight and another one third is obese, all conditions, especially the second one, which helps diabetes developing.

1.2 Physiology of Glucose and Insulin System

Insulin, a catabolic protein hormone, is secreted by beta-cells of pancreas. Its main function is to lower glycemia, i.e. the blood sugar (glucose) concentration when its high. Its antagonist is the glucagon, which is released by alpha-cells of pancreas with the function of raising the BG (blood glucose) whenever this is too low. This synergetic action is well regulated in healthy subjects. In fact when the glycemia raises, e.g after a meal, the glucose sensors, placed in many bodys parts, stimulate the release of insulin. This hormone gets the membrane of specific tissues more permeable: these tissues are called insulin dependent tissues. At this classes of tissues belong all tissues that need insulin for better using the glucose energy and store the glucose under glycogen, the human glucose reserve, lowering the BG (striated muscle, the heart, the lipid tissue and also could be the liver). It also, inhibits the gluconeogenesis in the liver and promotes the stocking of triglycerides as fat tissue (lip genesis). As the antagonist, glucagon stimulates the breaking of glycogen (glycolysis) and promotes the gluconeogenesis in the liver increasing the glucose fluxes on blood. Moreover there are tissue that are strictly dependent on glucose and independent on insulin action. The glucose dependent tissues (or insulin independent tissues) need glucose for survive and consume it almost-constantly during the day: they're the brain, the kidney and erythrocyte. Its important to remind that a meal is not usually only the ideal glucose dose but a mixed meal, in fact insulin rises in blood concentration with all sort of meal like proteins and fats not only with sugars[3].

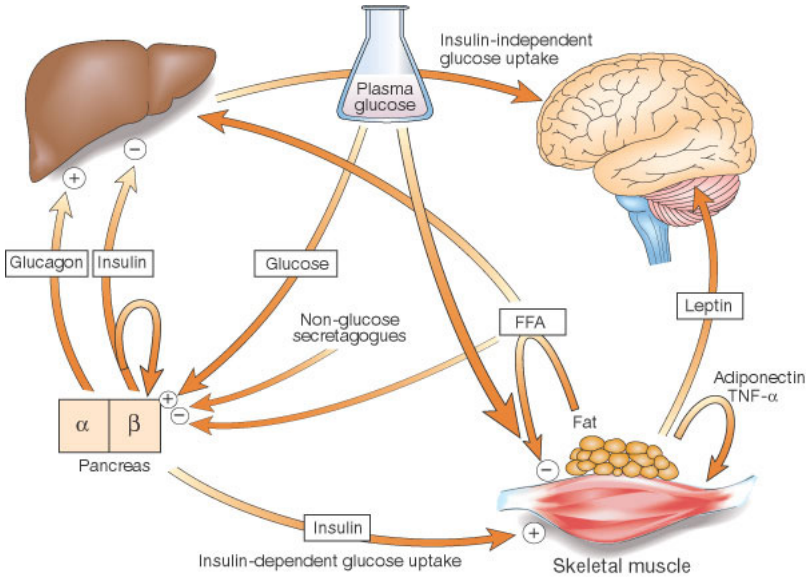

Figure 1.4: Glucose-Insulin regulation.

1.2.1 Hypoglycemia vs. Hyperglycemia

Hypoglycemia is a condition that occurs when the BG is too low. Throughout a 24-hour period blood plasma glucose levels are generally maintained between 4-8 mmol/L (72 and 144 mg/dL)

[3] .

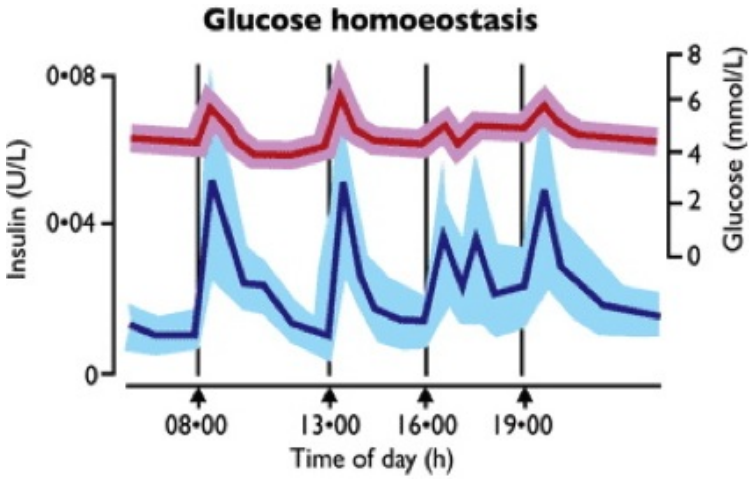

Figure 1.5: Glucose (red Line) and Insulin (blue line) patterns during a normal day in healthy subject.

Although 3.3 or 3.9 mmol/L (60 or 70 mg/dL) is commonly cited as the lower limit of normal glucose, symptoms of hypoglycemia usually do not occur until 2.8 to 3.0 mmol/L (50 to 54 mg/dL). The precise level of glucose considered low enough to define hypoglycemia is dependent on: the measurement method, the age of the person, presence or absence of effects, and the purpose of the definition. While there is no disagreement as to the normal range of blood sugar, debate continues as to what degree of hypoglycemia warrants medical evaluation or treatment, or can cause harm. Hypoglycemic symptoms and manifestations can be divided into those produced by the counter-regulatory hormones (epinephrine/adrenaline and glucagon) triggered by the falling glucose, and the neuroglycopenic[4] effects produced by the reduced brain sugar. Hyperglycemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood plasma. This is generally a glucose level higher than (200 mg/dl). Reference test range for blood tests are 11.1 mmol/l, but symptoms may not start to become noticeable until even higher values such as 250-300 mg/dl or 15-20 mmol/l. A subject with a consistent range above 126 mg/dl or 7 mmol/l is generally held to have hyperglycemia. Chronic levels exceeding 7 mmol/l (125 mg/dl) can produce organ damages. The damage of this ones are basically associated to a chronic status like diabetes mellitus. Fast BGs peaks are considerate not danger, but recent studies show how these isolates hyperglycemia episodes but frequently during a day or a week (in a short time generally and in p.p. period) comport microvascu-

[3]Glucose concentrations are expressed as milligrams per deciliter (mg/dL or mg/100 mL) in the United States, Japan, Spain, France, Belgium, Egypt, Saudi Arabia and Colombia, while millimoles per liter (mmol/L or mM) are the units used in most of the rest of the world. Glucose concentrations expressed as mg/dL can be converted to mmol/L by dividing by 18.0 g/dmol (the molar mass of glucose).

[4]Shortage of glucose in the brain. It affects the neuronal function altering brain functions and behavior. Recurrent or prolonged it could take the subject to brain damage, coma and then death.

lar diseases[5]. So its the aim of physicians and engineers maintain the BG in the normal range (euglycemic) lowering the peaks of glucose (especially after a meal) but absolutely avoiding the possibility of hypoglycemia. Also it will be good reducing the BG variability during short time therapy[4].

1.2.2 Gastric Emptying

Despite all glucose fluxes have been basically attributed to an interaction between insulin and glucagon, one of them, the rate of appearance of glucose on blood (i.e. how the sugar passes from the digestive system to blood), has not. Indeed only 50% can be explained with usual insulin-glucagon system. The other 40/50% can be explained with the gastric emptying system[7]. The gastric emptying is a complex body's feedback regulation that permits to control the rate of release of glucose from splanchnic organ to bloodstream (Rate of appearance, Ra). Indeed when the BG is too high, the Ra is slowed down, otherwise the Ra is speeded. Gastric emptying is modulated by feedback mechanisms arising from the interaction of nutrients with the small intestine. Diabetes type1 has a difficult gastric emptying regulation and the main reason is the lack of a hormone, the amylin[6]. This

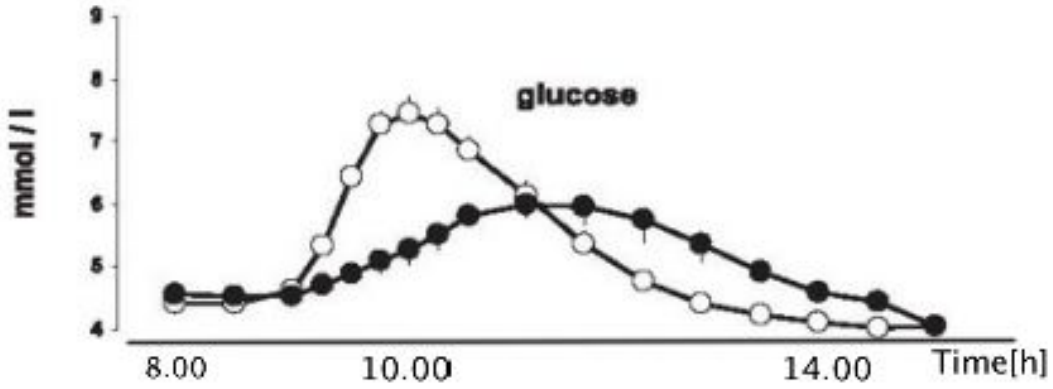

Figure 1.6: In the figure we see a quick rate of appearance in diabetes with exogenous insulin (white dotted) vs. normal subject (black dotted) in postprandial period (p.p., i.e. is the time, about 2 hours, after a meal. The opposite is preprandial period.) . In diabetes the fast Ra can produce a quick hyperglycemia which even if is not so dangerous its recently considered harmful.

entails a different Ra than healthy subject (Fig.1.6).

1.2.3 A1C

The American Diabetes Association (ADA) assesses a good diabetes therapy referring to A1C parameter. A1C or Glycated Hemoglobin or HbA1c is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way . This serves as a marker for average blood glucose levels over the previous months prior to the measurement. In the 2010 ADA Standards of Medical Care in Diabetes added the A1c greater equal to 48 mmol/mol (greater equal to 6.5% from normal value) as another criterion for the diagnosis of diabetes. In diabetes mellitus, higher amounts of glycated hemoglobin, indicates poor control of blood glucose levels. But A1C tests doesn't measure your day-to-day control. You can't adjust your insulin on the basis of your A1C tests. That's why your blood sugar checks and your lag of results are so important to staying in effective control. ADA fixes the AIC target at <7% for a good diabetes treatment[1].

1.3 Regular therapies for type 1

Type 1 is treated with insulin replacement therapy (insulin therapy), either via subcutaneous injection or insulin pump (or an insulin pump that inject subcutaneously, CSII), with attention to dietary management, typically including carbohydrate tracking, and careful monitoring of BG levels using glucose meters[5]. Today, the most common insulin are biosynthetic products produced using genetic recombination techniques; formerly, cattle or pig insulin were used, and even sometimes insulin from fish[8]. A more recent trend, from several suppliers, is insulin analogs, which are slightly modified insulin with different onset or duration of action times (mostly is used in combination: the fast insulin after the meal, and the slow one for night). As said before untreated type 1 diabetes commonly leads to coma, often from diabetic ketoacidosis, which is fatal if untreated. Continuous glucose monitors (CGM) have been developed and marketed. This device can alert patients to the presence of dangerously high or low blood sugar levels, in fact this technique allows the almost constant monitoring of BG and a minimum of invasiveness. But technical limitations have slowed the impact of these devices on clinical practice so far. Hypoglycemia is a very common occurrence in people with diabetes in treatment; usually its the result of a mismatch in the balance among insulin, food and physical activity, although the non-physiological method of delivery also plays a role[9]. In more extreme cases, a pancreas

[5]The most common are the home version (HBGM): a small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level (self test). The meter then displays the level in mg/dl or mmol/l.

transplant can restore proper glucose regulation. However, the surgery and accompanying immunosuppression required is considered by many physicians to be more dangerous than continued insulin replacement therapy, so is generally only used with or some time after a kidney transplant[10]. Experimental replacement of beta cells (by transplant or from stem cells) is being investigated in several research programs. Islet cell transplantation is less invasive than a pancreas transplant[11].

1.4 Pramlintide

Pramlintide[6] is the synthetic analog of human amylin. Amylin (IAPP) is a human hormone (protein) co-secreted with insulin by beta-cell islets in pancreas. It is the second beta-cells hormone[14]. The secretion pattern of both peptides during glucose or glucose plus arginine stimulation is identical (Fig. 1.8). The molar ratio of amylin amounts to 10% of that of insulin[15].

The pramlintide is a drug approved by FDA in 2005, and it's the only second drug approved for T1 diabetes after insulin that was approved in early twentieth century . Amylin has essentially two main functions (pramlintide either) in human body (Fig. 1.10):

1. MEAL DERIVED FUNCTION: slows the gastric emptying in response of meal;
2. LIVER DERIVED FUNCTION: participate in glucagon regulation[12].

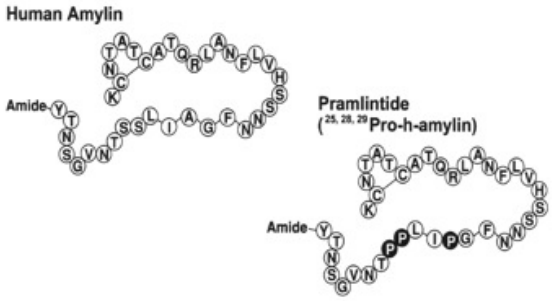

Figure 1.7: Amylin vs Pramlintide molecules: the twos are identical a part an amino acid substitutions.

Secreted in blood stream with a pattern similar to insulin amylin is absent in type1 patients as well as insulin as shown in Fig. 1.9 where there's a comparison between of daily pattern of insulin *vs* amylin in different amplitude scale.

In diabetes things change and it is well shown in the graphic (Fig 1.9) below where amylin concentration in T1 is basically not existent and in T2 is lower than healthy[13].

[6] Amylin Pharmaceutics Inc., San Diego, CA, USA.

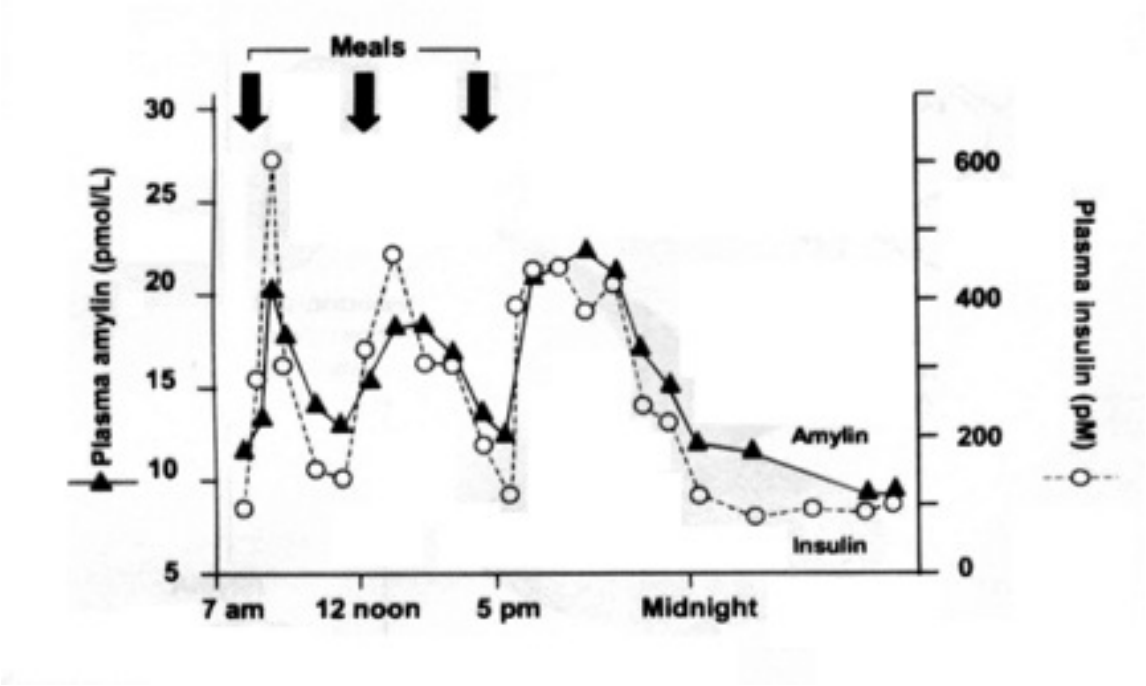

Figure 1.8: Insulin(empty dots) vs Pramlintide (triangle dots) with three meals.

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1.5 Therapies with pramlintide in type 1

Many type 1 diabetic patients, if not most, don't achieve and don't sustain, for long time, the optimal glucose profile just using injected insulin (even with CGM, CSII and insulin rapid and slow technologies) due to physiological barriers. The barriers are mainly these:

1. The exogenous insulin (subcutaneous and peripheral) has not the same dynamic of real one (plasmatic): the interstitial injection instead plasmatic and peripheral instead portal circulation creates delays and problems in control. For instance the injection in peripheral part of body gets liver unable to suppress appropriately the glucagon action (it doesn't have the insulin ready in its area), causing a systematic high BG. So the external controller injects more insulin increasing the risk of in hypoglycemia without counting that more insulin it's used more is the risk of weight gain.[7].

[7]Insulin helps the fat genesis[25]

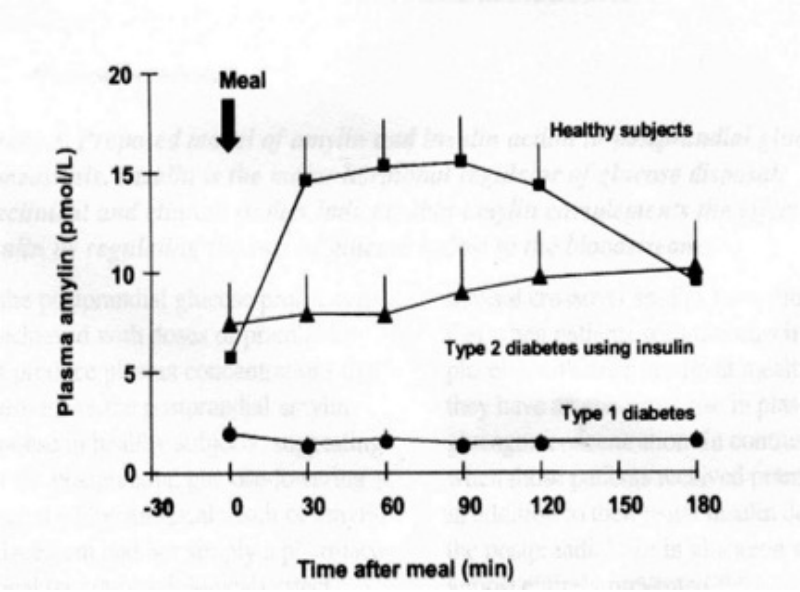

Figure 1.9: Amylin pattern in healthy, T2 and T1 subjects.

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2. In diabetes is well known the high level of glucagon in p.p. period. The cause is not so certain but this issue takes quickly the system in hyperglycemia in p.p. period until the external amount of insulin inhibits its production.

3. The less considerate contribution of gastrointestinal tract to glucose regulation: Ra, in fact, is a determinant factor of p.p. glucose pattern influenced by, not only the amylin, but also by secondary hormones, e.g. CCK, GLP-1 and GIP.

The pramlintide must be injected with regular insulin therapy but in different siring. It cannot be alone as a solo treatment[17]. The first studies about pramlintides benefits in 2003 showed how the injection of pramlintide with either regular or rapid insulin lowers glucose excursion after a meal[8]. But their main result was that diabetes must take this drug during or just before eating to see the best results (full squared and full dotted patterns, Figure 1.11)

It's noticeable that in insulin lispro (a brand of rapid insulin) the action of the rapid insulin with all sort of pramlintide injection take the system in hyper BG. This issue is probably due to the fast insulin, which disappears quickly than regular. Other thing: in pramlintide therapy it should be used less insulin, about 30% less at each meal, to lower the potential of weight gain and overall to avoid the hypoglycemic area. Moreover the AUC is reduced significantly: this parameter is important for set controller and parameters for diabetes (as

[8]Standard mixed breakfast at 7.00 am in 5 minutes after a fast night.

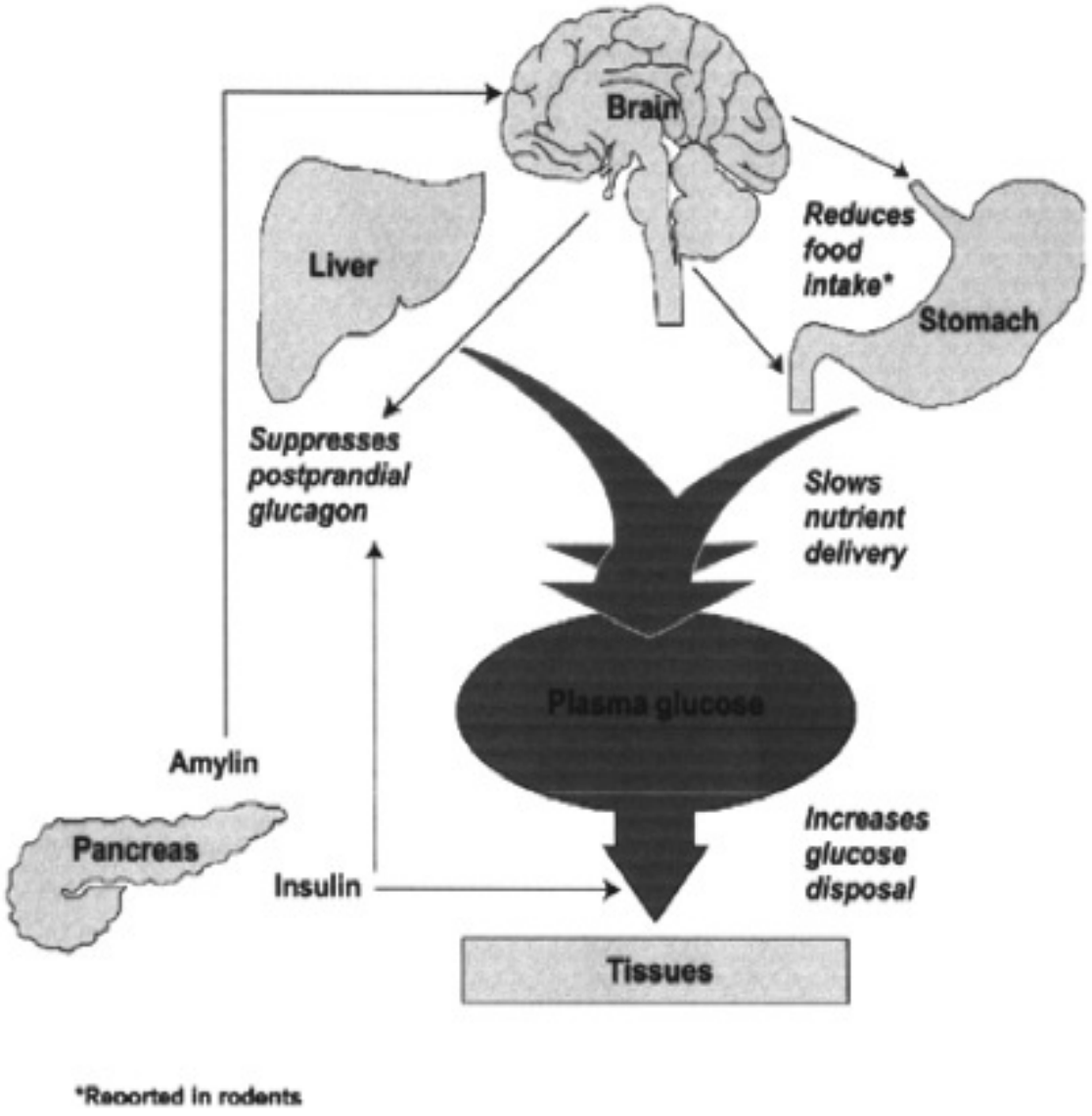

Figure 1.10: Amylin physiological system.

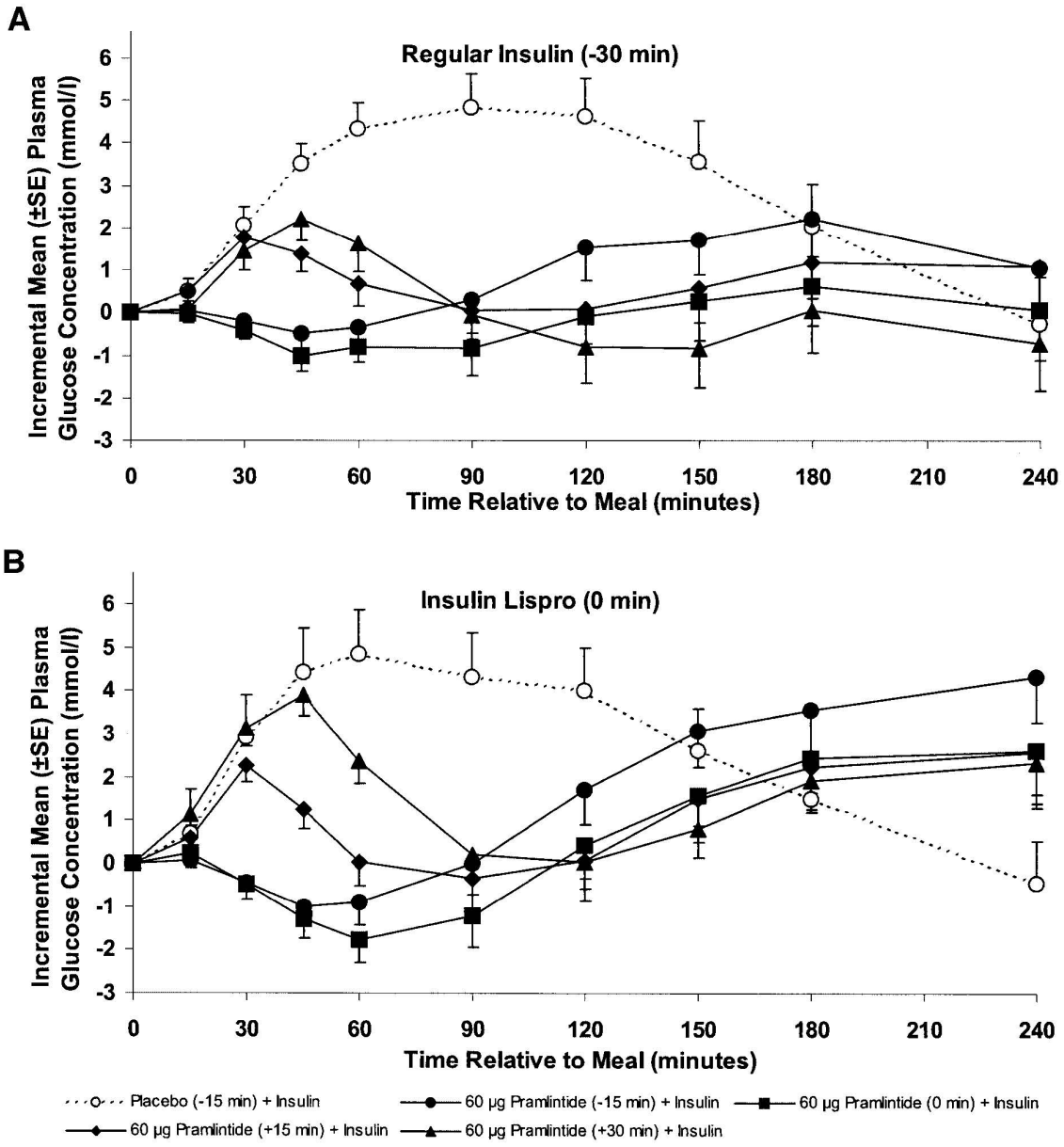

Figure 1.11: Different p.p. periods patterns for a pramlintide treatment. From either A (regular insulin) and B(rapid insulin) experiments, it's clear that the best pattern results from the administration of pramlintide at or just before the meal ingestion.

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insulin sensitivity). Lowering the pp glucose excursion is the main visible effect of pramlintide in diabetes T1.[18] This is physiologically due to the double action (meal derived and liver derived) of this drug, which simulating the amylin, delays the gastric emptying, allowing a slower glucose releasing on blood, and suppresses glucagon production, lowering the glucose production on blood. Experimental investigations in rodents indicate that the effects of amylin, and by inference pramlintide, on nutrient delivery (gastric emptying) are mediated via a central pathway that involves the area postrema and visceral efferents of the vagus nerve[26]. The area postrema in the brainstem contains a high density of amylin binding sites, and it is exposed to changes in plasma amylin and glucose concentrations because it does not have a blood-brain barrier[27]. Selective lesioning of the area postrema and/or bilateral vagotomy abolishes the effect of amylin on gastric emptying, demonstrating the importance of this central pathway in mediating amylins physiological functions[28], Fig.1.10. The second function is a very clever regulation; indeed the amylin begins to suppress glucagon production after a meal trigger. The suppression continues till the BG is in euglycemic range but stops when the BG goes in hypoglycemic status[29].

Another interesting finding was that the addition of pramlintide to insulin therapy also reduced postprandial triglyceride excursions, an effect that might be attributable to pramlintide's effect on gastric emptying. By slowing the delivery of all nutrients (carbohydrates, lipids, and proteins) from the stomach to the small intestine, pramlintide likely tempers not only the inflow of glucose into the circulation, but also the inflow of chylomicrons and other meal-derived lipoproteins. This may help to better match the patients capacity for lipoprotein clearance, thereby limiting the postprandial triglyceride excursion. Given that many patients with diabetes experience postprandial hyper- and dyslipidemia, and that this abnormality has been implicated as a potential cardiovascular risk factor, additional studies are warranted to further examine the postprandial lipid-lowering effect of pramlintide(Fig.1.12)[16].

1.5.1 Long Time Therapy

As defined before a good therapy must be within an A1C variation of <7%. In a 12-month study duration has been investigated the long-term glycemic control in patients with insulin-requiring T1 and T2 diabetes[20]. In all cases pramlintide were administered in addition to patients existing insulin regimens. These studies consistently demonstrated that the addition of pramlintide to pre-existing insulin therapy improved overall glycemic control in patients with either type 1 or type 2 diabetes, as evidenced by significant reductions in A1C of about 0.5-1.0% from baseline and about 0.3-0.5% compared to placebo . Furthermore, the proportion of patients who were able to achieve ADA glycemic targets (A1C <7%) were two- to threefold greater with pramlintide plus insulin than with insulin alone. Stratification by baseline body mass index (BMI) revealed that pramlintide tended to prevent weight gain in patients who were lean at study entry and induced increasing

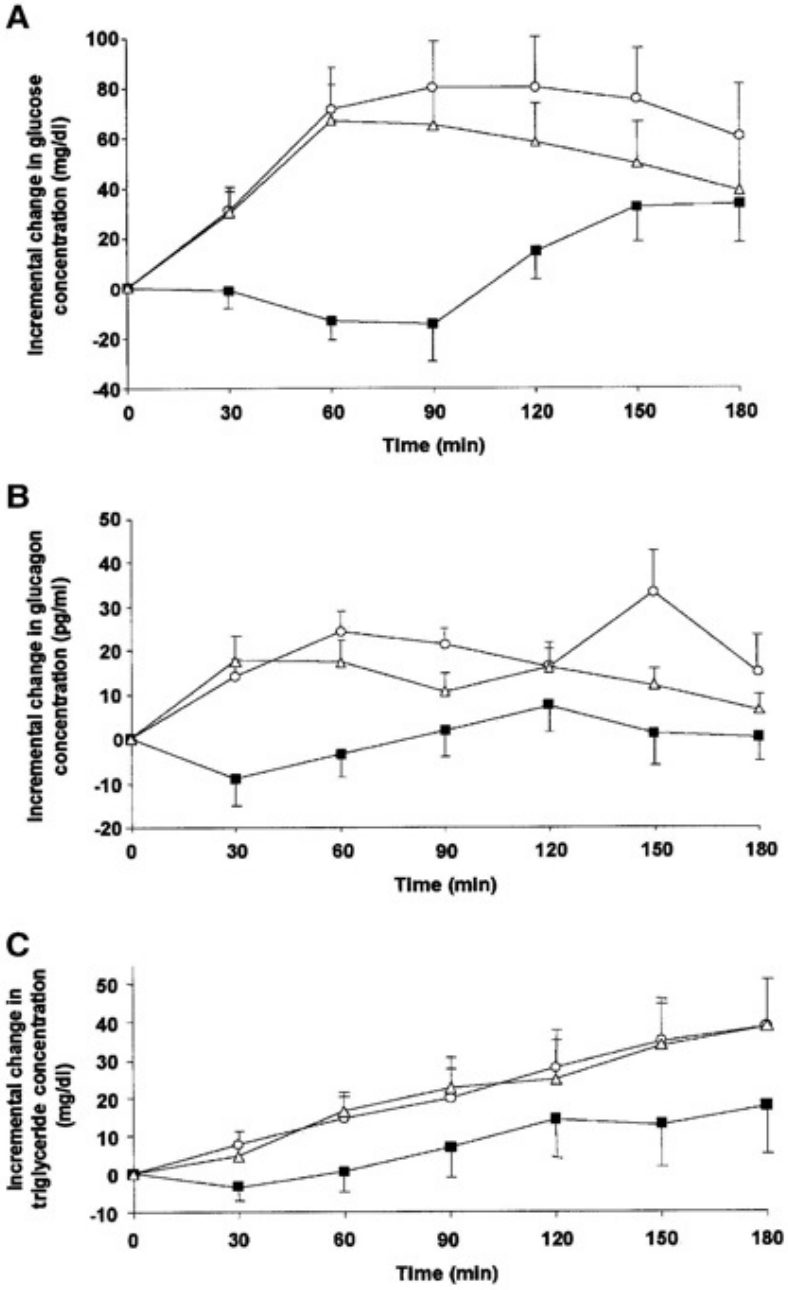


Figure 1.12: The circles line is the excursions at baseline (i.e. at the beginning of treatment, week 0), the squared line is in the middle of treatment (week 4) and the triangle one is the end of treatment (week 6). We observe that pramlintide effect lowers the glucose excursion (A), glucagon excursion (B) and triglycerides excursion (C), for a long term study.

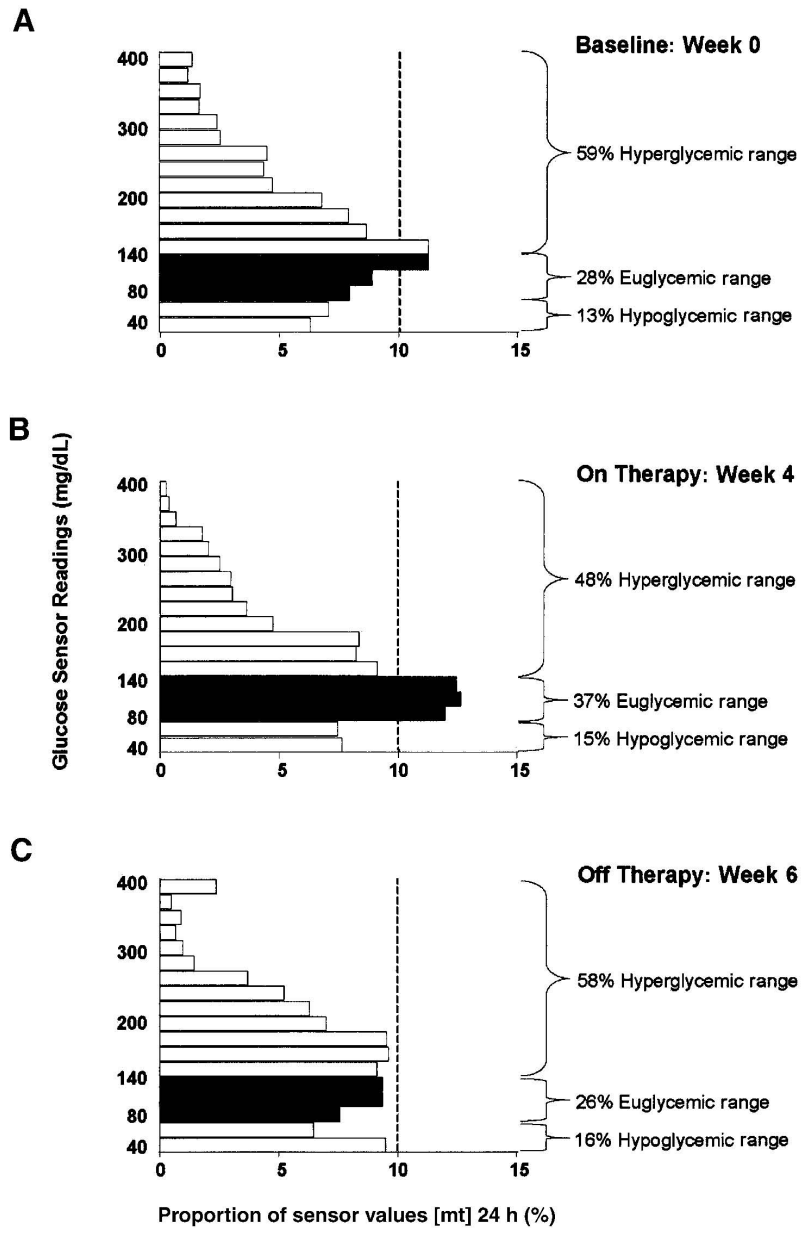


Figure 1.13: Reduction of Hyperglycemia status and increasing of euglycemic range, in week 4, i.e. in the middle of pramlintide treatment.

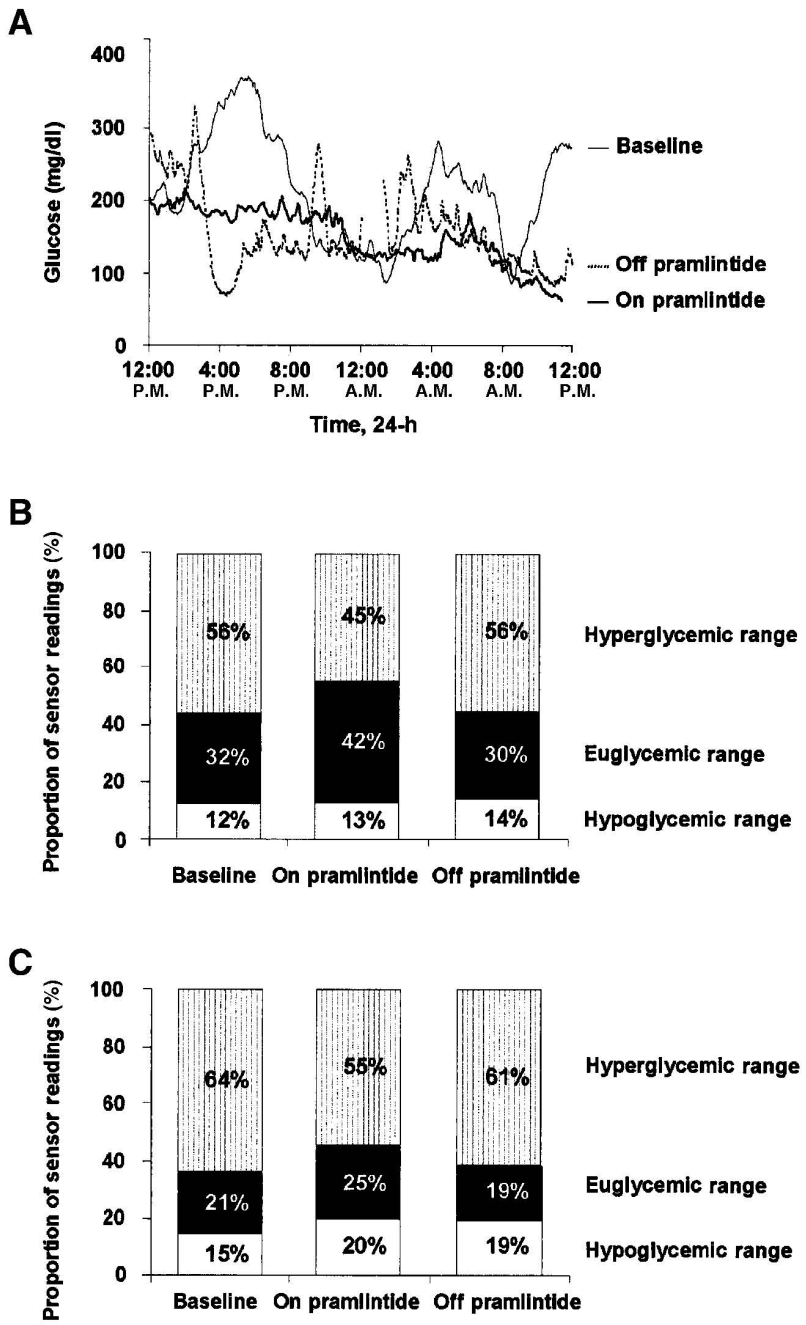


Figure 1.14: Sensor's point of view of a single subject. Similar results as shown in Fig 1.13.

amounts of weight loss in overweight and obese patients. This weight loss averaged 1.6 kg in patients with type 1 diabetes with a BMI >27 kg/m^2 and 2.4 kg in patients with type 2 diabetes with a BMI >35 kg/m^2 after treatment with pramlintide for 26 weeks. 56 pooled analyses of data from the long-term trials in patients with type 1 or type 2 diabetes showed that twice the number of pramlintide than placebo treated patients achieved a simultaneous reduction in both A1C and body weight. The long-term improvement of glycemic control with pramlintide was not associated with an increase in the overall event rate of severe hypoglycemia, as it is often seen when glycemic control is improved by intensification of insulin therapy [19](Fig 1.15).

1.5.2 BG Variability

A1c, like others measures of long-term average glycemia, is not designed to capture the rate and the magnitude of acute BG fluctuations, which affect the glucose variability. First, in clinic trial, only 7-point glucose profiles, taken at 3-month intervals, are usually available for analysis of glucose variability. Second glucose variability is quantified using standard deviation, which has been shown to be a poor measure of variability-associated risks. A recent report confirmed that: variability in blood glucose (BG) around a patients mean value has no influence on the development or progression of retinopathy or nephropathy. However, the conclusions of this report were confined to microvascular complications. A1c is so insufficient for determining the risk for the full spectrum of complications associated with diabetes. This is alarming because the hyperglycemic excursions, usually not detected by A1c, are a factor that contributes to morbidity associated with diabetes[9]. Therefore it's needed a therapy that lowers A1c but at same time minimizes acute BG extremes. Pramlintide could be an answer. It could be calculated, using the SMBG[10] readings, a quadratic risk function: the left wing of the quadratic risk function is LBGI, an estimator that calculates the risk of hypo., and the right wing is HBGI, which calculates the risk of hyper. Put them two together the quadratic function is still created, with the minimum value of 0 achieved at BG=6.25 mmol/L (112.5 mg/dL), which its a safe euglycemic reading. The readings are plotted in a scatterplot in Fig 1.16.

The BG is calculated like the difference between the y (the p.p. value) and x (the preprandial value) of a patient. We see from Fig 1.16 that the black cluster is moved down respect the placebo (white) one, which is a good thing, meaning that the pramlintide treated sub-

[9]Recently it has found a mood and cognitive disturbance when severe hyperglycemia occurs.
[10]Self Monitored Blood Glucose.

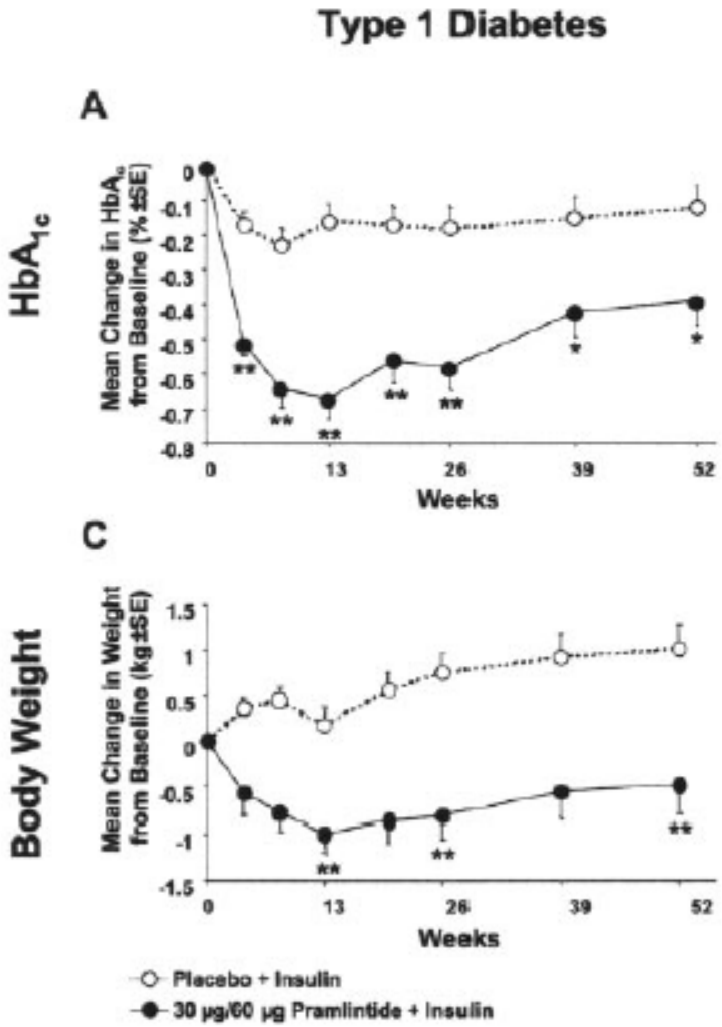


Figure 1.15: Statistical significance is denoted by P <0.05. In both types of diabetes, addition of pramlintide to existing insulin therapy led to significant and sustained reductions in both A1C (A) and body weight (B).

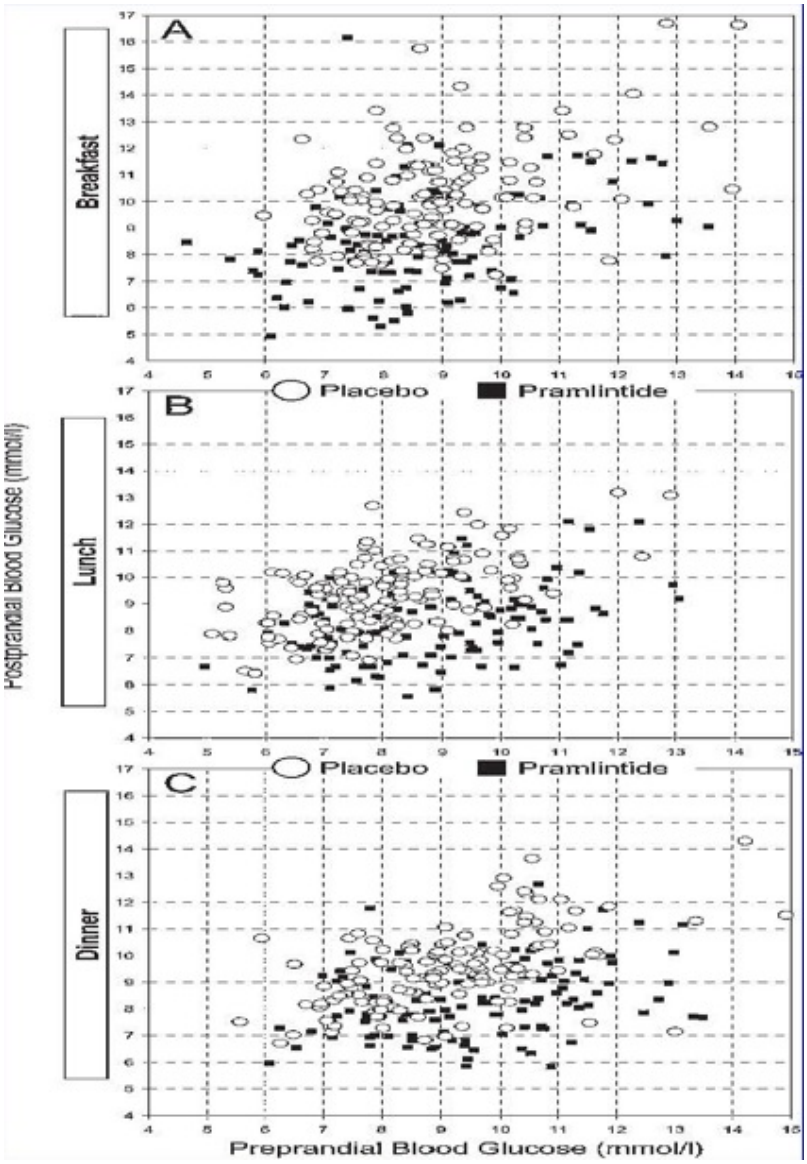


Figure 1.16: (AC) Scatterplots of the pre- to postprandial glucose excursions observed during the maintenance phase of the study at breakfast, lunch, and dinner, respectively. The data of the pramlintide group (black squares) are generally below the data of the placebo group (circles), indicating lower postprandial glucose excursions on pramlintide.

jects reduced their variability (below an imaginary bisector). Only breakfast has a little movement to left risking hypo. but its not relevant. The values are calculated and reported in Fig 1.17.

	Meal	Pramlintide		Placebo		Group effect (F and P level)[a]
		Pre-meal	Post-meal	Pre-meal	Post-meal	
A. Dynamics of postprandial glucose excursions						
Pre- to postprandial BG rate of change [(mmol/L/h)/(mg/dL/h)]	Breakfast	−0.12/−2.2		1.02/18.4		$F = 83.3$
	Lunch	−0.41/−7.2		0.93/16.7		$P < 0.0001$
	Dinner	−0.89/−16.0		0.27/4.9		
Average BG [(mmol/L)/(mg/dL)]	Breakfast	8.7/156.6	8.5/153.8	8.9/160.2	10.2/183.6	$F = 80.6$
	Lunch	8.8/158.4	8.3/149.4	8.0/144.0	9.3/167.4	$P < 0.0001$
	Dinner	9.6/172.8	8.4/151.2	9.1/163.8	9.5/171.0	
B. Risk analysis of pramlintide effect						
LBGI (risk for hypoglycemia)	Breakfast	3.1	—	2.7	—	$F = 10.3$
	Lunch	2.9	—	4.0	—	$P = 0.002$
	Dinner	2.1	—	2.6	—	
HBGI (risk for hyperglycemia)	Breakfast	5.4	5.1	5.6	8.2	$F = 73.1$
	Lunch	5.7	4.4	4.2	6.2	$P < 0.0001$
	Dinner	7.5	4.7	6.4	6.7	

[a]All analyses include average BG as a covariate; thus the significance level is independent of average glycemia.

Figure 1.17: Effects of pramlintide on the dynamics of pre-to postprandial BG excursions and on the risks of hypoglycemia and hyperglycemia.

It is important to point out that the reduction of BG variability is independent from the reduction of average glycemia. Indeed the glycemia average improvement (lowering) happens even in placebo group. Being A1c bounded to the BG average this is consistent to the previous consideration. Moreover the insulin injection amount s reduced during treatment of 30-50% and not raising the hypoglycemia risk [21].

Chapter 2

Meal Simulation Model of Glucose-Insulin system

2.1 Introduction to Global Model of Glucose/Insulin System

The important work, to which I will always refer, is the Meal Simulation Model of the Glucose-Insulin System in normal humans by Cobelli, Dalla Man, and Rizza[22]. The notable thing of this model is that the provided main glucose-insulin fluxes, e.g. Ra, endogenous glucose production, utilization of glucose and so on, are estimated in independent-model way, to estimate the sub-models parameters[23]. The whole system is decomposed into subsystems; each one has its estimated parameters with estimation methods (Fig.2.1). For example the Glucose System and Insulin System are represented in Fig.2.2 . The model is also good for simulating the daily life and takes care about the meal taken orally, which makes this model more complicated but more realistic as well (useful for oral test etc.). The identification process has been made using forcing function and using average datas of a population of 204 normal subjects. Also an addition study on 14 types 2 diabetes has been conducted.

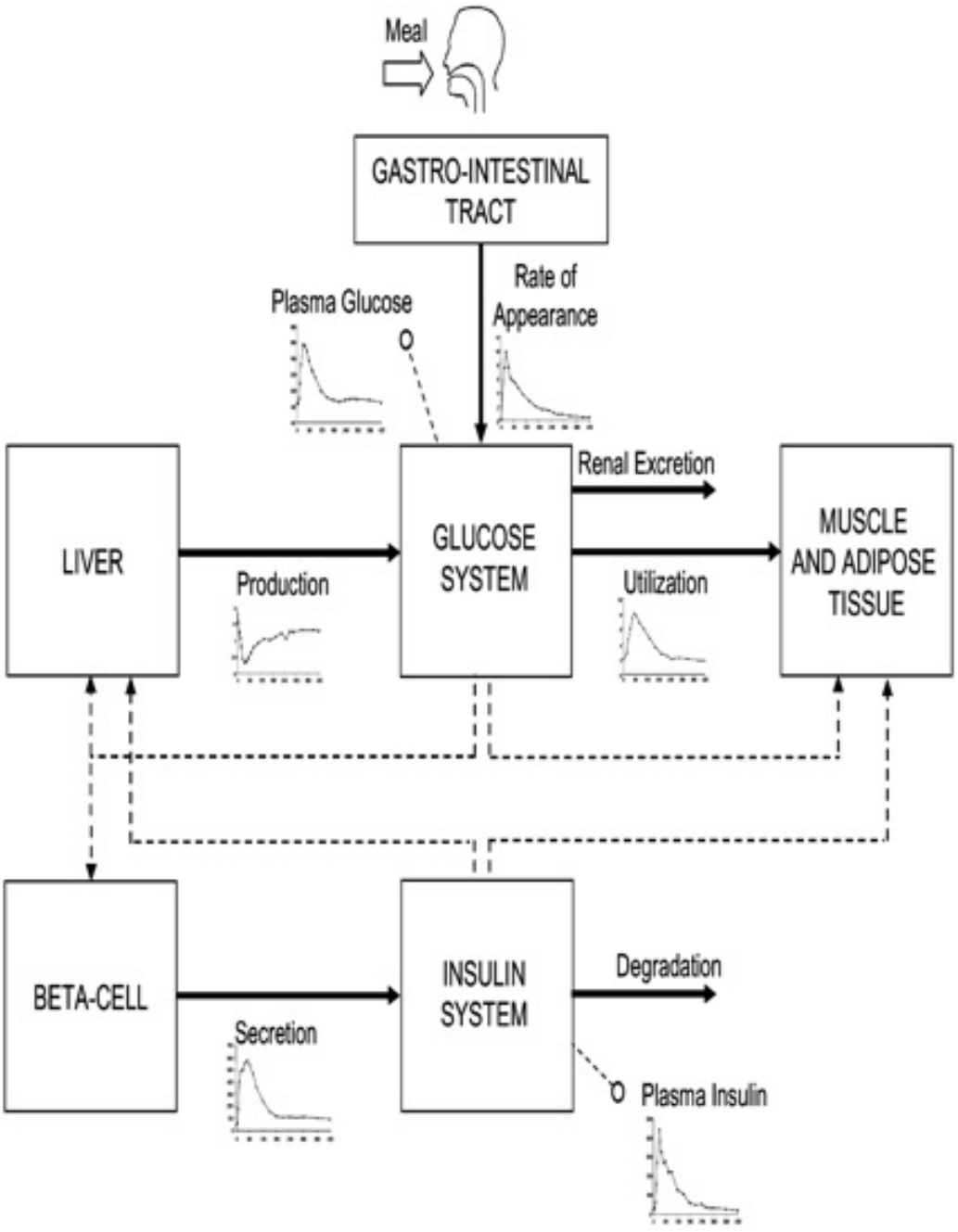


Figure 2.1: Scheme of the glucose-insulin system which puts in relation the measured plasma concentration, i.e. glucose G, and insulin I, and glucose fluxes: Ra, production EPG, utilization U, renal extraction E; and to insulin fluxes: secretion S and degradation D.

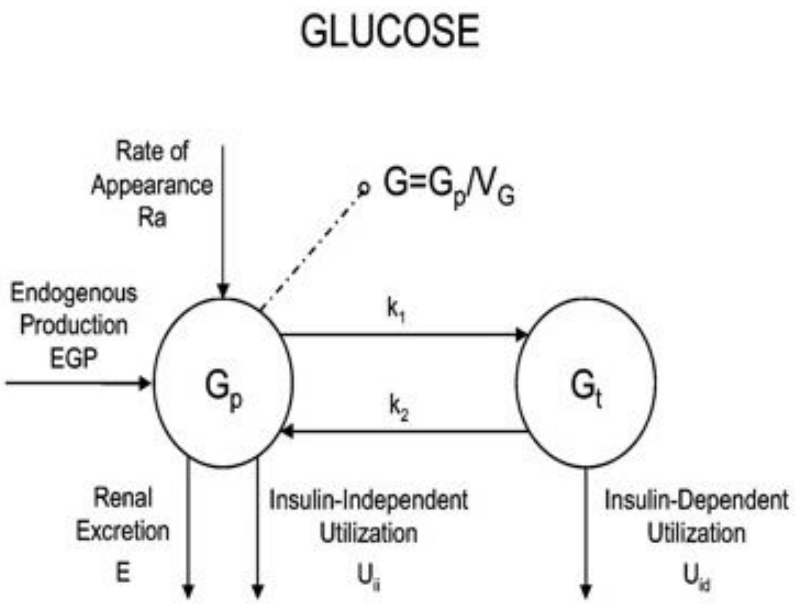


Figure 2.2: Scheme of glucose subsystem.

2.1.1 Glucose Subsystem

Here It is presented in detail only one subsystem. I present only one of the many subsystem's equations, the glucose subsystem (Fig. 2.2). By extension the other systems are described in the same way. The model identification is available in [32, 33]. The glucose model is composed by two compartments described by equations:

$$\begin{cases} \dot{G}_p(t) = EGP(t) + Ra(t) - U_{ii}(t) - E(t) - k_1 \cdot G_p(t) + k_2 \cdot G_t(t) & G_p(0) = G_{pb} \\ \dot{G}_t(t) = -U_{id}(t) + k_1 \cdot G_p(t) - k_2 \cdot G_t(t) & G_t(0) = G_{tb} \\ G(t) = \frac{G_p}{V_G} & G(0) = G_b, \end{cases} \tag{2.1}$$

where G_p and G_t are glucose masses in plasma: rapidly equilibrating tissue, and slowly equilibrating tissue respectively (mg/dl). G (mg/dl) is the plasma glucose concentration. Suffix "$_b$" denotes basal state; EPG is the endogenous glucose production (mg/kg/min); Ra is the glucose rate of appearance in plasma (mg/kg/min); E is renal excretion (mg/kg/min); U_{ii} is the insulin-independent tissue's glucose utilization and U_{id} is the insulin-dependent's glucose utilization (both mg/kg/dl). V_G is the distribution volume of glucose (dl/kg); k_1

and k_2 are the rate parameters (both $\min^{-1}$). At basal steady state endogenous production EGP_b equals glucose disappearance, i.e. the sum of glucose utilization and renal excretion (which is normally zero in normal subjects):

$$EGP = U_b + E_b. \tag{2.2}$$

Parameter values of V_G, k_1 and k_2 are reported in Fig 1.20, in glucose subsystem kinetic process. The fluxes of interest are estimated using forcing functions strategy as shown in Fig. 1.21, using nonlinear least squares.

The simulation of an entire day using the model is well presented in Fig. 1.22 for a normal human. Overall this model is based on virtually model-independent measurements of the various fluxes occurring during the day. So for the diabetes type 2 population studied is used the same model but a different parametric portrait. The model's limits, of course, exist and they are: the not concerning about other hormones influences (e.g. the glucagon action); the "glucocentric" nature of model (no fatty acids interactions); The parameters are quite fixed and sometimes they have daily changes, so their description should be improved. And finally, this is a mean model and, of course, does not account so well for inter subject variability.

Process	Parameter	Normal Value	Type 2 Diabetic Value	Unit
Glucose Kinetics	V_G	1.88	1.49	dl/kg
	k_1	0.065	0.042	min^{-1}
	k_2	0.079	0.071	min^{-1}
Insulin Kinetics	V_I	0.05	0.04	l/kg
	m_1	0.190	0.379	min^{-1}
	m_2	0.484	0.673	min^{-1}
	m_4	0.194	0.269	min^{-1}
	m_5	0.0304	0.0526	min ·kg/pmol
	m_6	0.6471	0.8118	dimensionless
	HE_b	0.6	0.6	dimensionless
Rate of Appearance	k_{max}	0.0558	0.0465	min^{-1}
	k_{min}	0.0080	0.0076	min^{-1}
	k_{abs}	0.057	0.023	min^{-1}
	k_{gri}	0.0558	0.0465	min^{-1}
	f	0.90	0.90	dimensionless
	a	0.00013	0.00006	mg^{-1}
	b	0.82	0.68	dimensionless
	c	0.00236	0.00023	mg^{-1}
	d	0.010	0.09	dimensionless
Endogenous Production	k_{p1}	2.70	3.09	mg/kg/min
	k_{p2}	0.0021	0.0007	min^{-1}
	k_{p3}	0.009	0.005	mg/kg/min per pmol/l
	k_{p4}	0.0618	0.0786	mg/kg/min per pmol/kg
	k_i	0.0079	0.0066	min^{-1}
Utilization	F_{cns}	1	1	mg/kg/min
	V_{m0}	2.50	4.65	mg/kg/min
	V_{mx}	0.047	0.034	mg/kg/min per pmol/l
	K_{m0}	225.59	466.21	mg/kg
	p_{2U}	0.0331	0.0840	min^{-1}
Secretion	K	2.30	0.99	pmol/kg per (mg/dl)
	α	0.050	0.013	min^{-1}
	β	0.11	0.05	pmol/kg/mirn per (mg/dl)
	γ	0.5	0.5	min^{-1}
Renal Excretion	k_{e1}	0.0005	0.0007	min^{-1}
	k_{e2}	339	269	mg/kg

Figure 2.3: Model parameters of normal and type2 average diabetic subject.

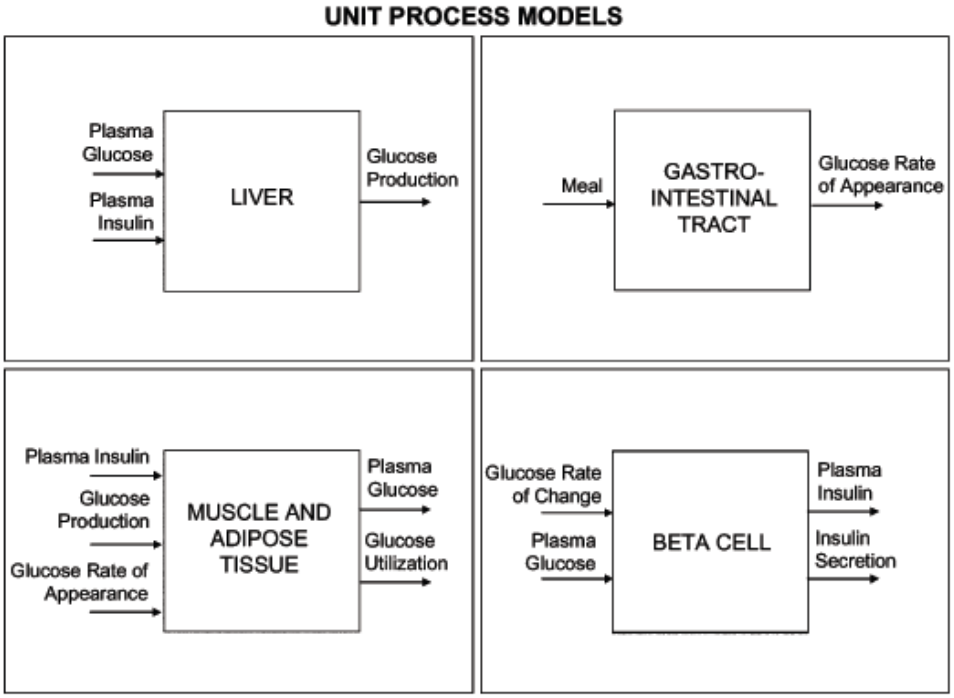


Figure 2.4: Endogenous Glucose Production (EGP) estimation strategy (top left panel); glucose Ra (top right panel); glucose utilization (bottom left panel); insulin secretion (bottom right panel). The input of each block is a forcing function, the outgoing arrows are model outputs.

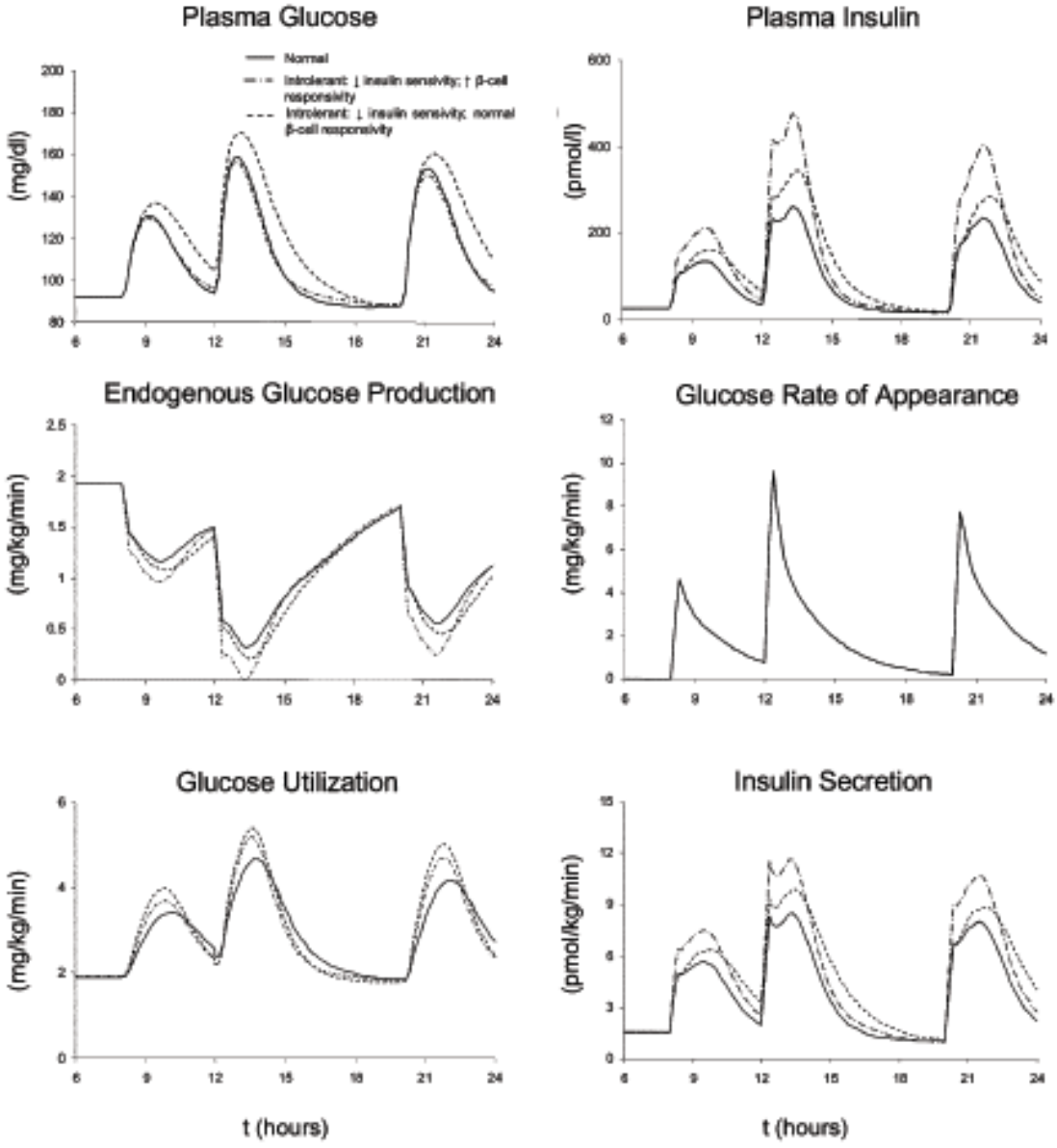


Figure 2.5: Whole day simulation of a normal (continuous) and of other experiments (modified some parameters). We see plotted the model prediction of plasma concentrations and fluxes with breakfast at 8.00 am (45g of glucose), lunch al 12 p.m. (70g), and dinner at 8 p.m. (70g).

2.1.2 Introduction to Oral Model System

The Oral Model System is a model which is integrated as a subsystem of the previous model, 2.1. It is called Gastro-Intestinal Tract (See Fig.2.1 and Fig.2.4 upper right)[24]. An oral ingestion model of glucose is very important because is used in many clinical test to assess glucose tolerance on humans (OGTT) and obviously about experiments with everyday meals. As we can see from Fig.2.4 upper right, the input is the meal ingested (amount of glucose in mg) and the output is the glucose rate of appearance Ra (mg/kg/min).

There are two old models proposed (model A and B of Fig.2.7, upper) but after the availability of gold standard data of Ra (Fig. 2.6) provided by [30, 31], the model C and D (Fig. 2.7, lower) are proposed. The physiology process, used in the models, is: the ingestion of glucose, then glucose is absorbed in the upper gastrointestinal tract, then it's transported to the splanchnic bed (mostly the liver) and, finally, reaches the peripheral circulation. Moreover many authors agree that the gastric emptying of liquids occurs exponentially and depends on the size of the meal, its energy and the amount of nutrient in stomach. But with increasing of nutrient caloric content, there is a deceleration from the exponential model to a closer approximation of linearity[34]. Thus, while liquid are non-linearly emptied, the solid part has a linear emptying. Therefore we need two compartments for stomach[35]. For obviously reasons, related to the non-linearity of gastric emptying, the model A (Lehman and Deutsh model), the model B (Elashoff Model) and, as we could see, the Model 1, are too simple and not good for achieve a good modeling result. It's considered as reference and most reliable model, the fourth, i.e. that one called Model D, in Fig.2.7, lower panel on the right. For having a more complete work it's considered the estimation for OGTT test, i.e the the input is just glucose(75g), and Meal test, i.e the intake of a mixed meal (45% carbohydrates, 90g of glucose) .

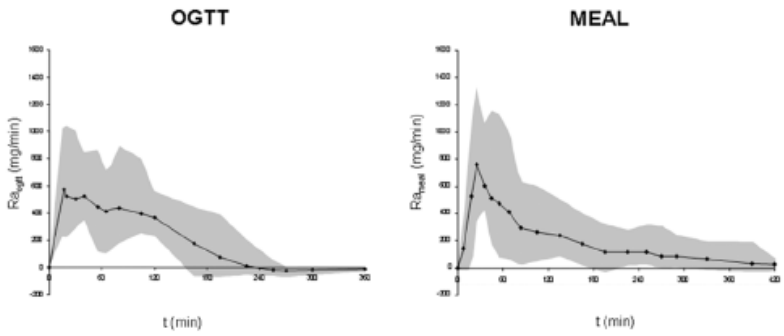


Figure 2.6: Rates of appearance measured with the multiple tracer tracer-to-tracee clamp technique during OGTT (left) and meal (right); grey area represents range of variability.

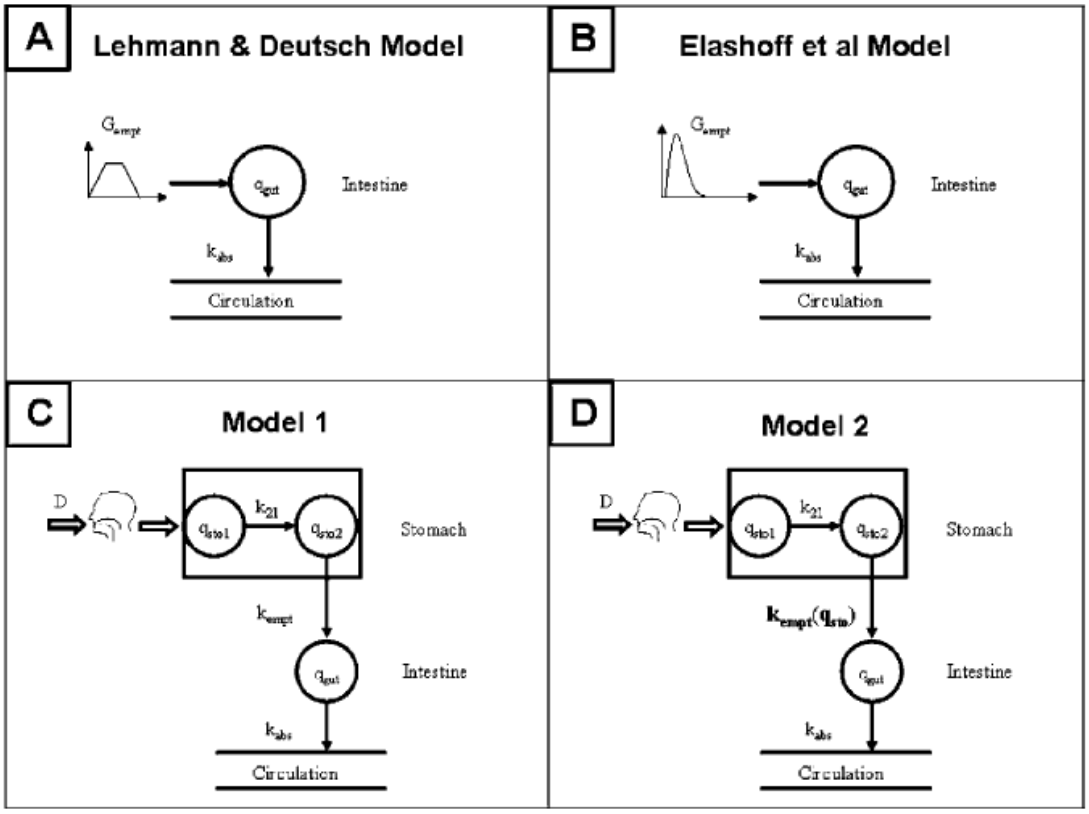


Figure 2.7: The four proposed models.

Gastro-Intestinal tract model

In Fig.2.7, model D, lower panel on right, as previously said, is the most reliable, and it is considered as reference of this work. It is composed by three compartments: glucose transits through the stomach, which is composed by two compartments, one for solid phase $sto1$ and one for liquid phase $sto2$, and it goes to small intestine. So the model is represented by a chain of three compartments, with equations:

$$\begin{cases} \dot{q}_{sto1}(t) = -k_{21} \cdot q_{sto1}(t) + D\delta(t) \\ \dot{q}_{sto2}(t) = -k_{empt} \cdot q_{sto2}(t) + k_{21} \cdot q_{sto1}(t) \\ \dot{q}_{gut}(t) = -k_{abs} \cdot q_{gut}(t) + k_{empt}(q_{sto}(t)) \cdot q_{sto2}(t) \\ Ra(t) = -f \cdot k_{abs} \cdot q_{gut}(t) \end{cases} \tag{2.3}$$

where q_{sto1} and q_{sto2} are the amounts of glucose in the stomach, solid and liquid respectively and q_{sto1}+q_{sto2}=q_{sto}. $\delta(t)$ is the impulse function, D is the amount of ingested glucose; q_{gut} is the glucose mass in the intestine; k_{21} is the rate of grinding, i.e. how the food passes from solid to liquid phase; k_{empt} is the rate of gastric emptying (it is linear in model C); k_{abs} is the rate of intestinal absorption; f is the fraction of the intestinal absorption which actually appears in plasma (in fact a part of glucose is "sequestered" by splanchnic organ for their activity) and it is expressed as follows:

$$f = \frac{\int_0^\infty Ra_{ogtt/meal}(t)}{D} \tag{2.4}$$

where the numerator is the area under the curve (AUC) of Ra (Ra_{ogtt} or Ra_{meal}). We note from the gold datas that Ra_{ogtt} has three phases in its graph and Ra_{meal} has two (Fig. 1.23): OGTT shows a peak at 30 min, a plateau at 120 min and a rapid decrease to zero in the last part; in the MEAL Ra we observe the peak at 60 min (reflecting the slower emptying of mixed meal components), and the the curve goes to zero without a plateau. Clearly a linear model cannot capture these features. So the Model 2 has to be a non-linear approach. It's described by the equations (1.3) and (1.4), in which it contains only a non-linearity in the gastric-emptying definition, i.e. in the k_{empt} parameter, which is not a constraint but it's dependent on the total amount of glucose in the stomach q_{sto} as follows:

$$k_{empt}(q_{sto}) = k_{min} + \frac{k_{max} - k_{min}}{2} \cdot \{\tanh[\alpha(q_{sto} - b \cdot D)] - \tanh[\beta(q_{sto} - c \cdot D)] + 2\} \tag{2.5}$$

with

$$q_{sto}(t) = q_{sto1}(t) + q_{sto2}(t) \tag{2.6}$$

where k_{empt} is maximum ($=k_{max}$) when the stomach contains the amount of the ingested glucose D, i.e. at the beginning of the experiment, then it decreases with rate α to a minimum, k_{min}, then it recovers back again to its maximum k_{max} with a rate of β, when the stomach is empty (Fig.1.25). Then, b is the percentage of dose for which k_{empt} decreases at $(k_{max}-k_{min})/2$ and c is the percentage of dose for which k_{empt} is back to $(k_{max}-k_{min})/2$. They both correspond to flexes points. All the measure units (Model 1 and 2) are of course the same of (1.1). For the estimation of α and β it's imposed, considering that k_{empt}=k_{max} for both $q_{sto} = D$ and $q_{sot} = 0$, the following equations:

$$\alpha = \frac{5}{2 \cdot D \cdot (1-b)} \tag{2.7}$$

$$\beta = \frac{5}{2 \cdot D \cdot .c} \tag{2.8}$$

.

If c is very small k_{empt} remains at level k_{min} till the stomach is completely empty and (1.5) simplifies as:

$$k_{empt}(q_{sto}) = k_{min} + \frac{k_{max} - k_{min}}{2} \cdot \{\tanh[\alpha(q_{sto} - b \cdot D)] + 1\}. \tag{2.9}$$

The Model 2 fits very well Ra_{ogtt} and Ra_{meal} profiles in both average as well as in each individual (Fig. 1.26).

The estimations are made by nonlinear least squares, Ra error is assumed independent and Gaussian with zero mean and unknown standard deviation (*posteriori*-estimated) and negative Ra values are not considerate. To favor identification of Model 2, especially for the meal studies, the constraint $k_{21} = k_{max}$ has imposed.

Having c very small means that the k_{empt}'s curve doesn't recover the initial rate, in other words, the $k_{empt} = k_{min}$ when the stomach is empty. As result for a MEAL test when it's used either (1.5) and (1.9) we have an average k_{empt} as shown in Fig.1.27., right panel, where for q_{sto}=0 we have a k_{empt} different from k_{max}.

Overall the maximum gastric emptying is similar in the two tests but k_{min}, k_{abs}, b and c are lower in MEAL than OGTT. This agrees with the knowledge that glucose absorption is slower if I eat a meal than a OGTT due the presence of other components, mainly fats,

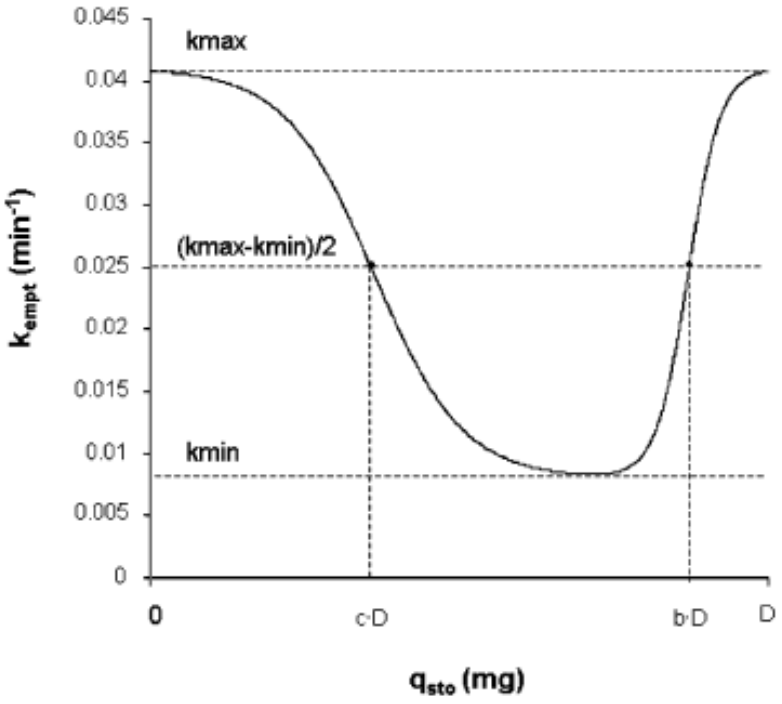


Figure 2.8: Qualitative plot of k_{empt} as function of the amount of glucose in the stomach q_{sto}: it's k_{empt}=k_{max} for both $q_{sto} = D$ and $q_{sto} = 0$, i.e when stomach is full or empty.

which significantly slow down gastric digestion : k_{min}^{MEAL}<$k_{min}^{OGTT}$and intestinal absorption is consequently k_{abs}^{MEAL} <k_{abs}^{OGTT}.
The interesting feature of Model 2 is that its parameters are potentially usable to quantitatively characterize the different Ra pattern observed in various conditions, e.g., young vs elderly, men vs women, diabetic vs non diabetic, even **pramlintide treated diabetic type 1 subjects vs regular treated diabetic type 1 subjects**.

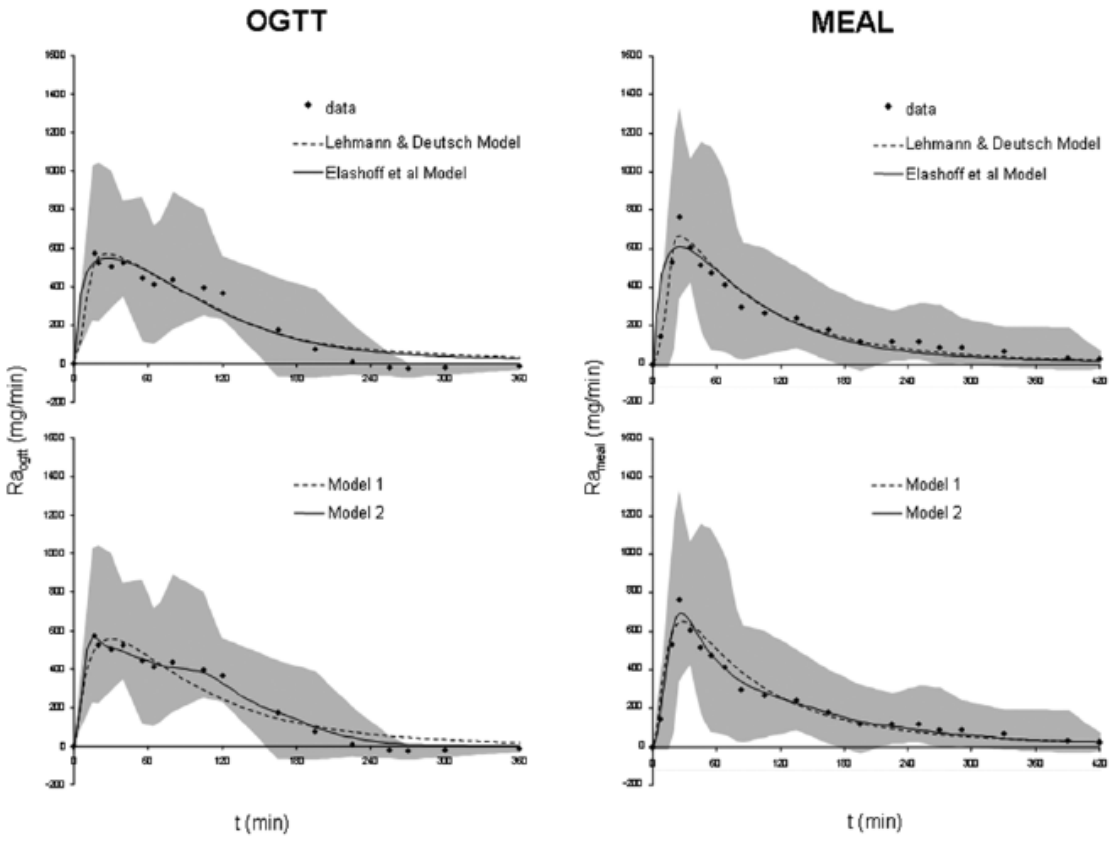


Figure 2.9: Left panel is OGTT test and right is MEAL test. Upper panel is Lehman and Deutsch model (broken line) vs the Elashoff model (solid line) vs data (black dots). Lower panel is Model 1 (broken line) vs Model 2 (solid line) vs data (black dots).

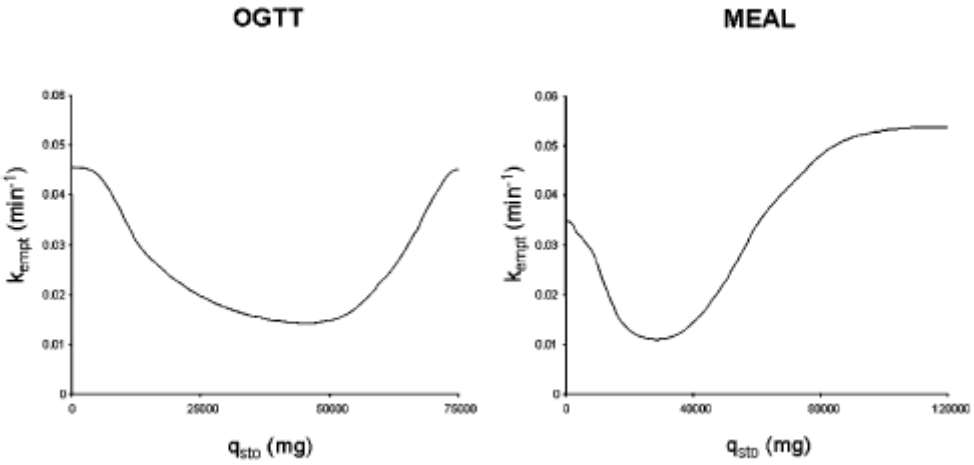


Figure 2.10: Average gastric emptying rate estimated during OGTT test (left panel and similar to the ideal one in Fig.1.25) and during MEAL (right panel). For the estimation of the k_{empt}^{MEAL} is used for 50% of patients (1.5) equation and for the others 50% the (1.9) one.

Chapter 3

Dataset

The aim of the thesis is is to find a parametric estimation model of the average patient treated with Pramlintide using the clinic protocol written below, in this chapter, obtaining a model to include in a simulator to prove the Pramlintide effect in silico. In fact, my new subsystem model will be integrated to the model described in section 1.6, for a characterization of the T1 diabetes regularly treated with insulin with the Pramlintide addition.

Major findings of gastric emptying studies are that the physiological defense mechanism to delay gastric emptying in response to postprandial hyperglycemia is impaired in patients with type 1 diabetes[36]. The IAPP(amylin) in healthy subjects delays the gastric emptying, leading BG to a slower Ra avoiding high glucose peaks. The important study of Woerle and others offers a good protocol for my experimentation[37].

3.1 The Experiment Protocol

The study, issued in 2008, is about an healthy population (7 men and 3 women, n=10, 39±4 years of age, BW 80±4kg) and a diabetics type 1 population (8 men and 7 women, n=15, 37±2 years of age, BW 76±3kg). Both populations has normal physical examination and no gastric problems or nephropathy. All type1 patients receive continuos subcutaneous insulin infusion therapy for at least 3 months before the study with a good glycemic control (A1C 7.3 ± 0.2%) without severe hypoglycemic episodes. Three days before the test no alcohol, no smoke or exercises with 200 g of carbohydrates daily. The healthy population has been chosen not to have history of diabetes and to have a normal glucose response. No food intake before 10 hours before the meal test. The breakfast time is from 7.00 am to 7.30 a.m., and possibly within five minutes. The T1 subject has the SMBG at 10 p.m.

and 2 a.m. at the night before test due the adjustment of basal insulin to achieve the BG concentration of 5 and 10 mmol/l respectively. The pramlintide amount for the designed patients (n=15) is 30μg is injected subcutaneously in the lower abdominal wall and taken at meal time. The postprandial Insulin infusion for euglycemic and hyperglycemic conditions are: 6.4±0.9, 6.8±0.8, 3.5±0.8, 1.4±0.3 and 0.9±0.1islet equivalents/h at the times: 0-30, 35-60, 65-120, 240-330 minutes. The mixed meal is about 450 kcal (45% carbohydrates, 30% fats, 25% proteins) with glucose amount (D) of 50g (50000mg) containing tracers. For the health subjects in hyperglycemia status other sugar is added in postprandial period but for type 1 experiments is not.

3.1.1 Results

They induced hyperglycemia status in health and diabetes T1 subjects finding that the feedback delay mechanism is completely absent in T1 subjects, as expected. In fact in normal subjects when hyperglycemia was induced the amylin concentration went from 9 to 43 pmol/l postprandially so the gastric emptying was strongly delayed. For T1 diabetes the same gastric emptying profile is detected for either euglycemic status and hyperglycemic status due to the lack of amylin Fig(3.1).

Note that initial gastric emptying is greater in T1 patients. Hyperglycemia delays the percentage of emptying rate only in healthy subjects (blue vs green plots), but there's no difference between the two diabetes emptying plots (euglycemic vs hyperglycemia, red vs yellow plots). The pramlintide treated patients have a strong emptying delay, the gray line plot, which is similar to the normal subject hyperglycemic pattern (green plot).
The others average fluxes of all kind of populations are illustrated in Fig.3.2. where I note that the subcutaneous pramlintide reduces the postprandial glucose in type 1 diabetic patients (as expected). Moreover plasma insulin is not different between T1 with and without pramlintide.

3.1.2 Rate of Appearance in type 1 diabetes treated with pramlintide

A good observation is the rate of appearance, which is composed by two complementary components: the endogenous, i.e. the internal glucose fluxes appearing on blood, and exogenous, i.e. the external glucose flux appearing on blood. Amylin, so pramlintide, effects both fluxes (liver derived function and meal derived function respectively, section 1.4). The endogenous production is strictly correlated to glucagon action and recent studies showed that IAPP and pramlintide suppress postprandial glucagon secretion[38, 39]. Indeed, they found greater suppression of postprandial glucagon in type 1 diabetic patients

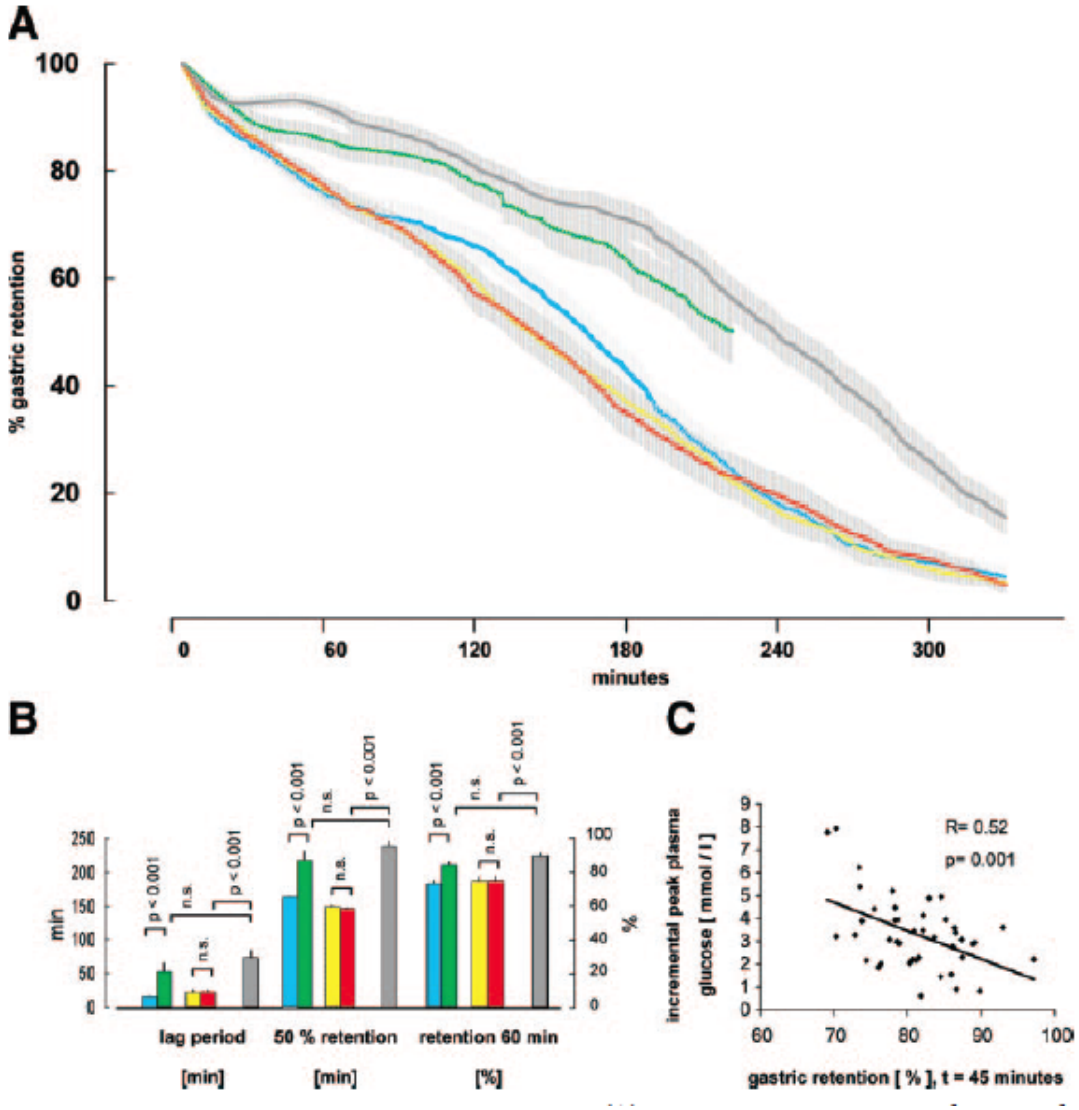


Figure 3.1: A: gastric emptying in function of time after a meal test. *Blue* line is normal subject in euglycemic condition; *green* line is normal subject in hyper.; *yellow* line is T1 diabetes in euglycemic; *red* line is T1 diabetes in hyper.; *gray* line is T1 in hyperglycemia condition treated with pramlintide. B: Percent gastric retention, lag periods, 50% retention time, percent retention at 60min. C: Inverse correlation of gastric retention at 45 min and incremental plasma glucose concentration at 60 min. Comparison made using paired and unpaired t tests within and between patients groups.

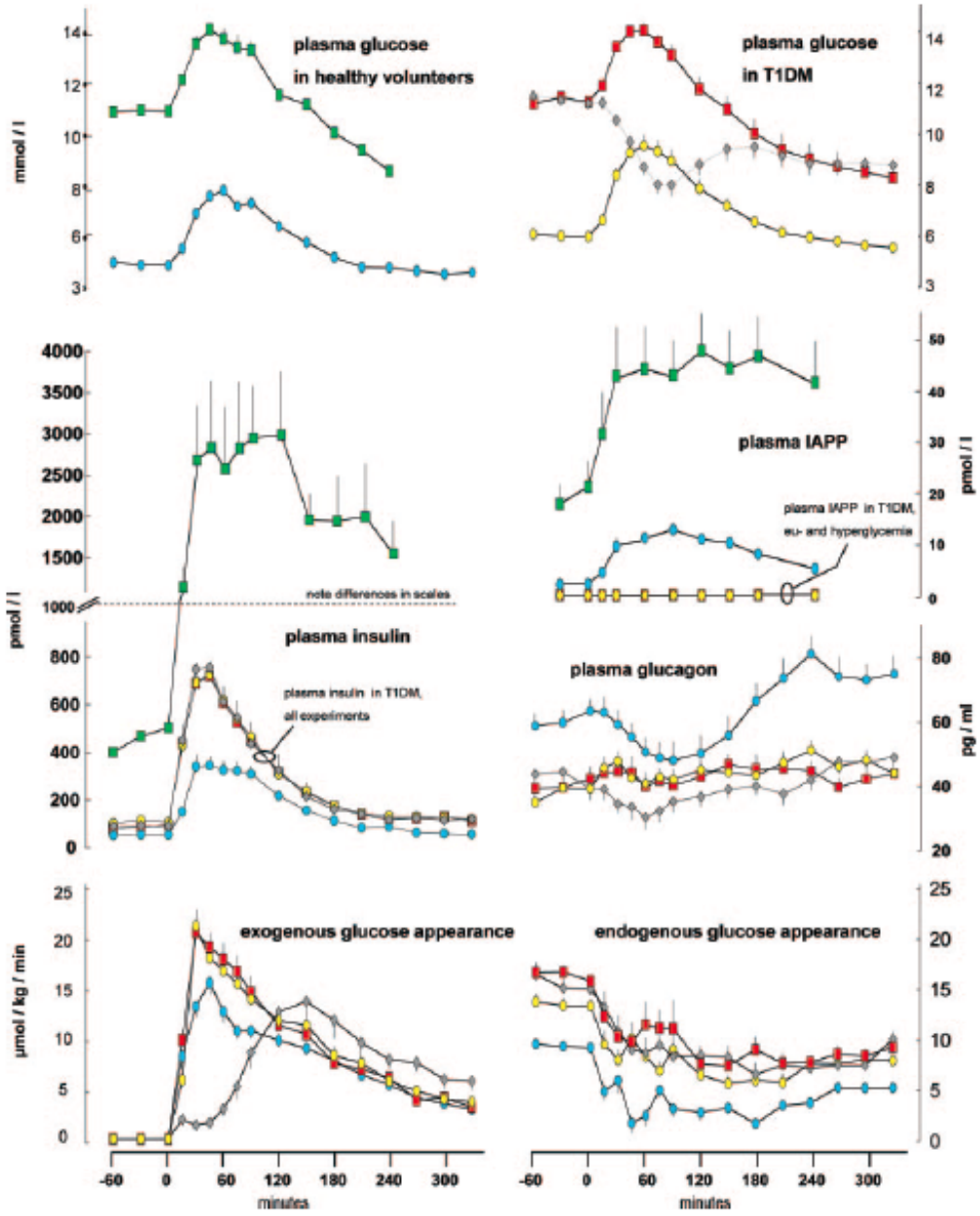


Figure 3.2: Glucose concentrations on plasma at top panel; insulin plasma concentrations at middle left panel; glucagon plasma concentration and amylin concentration at middle right panel; Ra plots on the bottom panel. The blue circle line is the average healthy subject; the green squared line is the average healthy subject in hyperglycemia; the yellow line is average euglycemic T1 subject; red squared line is the average T1 subject in hyper.; the gray line is the average T1 subject in hyper. treated with pramlintide. Comparison made using paired and unpaired t tests within and between patients groups.

when pramlintide was given (Fig.3.2, middle right panel), and a lower endogenous production in pramlintide treated. But looking at the plots (Fig.3.2, bottom right panel) the endogenous Ra is not affected by pramlintide usage! The reason why the endogenous production is so similar using pramlintide or not is not so clear but the glucagon suppression may have occurred or directly by an inhibitory effect of pramlintide on the pancreatic-cell or indirectly via reduced efflux of nutrients from the gut, because amino acids such as arginine are known to stimulate glucagon secretion[40]. Thus, it remains unclear whether the greater suppression of glucagon secretion is attributable to a direct inhibition of the pancreatic-cells or to reduced influx of nutrients from the gut. However, because endogenous glucose production is comparable in the placebo and pramlintide experiments in type 1 diabetic patients, the physicians believe that the pramlintide induces reduction of postprandial glucose concentrations and it is primarily due to the delay in gastric emptying. So they skip the pramlintide's meal derived function considering only exogenous Ra as effective Ra. On the other hand Ra (exogenous Ra) which was greater in diabetics than healthy, with pramlintide action it is now reduced (bottom left panel 3.2, gray diamonds) and the shorter peak is shifted to the right marking an important delay.

Chapter 4

Oral Glucose Absorption Model Identification

The reference model is the Model 2 illustrated at 1.6.2. The model is prior identifiable. The estimation method is the non-linear weighted squares with or without MAP[1] estimator. The all identification is implemented with MATLAB[2] and/or SAAM II[3]. The two identifications are for the average patient from clinical data test previously described (section 1.1, Fig.4.1) in a first place, then It's estimated the single patient's Ra.

[1]MAP: Maximum At Posterior: it's a Bayesian punctual estimator.
[2]MATLAB software by MathWorks Inc., R2012a.
[3]The Epsilon Group, University of Washington, 2011.

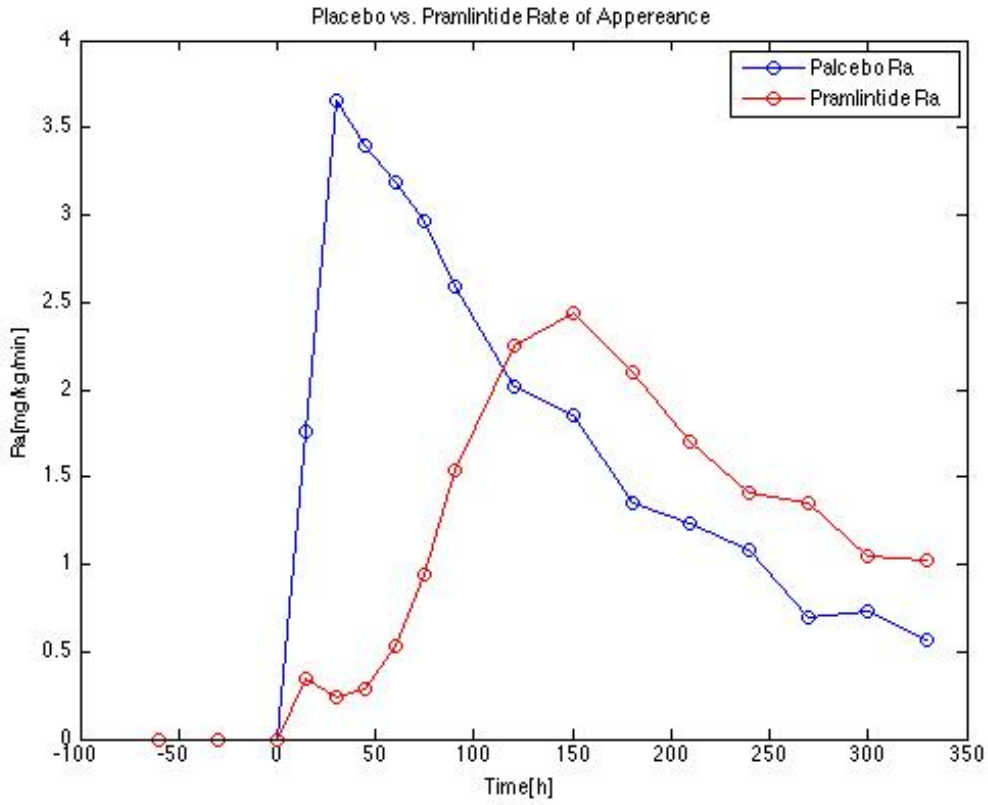


Figure 4.1: The average Ra's pattern for T1 hyperglycemic patient (blue line); the average Ra's pattern for T1 hyperglycemic patient treated with pramlintide.

4.0.3 Identification of the average type1 diabetic in hyperglycemia (only insulin treated), PLACEBO

Recalling the Model's 2 equations (2.3) with the k_{empt} as described in (2.5), the unknown model's parameters are: k_{max}, k_{min}, k_{abs}, b and c. The initial values, according with Fig.2.3 are: $k_{max} = 0.06$; $k_{min} = 0.008$; $k_{abs} = 0.08$; $b = 0.8$; $c = 0.4$ (for the measurements units see Fig.2.3).

The model's portrait is (Value, Estimation Precision) in following table:

with $f = 0.83085$ calculated as in formula (2.4). The prediction model is in Fig.4.2, upper panel. The WRSS (Weighted Residual Sum Squared) is 20, whose weights residuals are in Fig.4.2 lower panel.

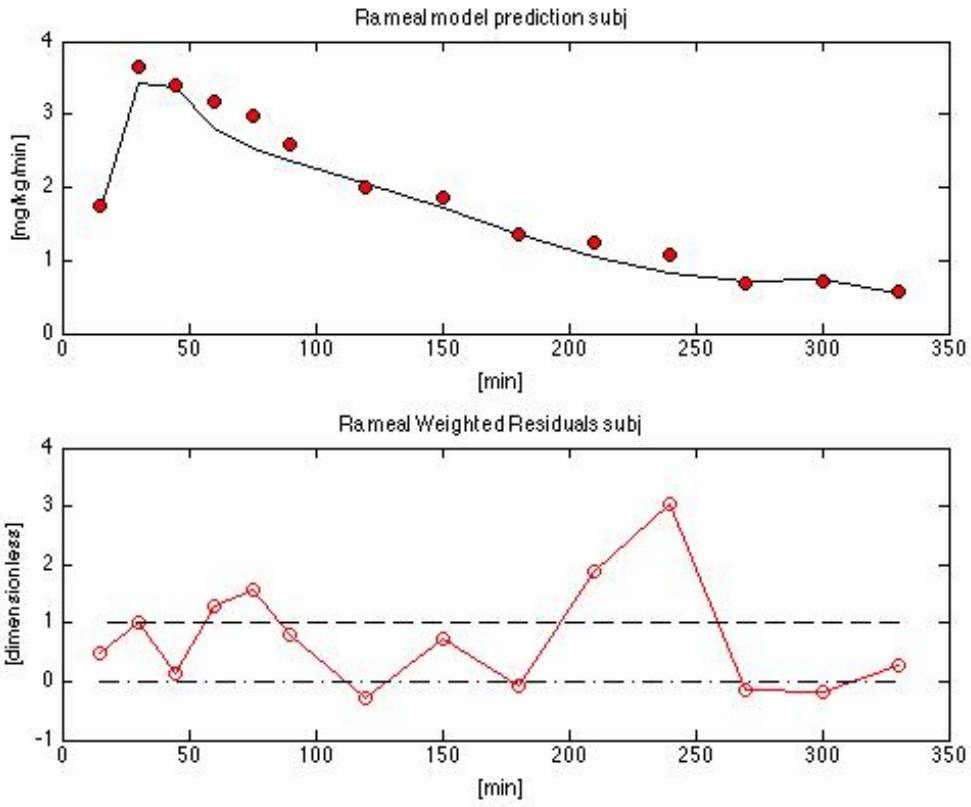


Figure 4.2: The model prediction (continuous line) vs data (red points), upper panel. Weighted normal residuals, lower panel.

Parameters	Values	CV [%]
k_{max}	0.0242 [min^{-1}]	19
k_{min}	0.0095 [min^{-1}]	6
k_{abs}	0.094 [min^{-1}]	58
b	0.79 [unit-less]	3
c	0.0817[unit-less]	13

Table 4.1: Average Diabetic subject, regularly treated with insulin, in hyperglycemia status, i.e. placebo (PBO), identification.

As I can see from Fig.3.4 the fit is good and the gastric emptying's plot is similar to the healthy one, but obviously different in parameter's value. In fact the k_{abs} is bigger than in healthy subject: ($k_{abs}^{PBO} = 0.094 min^{-1} > k_{abs}^{HE} = 0.071 min^{-1}$), showing that diabetes T1 in hyperglycemia has a faster glucose absorption. Then the c and b values are lower than healthy ones: ($b^{PBO} = 0.69 < b^{HE} = 0.79$ $c^{PBO} = 0.008 < c^{HE} = 0.17$), which they give a sort of slopes to the curve provoking different plot of the emptying. Even k_{max} is different than healthy one but this gastric emptying respects the fact that the k_{empt} is k_{max} either with full and empty stomach.

4.0.4 Identification of the average type1 diabetic in hyperglycemia with Pramlintide addiction

The average data for average T1DM in hyperglycemia and treated with Pramlintide is the red plot in Fig.4.1. The initial values, according with Fig.2.3 is: $k_{max} = 0.06$; $k_{min} = 0.008$; $k_{abs} = 0.08$; $b = 0.8$; $c = 0.4$ (for the measurements units see Fig.2.3). The population value are (prior information):

Parameter	Bayesian Prior Value	Bayesian Prior SD (CV %)
k_{max}	0.04 [min^{-1}]	100%
k_{min}	0.004 [min^{-1}]	10%
k_{abs}	–	–
b	–	–
c	–	–

Table 4.2: Priors used in the Pramlintide average subject's Ra identification.

Recalling the model's equations (2.3) with the k_{empt} as described in (2.5), the unknown model's parameters are: k_{max}, k_{min}, k_{abs}, b and c. The f is fixed to the population value [41], Fig.4.3, and the reasons are explained later in this section.

$$f = 0.9. \tag{4.1}$$

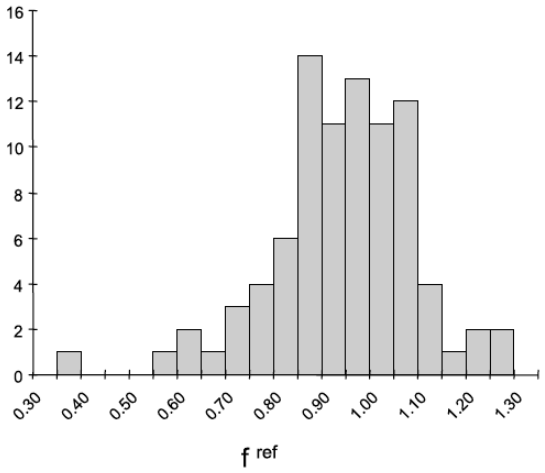


Figure 4.3: The f distribution for an healthy population. The mean is round 0.9 and it's chosen as reference in simulation A.

The model's identification portrait is (Value, Estimation Precision):

Parameters	Values	CV [%]
k_{max}	0.043 [min^{-1}]	50
k_{min}	0.0038 [min^{-1}]	60
k_{abs}	0.0056 [min^{-1}]	11
b	0.99 [unit-less]	3
c	0.56[unit-less]	22

Table 4.3: Average Diabetic subject, regularly treated with insulin and Pramlintide, in hyperglycemia status, i.e. PRAM, identification.

The prediction model is in Fig.4.4, upper panel. The WRSS (Weighted Residual Sum Squared) are equal to 14 and they are at central panel, and the rate of emptying is lower panel.

Assessment of the fraction of ingested glucose f with Pramlintide

The reason why the f is fixed and not calculated with (2.4) from the available Ra datas is because the observation window (from -60min to +330min) is not sufficient to see the complete evolution of Ra. Indeed the integral of the formula should calculate the AUC from the initial time zero (steady state) to a big value (virtually infinite), in which the Ra returns to zero (back to steady state). But as shows Fig.4.5 the final part is missing. A

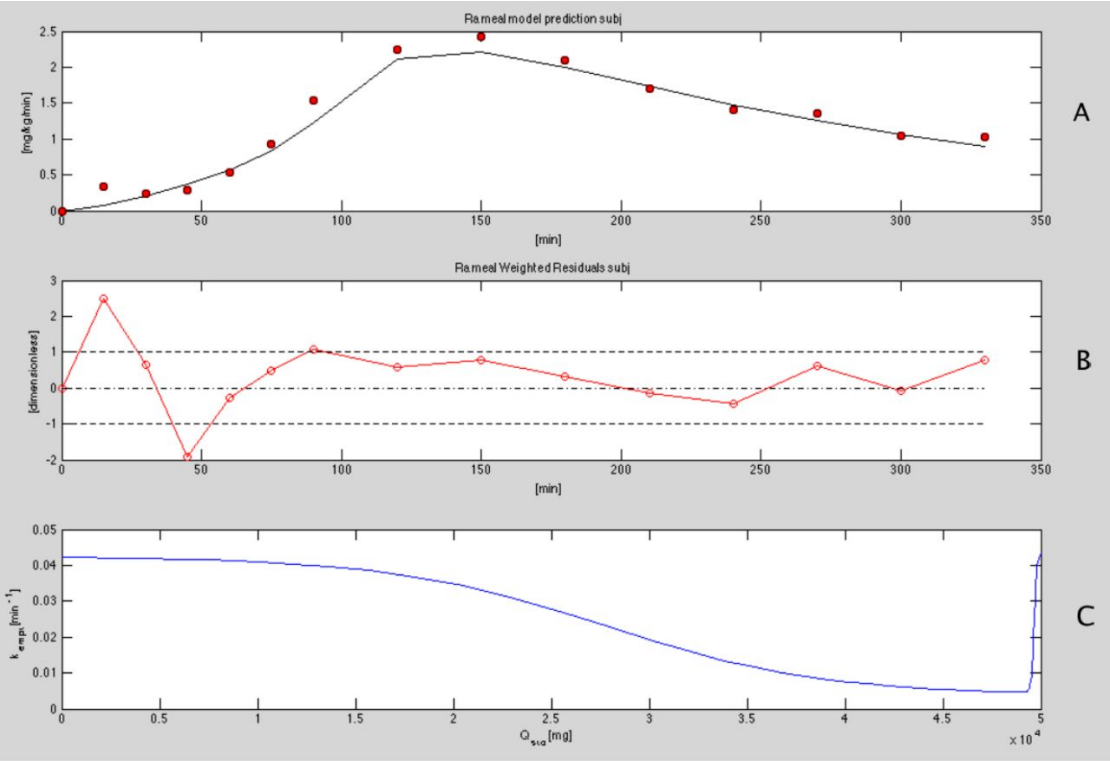


Figure 4.4: A: Prediction model (continuos data) vs Prediction (red dots) of average Pramlintide patient. B: The weighted residuals of prediction model in A. Gastric emptying in function of stomach glucose amount.

solution could be an interpolation using the last sample (Ra=1.022 mg/kg/min,t=330min) and a virtual final null samples when the digestion is supposed to be actual complete (t=480min=8h), but It'll be difficult to choose what kind of interpolation is good, and if a linear is chosen it's not clear how the slopes are (in Fig.4.5 it's chosen a random interpolation, just for illustration purpose). So It's not a good way to proceed.

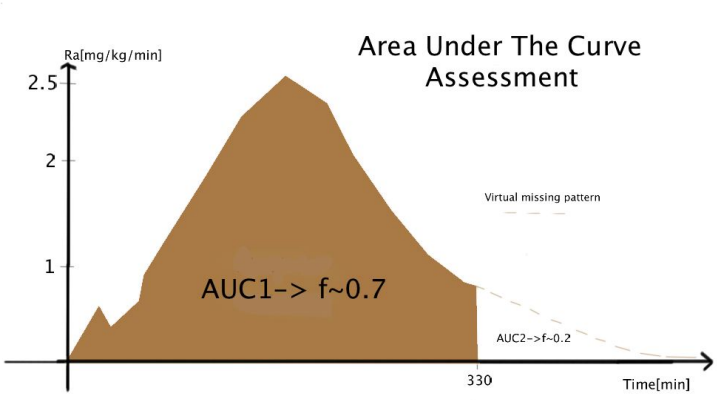


Figure 4.5: AUC assessment. The virtual pattern could go approximately to zero (end of digestion) and its AUC (white AUC) gives the missing part of f which is not calculated using the data, and according with theory should be about 0.2, to get the its actual final value of 0.9.

Calculating the f value using (2.4) from the Ra average data of T1DM Pramlintide treated, in the dominium of my data (the brown AUC on Fig.4.5):

$$f^{PRAM} = 0.6898.$$

It should be expected that the f of a Pramlintide Ra is similar to a f of a healthy Ra subject. Indeed the Pramlintide purpose is to bring the placebo Ra having a Ra similar to the healthy one! Calculating the f from the average healthy subject:

$$f^{healthy} = 0.6959.$$

It's the value that it's expected, i.e. similar to f^{PRAM},(p<0.5). So the two f belong to the same statistical population showed in Fig.4.3. That's why choosing f fixed to the mean

of population makes sense.
The identified model feel predicts Ra data and provides precise estimates of model parameters. The AIC and WRSS are the best related to the others simulation I've made and residuals are a good representation of a white noise. The gastric emptying rate is different from healthy one. Indeed at the beginning of the experiment, when the emptying rate of stomach $k_{empt}(q_{sto})$ promptly decreases from k_{max} to k_{min}. This means that the k_{empt} is immediately slowed down resulting in a delayed gastric emptying. Observing the b value, which close to one, it represents the fact that k_{empt} is immediately slowed down to k_{min}. Then the gastric emptying rate slowly increases and it recovers back to a constant value, similar to k_{max}, till the stomach is empty. Its plot is consistent with a gastric emptying delayed action of pramlintide respect to the gastric emptying in a type 1 diabetic regularly treated with only insulin (Fig.2.4, lower panel).

4.1 Identification on single T1DM patient treated with Pramlintide

The T1DM Pramlintide average model, as presented previously, is well identified. But what happened if I try to identify the model for a single patient? Is the model still good for a individual patient, or is reliable only for the average patient? All this questions aim to the same goal, i.e. the application of the average model to the single patient in order to incorporate the effect of Pramlintide into the Meal Simulation Model of the Glucose-Insulin system.

Before going into the single identification activity, many issues have to be underscored. The single patients is affected by more noise than average patient, e.g measurement, environment, physical noises. In this general situation the bayesian tools are fundamental. I use the MAP estimator and I modify the prior information for a better fit and estimation precision at the same time. It must be noticed that the individual Ra are sometimes very different among one other, pointing out how much the physiology is variable and complicated. Moreover the data available for a patient is only the Ra measurement and not its variance. So the identification of each patient will be made with unknown measurement error, in which the SD will be estimated a *posteriori*. Other topic is the f constraint: it's not possible to fix the f to 0.9 as done before because it is a personal parameter, which in this case cannot be fixed to mean population value. Due the fact that f is priori unknown I calculate it using (2.4) equation (for a 15 subjects' f distribution, see Fig.4.6).

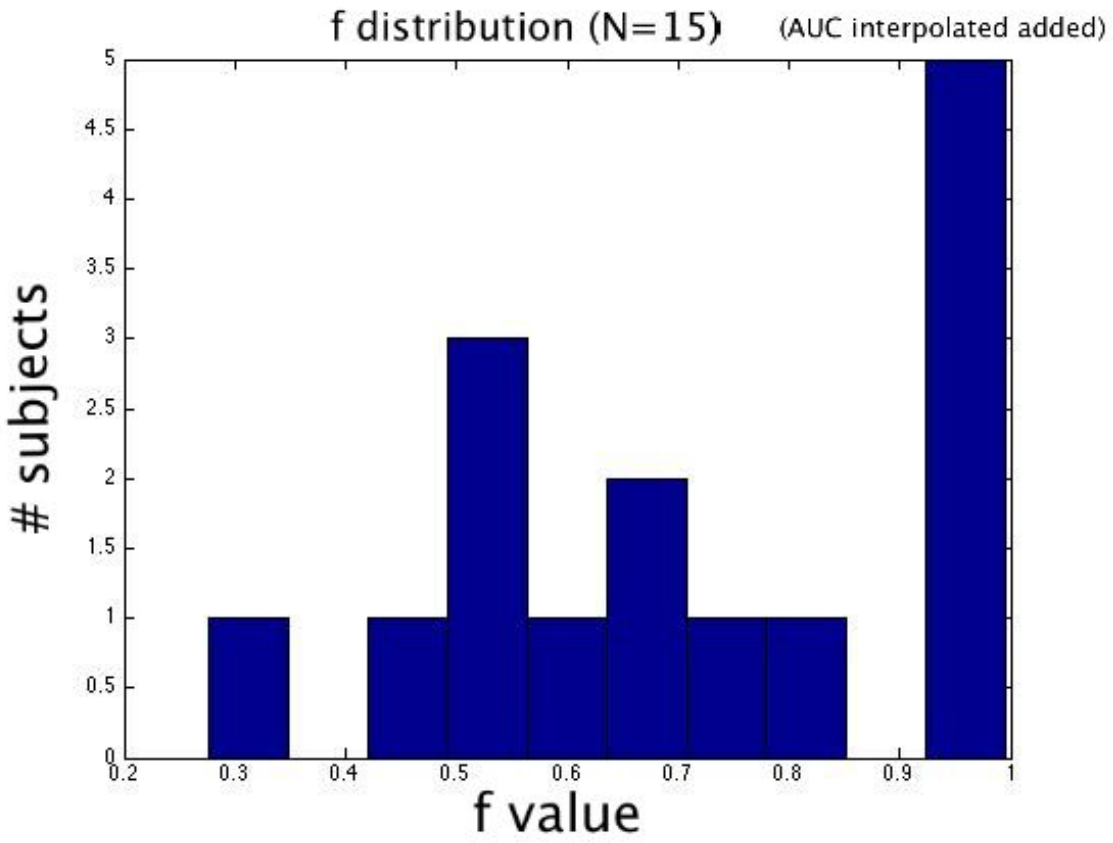


Figure 4.6: The f distribution; linear interpolation AUC added.

4.1.1 Identifications of single patients: PLACEBO *vs.* PRAMLINTIDE

According with the identification theory the bayesian is avoided where not necessary.

Placebo, PBO

The initial values are: $k_{max} = 0.06min^{-1}$; $k_{min} = 0.01min^{-1}$; $k_{abs} = 0.08min^{-1}$; $b = 0.8$; $c = 0.2$ and used for subjects: 1,2,3,4,6,7,10,11,12,13,14,15; The initial value for subjects 5,8,9 and 13 are the same above but c=0.6. The Placebo (PBO) bayesian prior set is in table 4.4 and 4.5.

Parameter	Bayesian Prior Value	Bayesian Prior SD (CV %)
k_{max}	–	–
k_{min}	0.001 [min^{-1}]	10%
k_{abs}	0.08 [min^{-1}]	100%
b	–	–
c	0.2	10%

Table 4.4: Bayesian Priors used in the placebo (PBO) identifications for subjects 3,7,12 and 15.

Parameter	Bayesian Prior Value	Bayesian Prior SD (CV %)
k_{max}	–	–
k_{min}	–	–
k_{abs}	0.08 [min^{-1}]	100%
b	–	–
c	0.7	100%

Table 4.5: Bayesian Priors used in the placebo (PBO) identifications for subjects 5,8,13.

Pramlintide, PRAM

Parameter	Bayesian Prior Value	Bayesian Prior SD (CV %)
k_{max}	–	–
k_{min}	0.001 [min^{-1}]	10%
k_{abs}	–	–
b	–	–
c	–	–

Table 4.6: Bayesian Priors used in the Pramlintide (PRAM) identifications. Used for subject 1,3,4,8,9,12,15.

Parameter	Bayesian Prior Value	Bayesian Prior SD (CV %)
k_{max}	0.06 [min^{-1}]	100%
k_{min}	0.001 [min^{-1}]	10% or 50%
k_{abs}	–	–
b	–	–
c	–	–

Table 4.7: Bayesian Priors used in the Pramlintide (PRAM) identifications. Used for subject 2,5,6,7,10,13. Subjects 11 and 14 have the prior SD at 50% variation.

Predictive Models

As shown in Fig 4.15-16, where there are the identifications portrait for all the 15 subjects, the model can be identified with a good precision and as shown from the Fig.4.7 to 4.14, the fit is also good and satisfactory. In the PRAM identification, b is close to one value in every subject. This means that the gastric emptying is very fast to decrease from its maximum to its minimum, according to the Pramlintide's effects. In the fig. 4.17-4.22 are reported the parameters distributions of 15 subjects. It is a small set of subjects for extract some important information from the histograms. However from these distribution it's very clear how the gastric emptying rate is modified after a Pramlintide addiction. Looking into the parametric values of the two kind of patients it could be observed that the parameters are quite different in values. This means a change of shape of the two k_{empt} plots. The Pramlintide's gastric emptying is the usual seen before for the average subject, with a rapid decreasing from k_{max} to k_{min} and a slow increase back to the maximum value. This means that b is decreased, close to one, and c is decreased. The p-values could be calculated but it's clear that the differences are not due casualty, but to a remarkable drug effect.

Subj	Kmax PBO [1/min]	Kmin PBO [1/min]	Kabs PBO [1/min]	b PBO [unit-less]	c PBO [unit-less]
	cv %	cv %	cv %	cv %	cv %
1	0.04	0.006	0.039	0.54	0.1
	13	24	18	8	16
2	0.039	0.007	0.03	0.48	0.09
	25	39	34	22	38
3	0.037	0.001	0.05	0.66	0.32
	28	8	45	8	19
4	0.032	0.012	0.04	0.86	0.12
	36	16	39	10	13
5	0.029	0.006	0.09	0.77	0.13
	22	25	46	6	33
6	0.036	0.009	0.07	0.79	0.059
	26	19	46	6	52
7	0.022	0.012	0.099	0.84	0.19
	30	22	79	13	9
8	0.056	0.0011	0.03	0.79	0.1
	58	24	65	21	10
9	0.045	0.008	0.031	0.64	0.047
	29	37	40	18	64
10	0.05	0.009	0.029	0.69	0.068
	33	23	50	14	41
11	0.04	0.01	0.029	0.60	0.098
	37	59	57	33	52
12	0.04	0.008	0.072	0.73	0.081
	9	6	14	3	10
13	0.041	0.09	0.084	0.81	0.1
	49	26	57	15	58
14	0.033	0.04	0.033	0.48	0.13
	27	54	45	25	38
15	0.051	0.006	0.069	0.53	0.09
	22	52	29	13	27

Figure 4.7: The 15 subjects Placebo's identification parameters.

Subj	Kmax PRA [1/min]	Kmin PRA [1/min]	Kabs PRA [1/min]	b PRA [unit-less]	c PRA [unit-less]
	cv %	cv %	cv %	cv %	cv %
1	0.1680	0.0030	0.0093	0.9999	0.4880
	26	2	14	0	42
2	0.0424	0.0010	0.0149	0.9900	0.5456
	52	4	33	0	10
3	0.0440	0.0010	0.0127	0.9995	0.5851
	42	3	21	3	11
4	0.0901	0.0010	0.0080	0.8582	0.4599
	68	3	20	9	23
5	0.0591	0.0010	0.0090	0.9900	0.5495
	50	5	22	0	9
6	0.0208	0.0010	0.0120	0.9900	0.7457
	82	4	83	0	29
7	0.0389	0.0010	0.0078	0.9998	0.5875
	52	4	35	8	17
8	0.2301	0.0010	0.0099	0.9361	0.3988
	15	6	30	6	13
9	0.0436	0.0010	0.0090	0.9825	0.6870
	80	5	25	5	25
10	0.0282	0.0001	0.0149	0.9900	0.6766
	99	7	75	0	26
11	0.0461	0.0001	0.0130	0.9900	0.6047
	77	12	46	0	16
12	0.0266	0.0010	0.0111	0.9794	0.6849
	85	3	46	0	32
13	0.0656	0.0001	0.0097	0.9706	0.5370
	28	3	20	4	9
14	0.0252	0.0001	0.0136	0.9759	0.6995
	59	10	87	8	32
15	0.1748	0.0010	0.0122	0.9413	0.4444
	19	2	10	2	6

Figure 4.8: The 15 subjects Placebo's identification parameters.

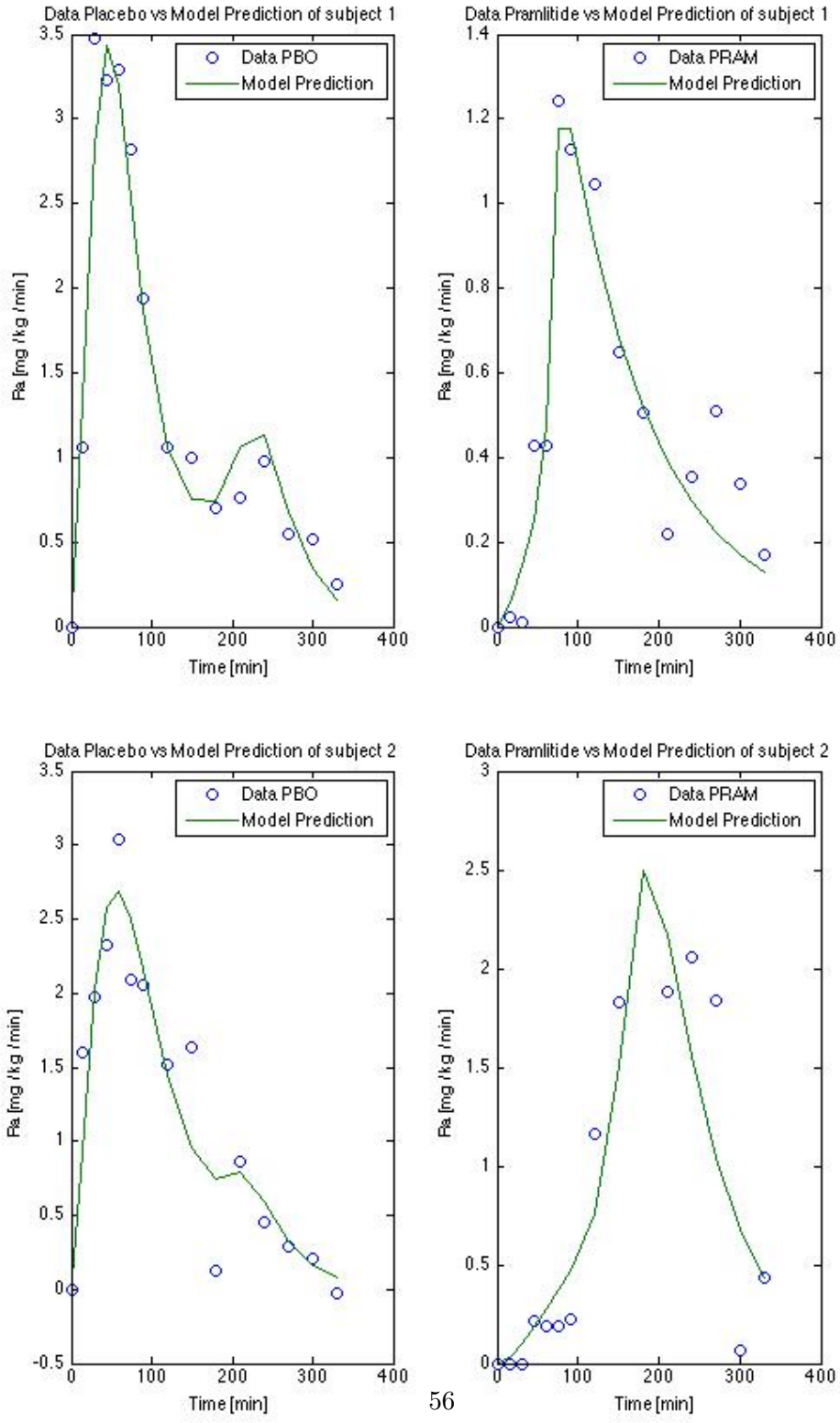


Figure 4.9: Upper: PBO vs PRAM identification of subject 1. Lower : PBO vs PRAM identification of subject 2.

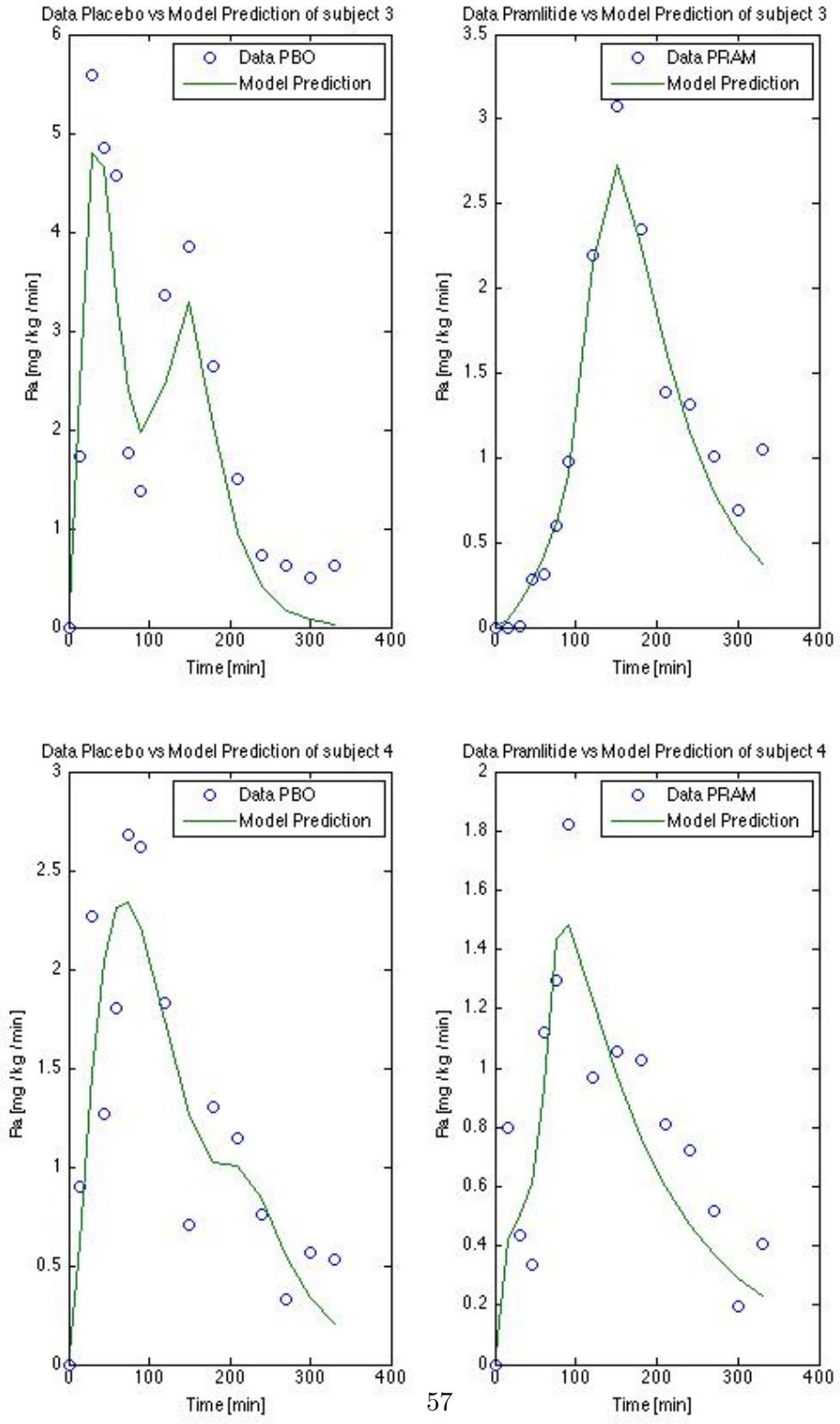


Figure 4.10: Upper: PBO vs PRAM identification of subject 3. Lower : PBO vs PRAM identification of subject 4.

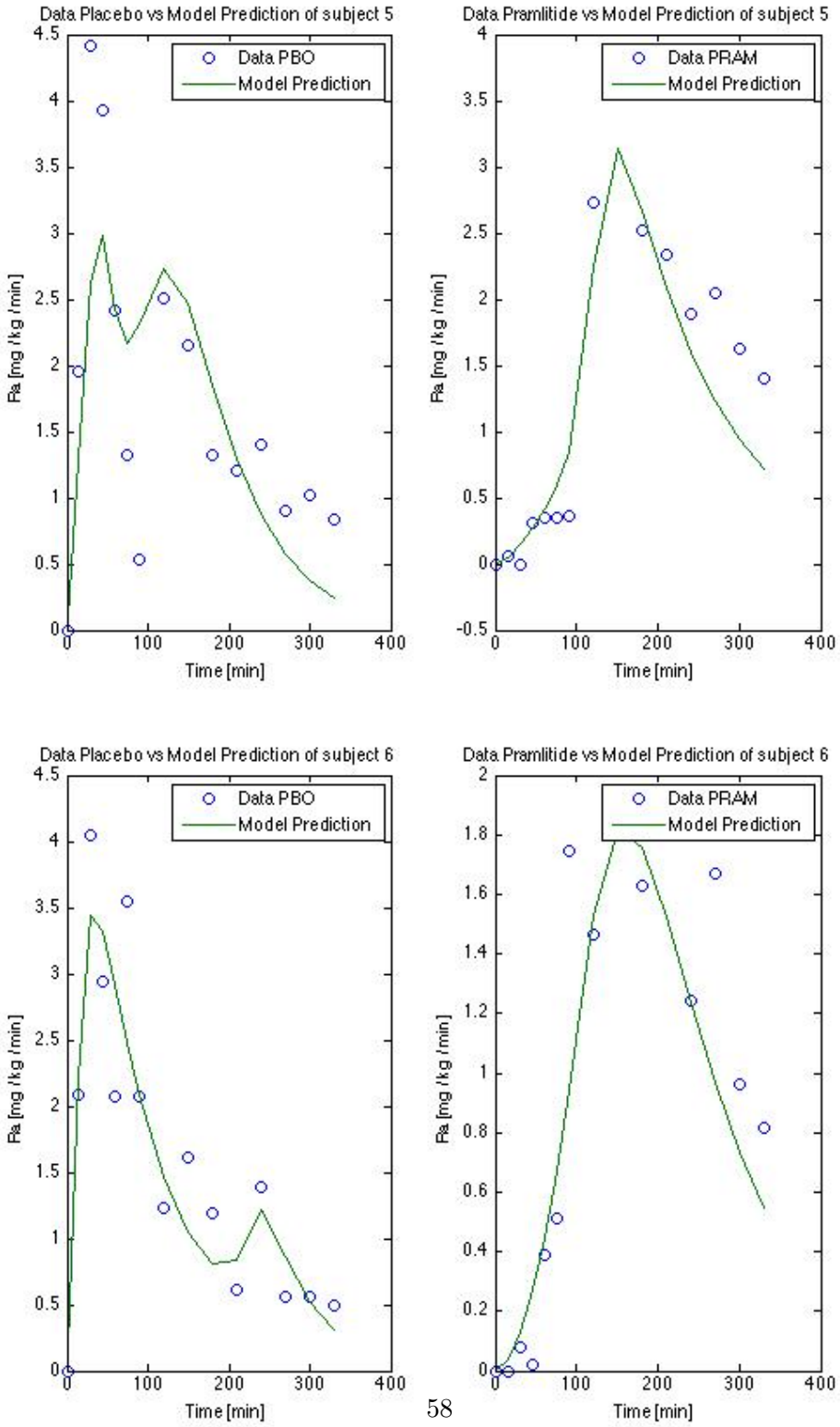


Figure 4.11: Upper: PBO vs PRAM identification of subject 5. Lower : PBO vs PRAM identification of subject 6.

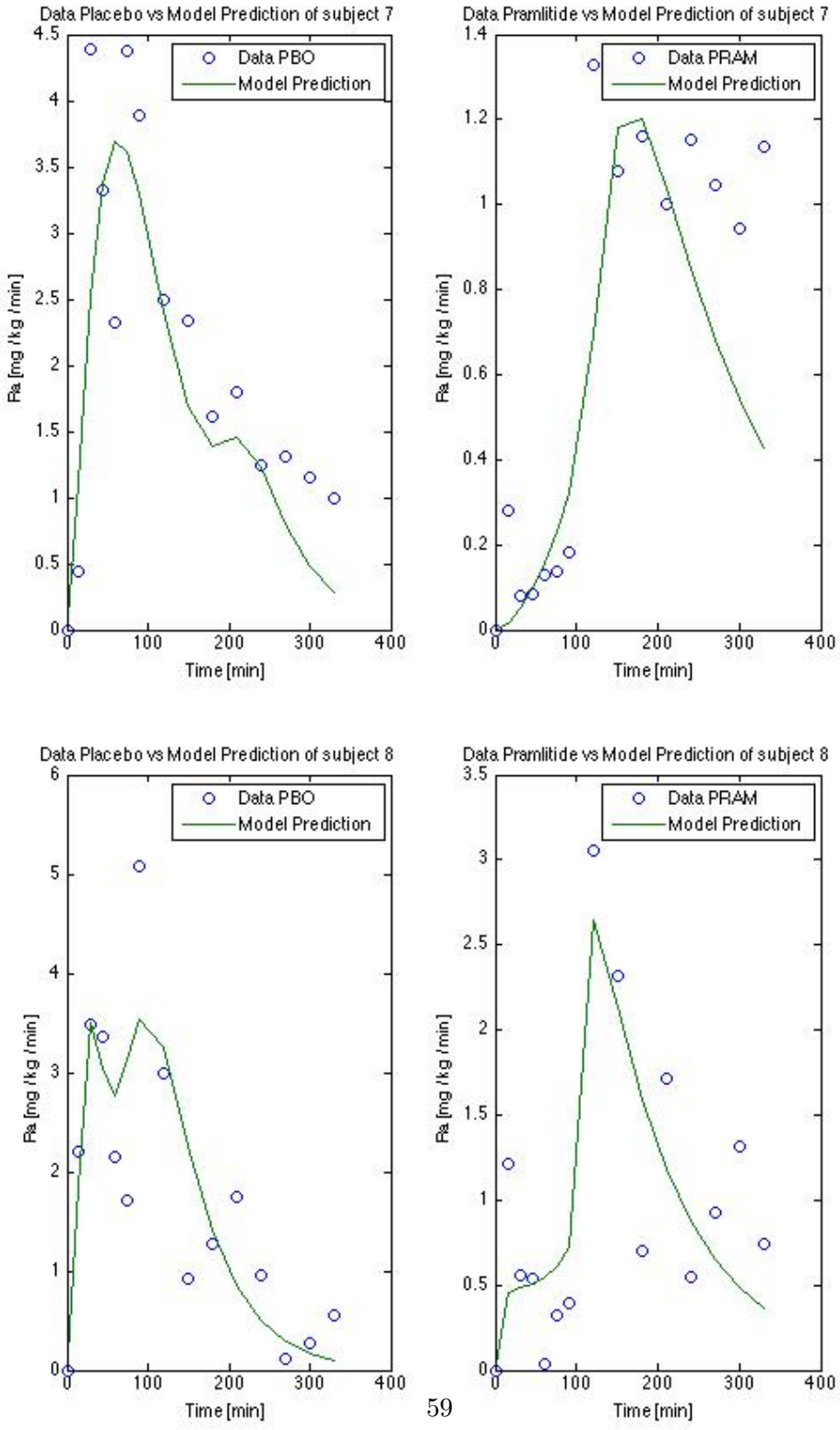


Figure 4.12: Upper: PBO vs PRAM identification of subject 7. Lower : PBO vs PRAM identification of subject 8.

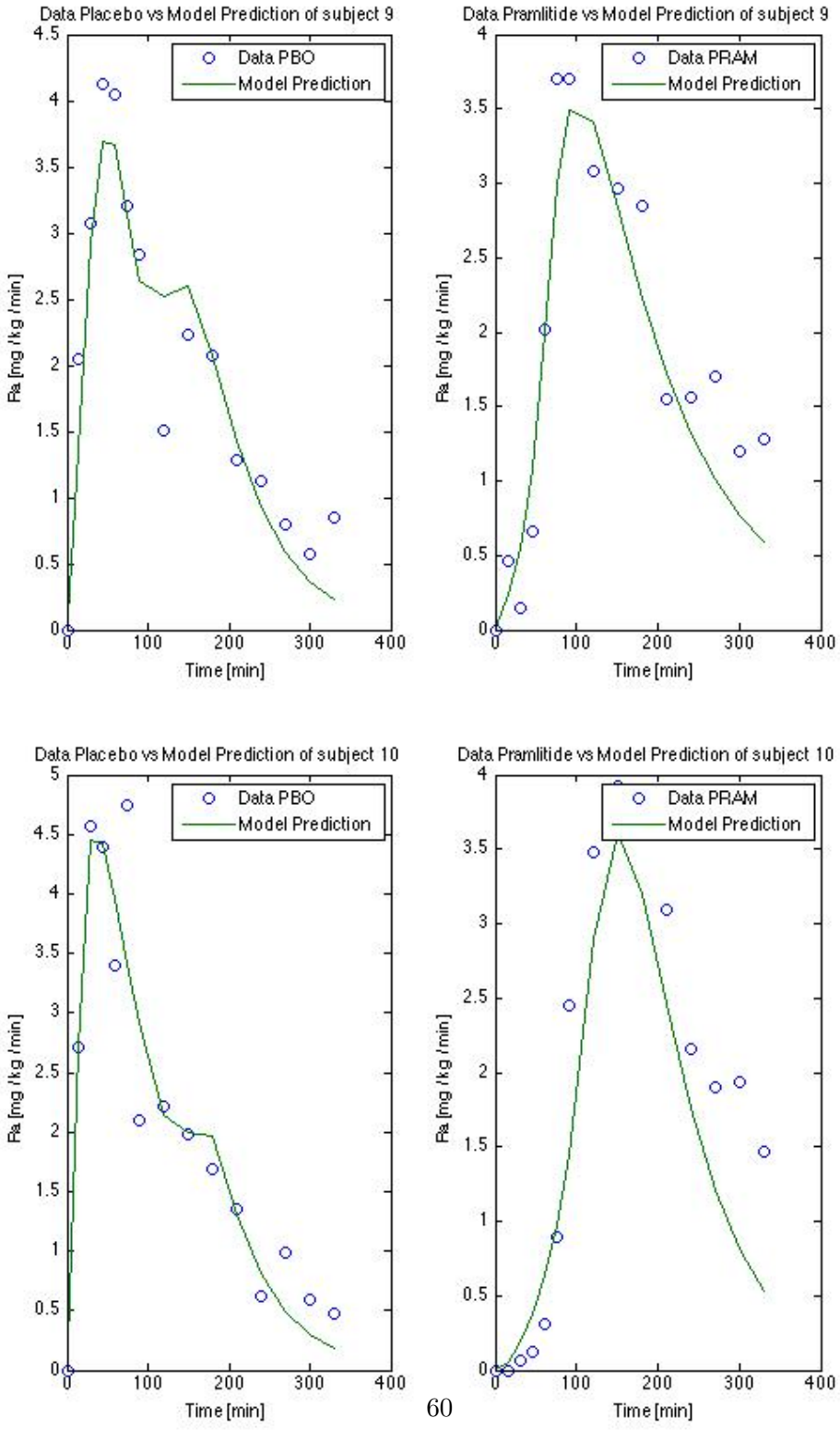


Figure 4.13: Upper: PBO vs PRAM identification of subject 9. Lower : PBO vs PRAM identification of subject 10.

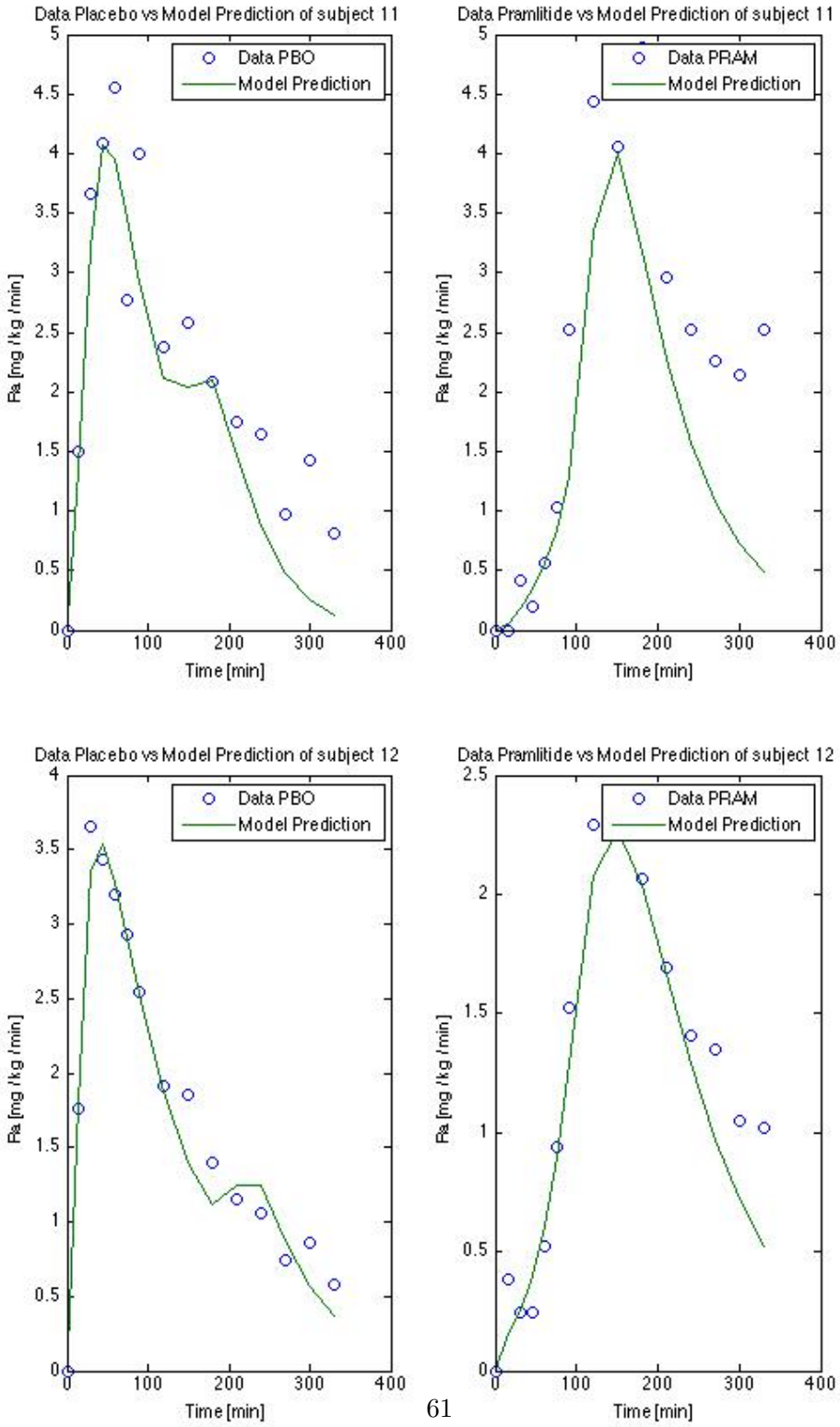


Figure 4.14: Upper: PBO vs PRAM identification of subject 11. Lower : PBO vs PRAM identification of subject 12.

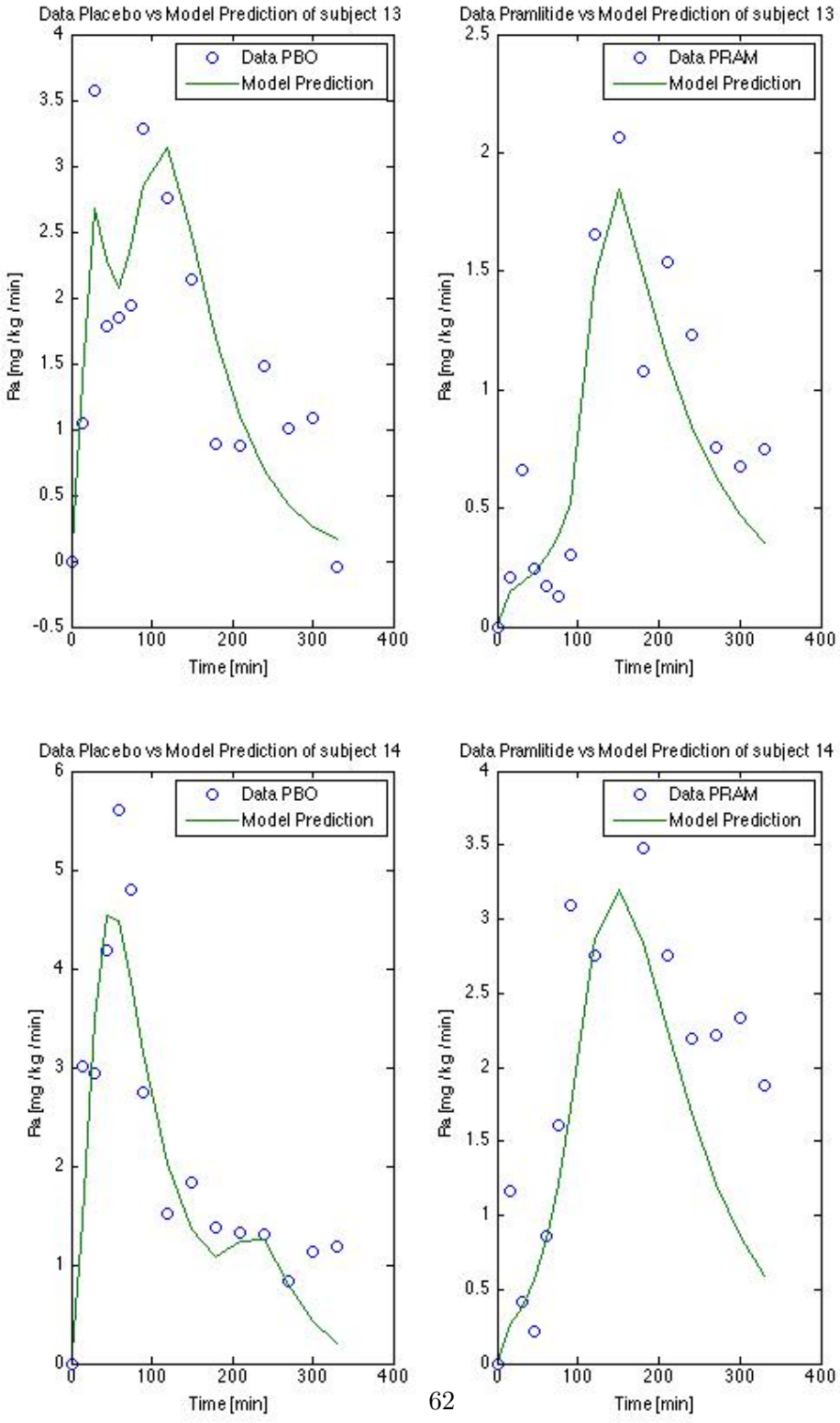


Figure 4.15: Upper: PBO vs PRAM identification of subject 13. Lower : PBO vs PRAM identification of subject 14.

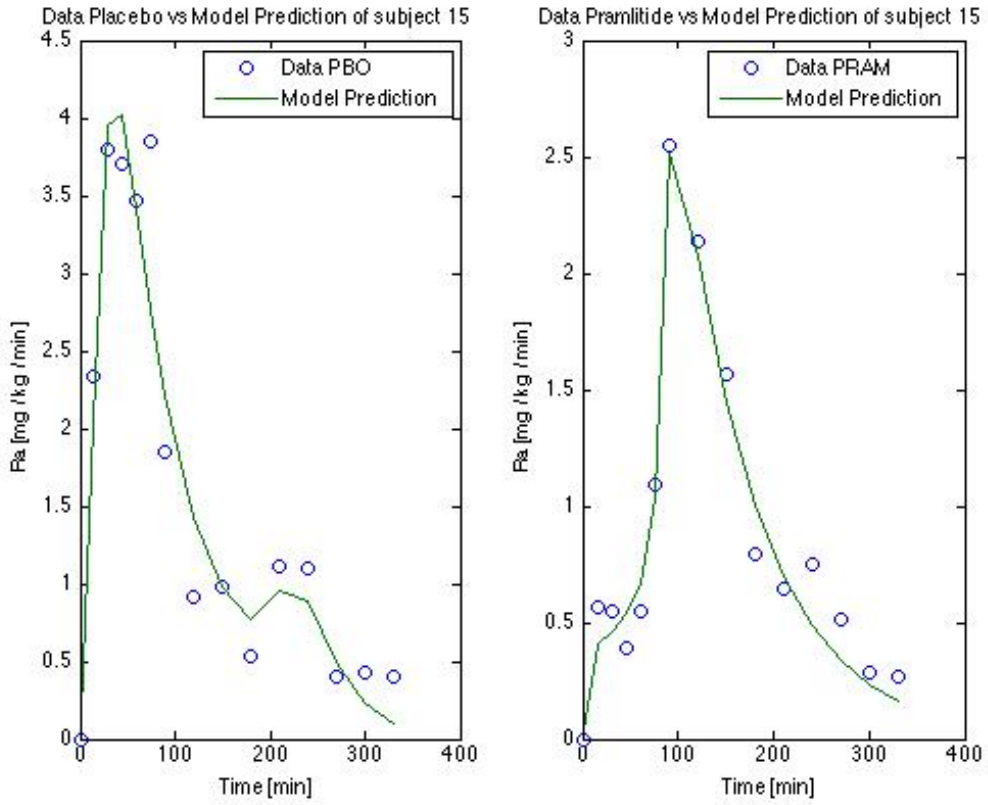


Figure 4.16: PBO vs PRAM identification of subject 15.

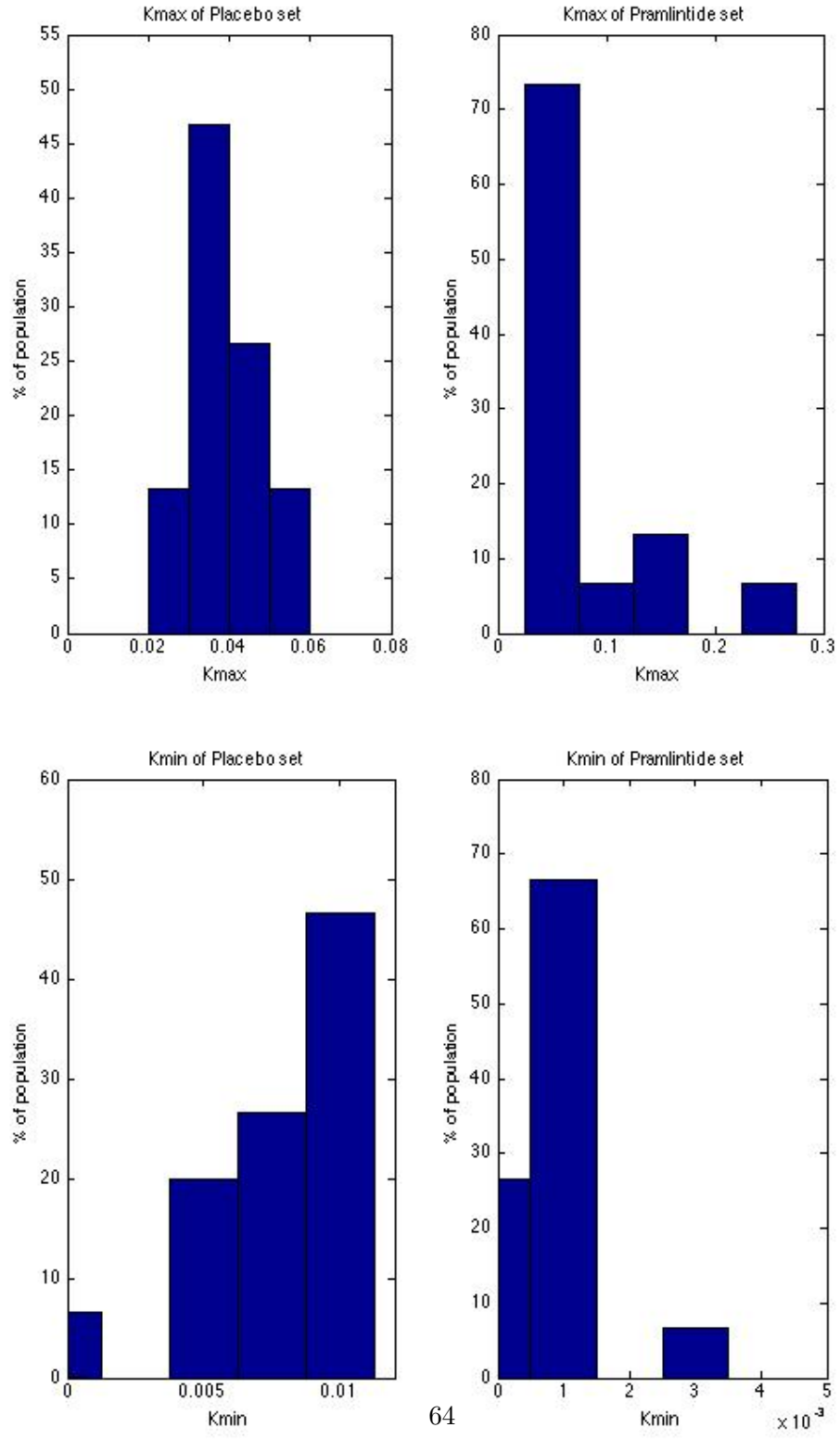


Figure 4.17: Upper: k_{max} distribution, PBO vs. PRAM. Lower: k_{min} distribution, PBO vs. PRAM. Set of 15 T1DM subjects.

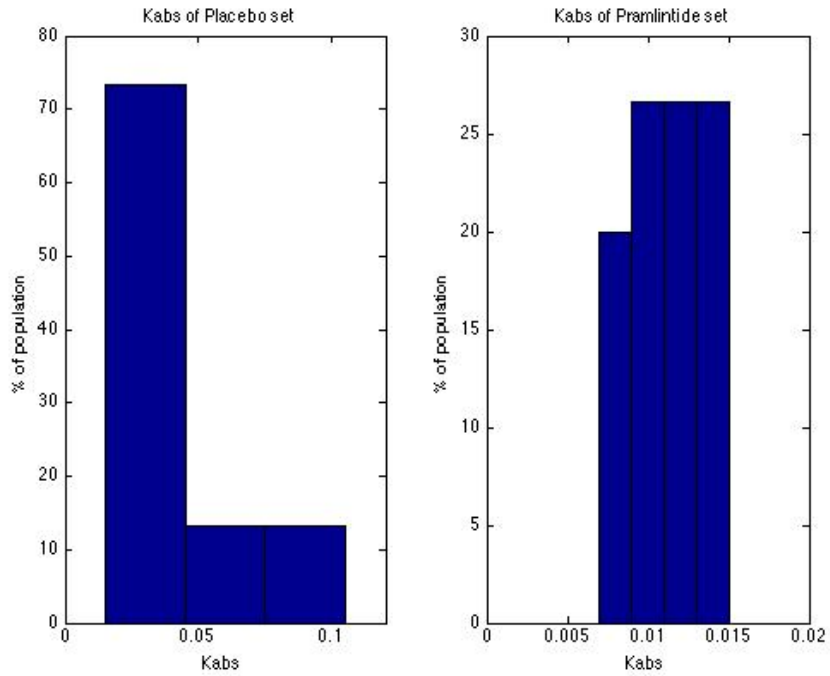


Figure 4.18: Upper: k_{abs} distribution, PBO vs. PRAM. Set of 15 T1DM subjects.

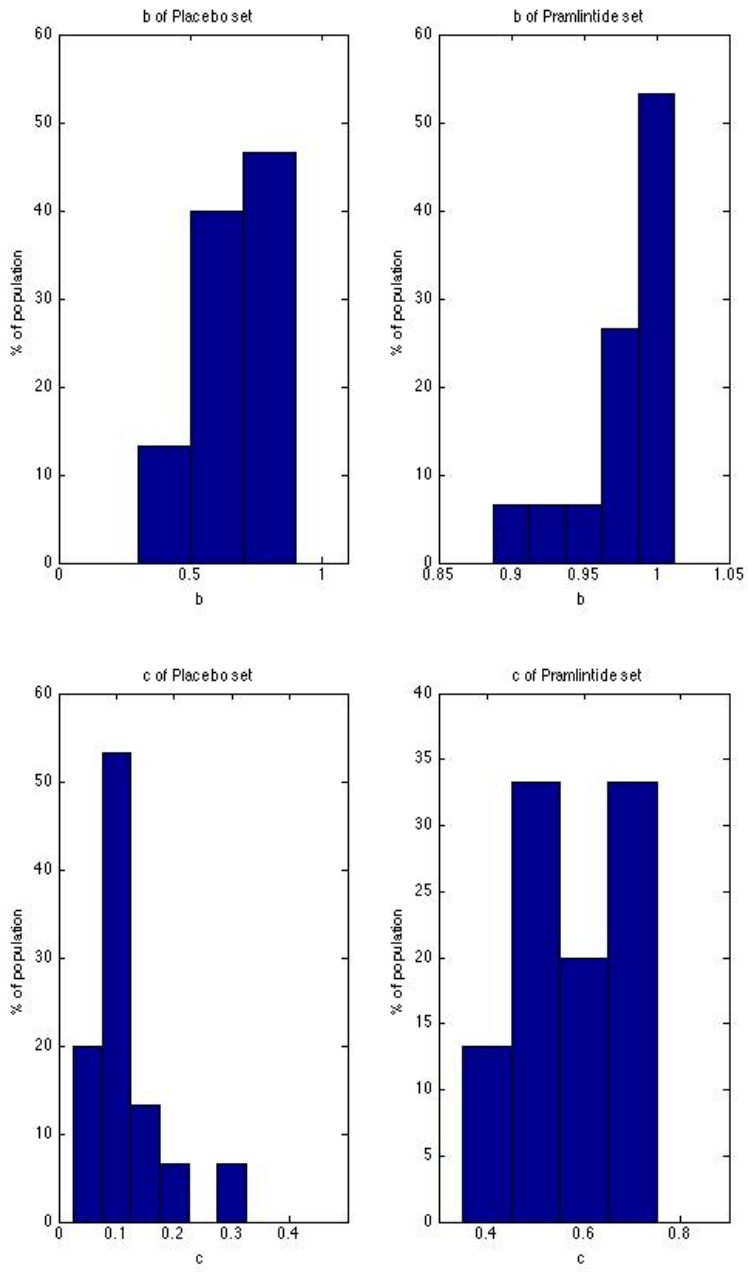


Figure 4.19: Upper: b distribution, PBO vs. PRAM. Lower: c distribution, PBO vs. PRAM. Set of 15 T1DM subjects.

Chapter 5

In Silico Experiments

The aim of the following chapter is to try to include the Pramlintide effect into a Glucose-Insulin simulator for diabetes type 1[42]. This simulations, are also called in silico because it's a simulation of a biological system on a calculator, i.e. using numerical strategies.

5.1 Placebo *vs.* Pramlintide

The effect of Pramlintide is to try to lead the diabetic subject to have the closest BG control as a normal subject. To see the difference between the reference, placebo, and the Pramlintide treated, we must observe the parametric difference as shown in Fig.5.1. The difference between placebo and pramlintide (with 30μg dose) is expressed by the following formula:

$$\mathbf{\Delta p}_i = \frac{\mathbf{p^{PRAM}}_i - \mathbf{p^{PBO}}_i}{\mathbf{p^{PBO}}_i} \cdot 100 \tag{5.1}$$

, where $\mathbf{\Delta p}_i$ is the vector 5x1, containing the differences in % between the parameter vector of placebo and the parameter vector of Pramlintide action, with dose at 30μg, for the subject i. Applying (5.1) to each subject the Fig. 5.1 is obtained.

In Table 5.1 are reported in the bottom the mean differences collected among 15 T1DM subjects. it could be seen that the k_{max} of a Pramlintide is greater in average of 79% than a k_{max} of placebo. The same is for b and c of Pramlintide, greater in average than placebo respectively of 49% and 529% . On the contrary the k_{abs}^{PRA} is lower than placebo of 93%. It's well expected, that in Pramlintide either b and c grow.

subject #	Δkmax	Δkmin	Δkabs	Δb	Δc
1	317	-26	-94	84	378
2	8	-49	-98	110	509
3	19	-20	-96	52	82
4	178	-35	-97	-1	277
5	103	-40	-89	30	323
6	-42	-23	-81	27	1163
7	80	-52	-91	20	196
8	310	-46	-95	18	299
9	-4	-16	-90	54	1355
10	-43	-17	-89	45	892
11	15	-50	-96	66	519
12	-33	-18	-98	35	743
13	59	-22	-99	20	412
14	-23	-27	-90	103	433
15	243	-9	-86	76	359
Δmean	**79**	**-30**	**-93**	**49**	**529**
ΔSD	**125**	**14**	**5**	**33**	**359**

Figure 5.1: Differences, in %, of the two parameters vectors, for each subject. Formula 5.1 is used. The minus means that PRAM is increased from PBO and *vice versa*.

5.2 In silico Open-Loop experiments

The next step is to evaluate Pramlintide effect on a simulation of 100 in silico subjects. In other words including the Pramlintide action on the GIM: Glucose-Insulin Model, developed by Chiara Dalla Man, Davide Raimondo, Robert Rizza and Claudio Cobelli[42]. The GIM version which is running is the one approved by FDA and developed by Dalla Man et al.[43]. An important matter is that the simulation is in OPEN LOOP strategy control because it's a simulation to underline the Pramlintide's effects more than controlling purposes. A population of 100 T1DM is created by simulation GIM[1]. The simulations time is a day (24h) and there is one meal: at 8.00 a.m. 50g of sugar. The insulin control is always in OPEN-loop method for the whole day (Fig.5.2). Using as working condition the Pramlintide's dose of 30μg for all the 100 subjects the plot is represented in Fig.5.3. To quantitatively determine the safety and efficacy of the Open loop therapy with and without Pramlintide, I will use the CVGA (Control-Variability Grid Analysis) grid-plots. It is a population index which shows how good is a treatment over a period of time (in this case a day)[46]. Each dot on the grid represents a subject and can be on different areas of the grid: A: Accurate Control (bright green); Lower B: Begin deviations into hypoglycemia; B: begin control deviations; Upper B: begin deviations int hyperglycemia; Lower C: over-correction of hyperglycemia; Upper C: over-correction of hypoglycemia; Lower D: failure to Deal with hypoglycemia; Upper D: failure to Deal with hyperglycemia; E: Erroneous control (red);

it is clear form Fig.5.4 upper, that a normal treatment, placebo, the cluster is on A and B zone, i.e. the therapy is accurate with important deviations on hyperglycemia. Form Fig.5.4, lower, it is clear that the the therapy, Pramlintide at 30μg, maintains the main cluster in A, but takes some subjects in Lower B and Lower C, showing a deviation to hypoglycemia. Moreover is evident from the BG plots how the Pramlintide mean (bold line of Fig.5.3, upper) is lower and smoother than the average placebo BG pattern (bold line in Fig.5.2), according to the Pramlintide effects on BG control.

The empirical method to include Pramlintide's effect, it is to modify the subject's placebo parameters (originally loaded), and multiply them for the differences, that they have been founded in Table 5.1. So for subject i, its new parameters' vector, which is loaded in the simulator to include the Pramlintide's effects is equal to: $\mathbf{p_i}^{PRA} = \mathbf{p_i}^{PBO} \cdot \mathbf{\Delta p}/100$, where $\mathbf{p_i}^{PBO}$ is the subject parameters vector of the rate of appearance (Ra_i), and $\mathbf{\Delta p}$ is the vector represented in Table 5.1 containing the average parameter differences.

[1]In order to simulate a type 1 subject, in the simulator GIM ,the insulin secretion module (present in healthy or type 2) is substituted by a subcutaneous insulin infusion module and higher endogenous glucose production is induced to create a typical basal glucose of a type 1 diabetic. All other parameters are kept at values of the normal subject (subject assumed in a good control)[44].

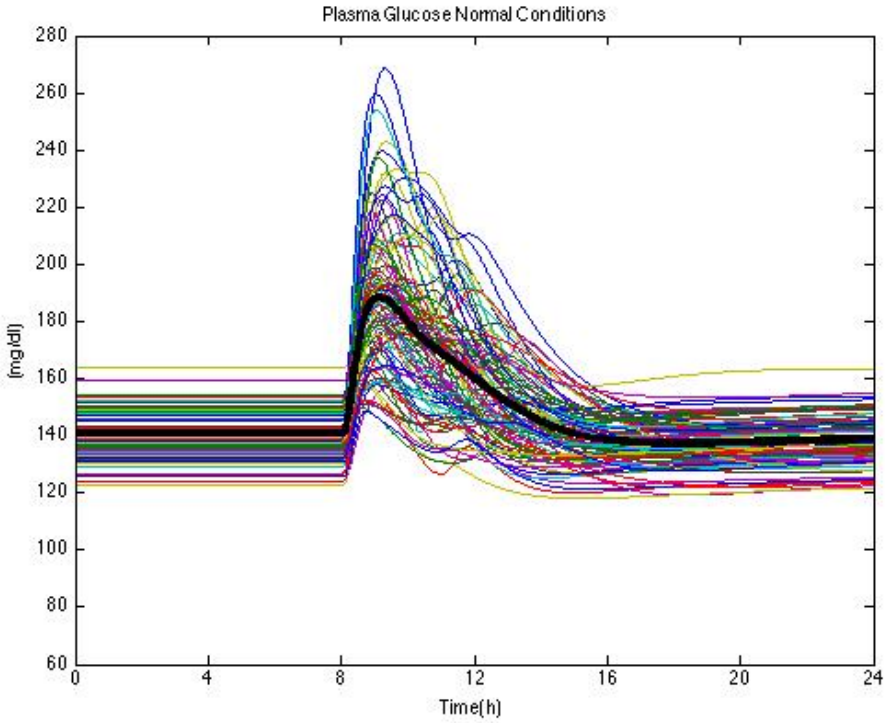
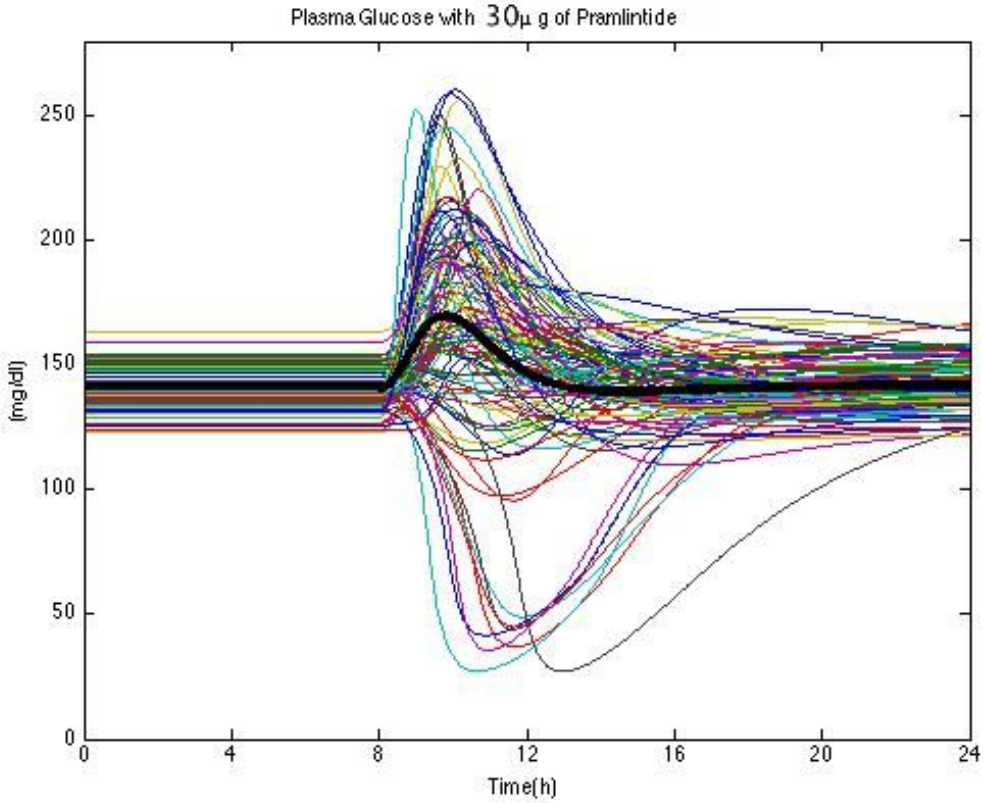


Figure 5.2: Blood Glucose patterns for 100 in silico T1DM subjects normally treated with insulin,i.e. placebo. The bold line is the mean blood glucose path.



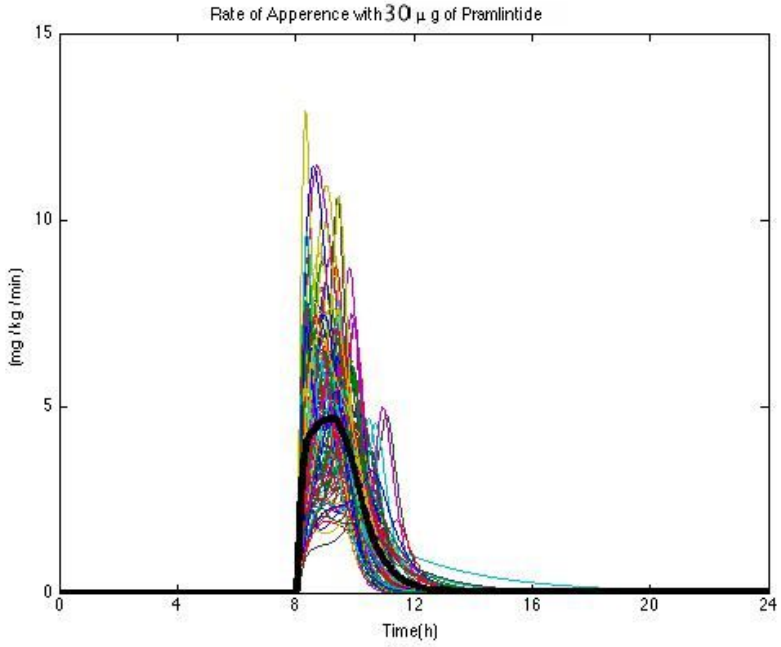


Figure 5.3: Upper: Blood Glucose patterns for a 100 T1DM subjects treated with 30μg of Pramlintide. Lower: Rate of Appearance of 100 T1DM subjects treated with 30μg of Pramlintide. The bold lines are the mean patterns.

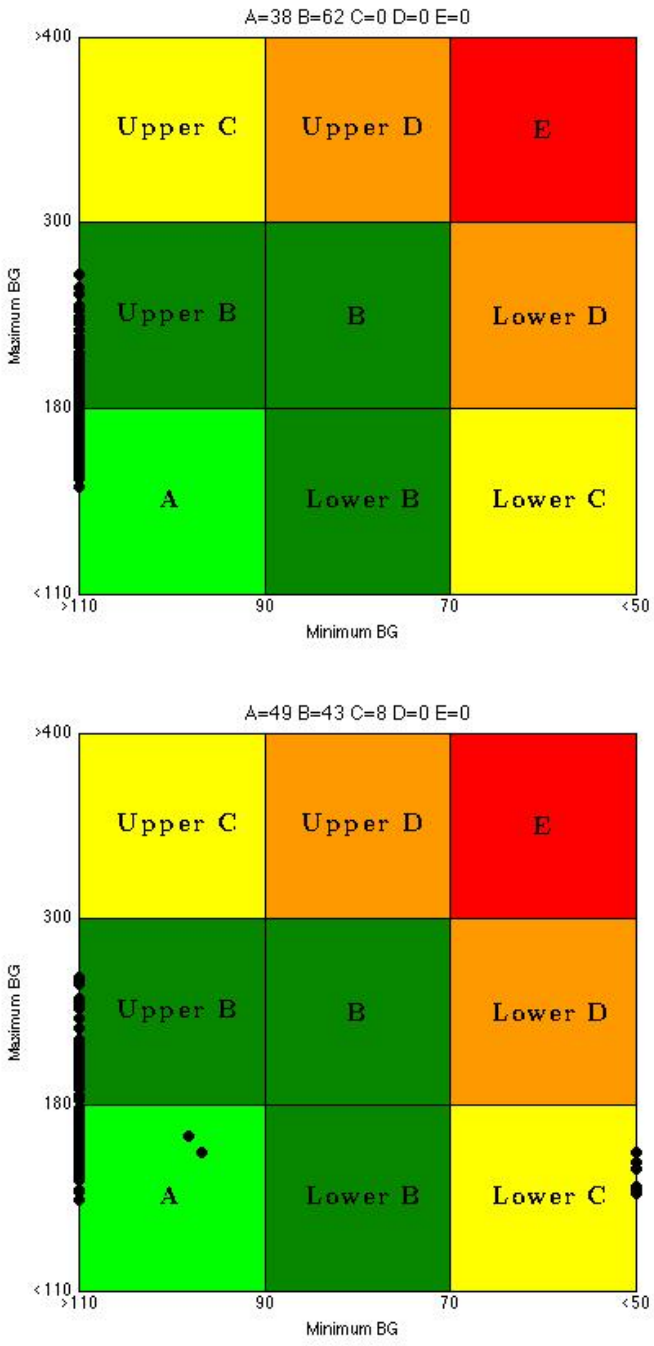


Figure 5.4: Upper: cvga grid of Placebo treated set. Lower: cvga grid of 30μg of Pramlintide treated set. See the Pramlintide set's cluster shifting to the bottom right, indicating a lowering of BG respect the placebo.

Chapter 6

Dose-Response

The dose-response relationship describes the change in effect on an organism caused by differing levels of exposures (doses of Pramlintide) to a stressor (T1DM) after a certain exposure time. In other hand it'll be evaluated the effect of different amounts of Pramlintide to a T1DM in silico, using different doses from the usual reference of $30\mu g$. The

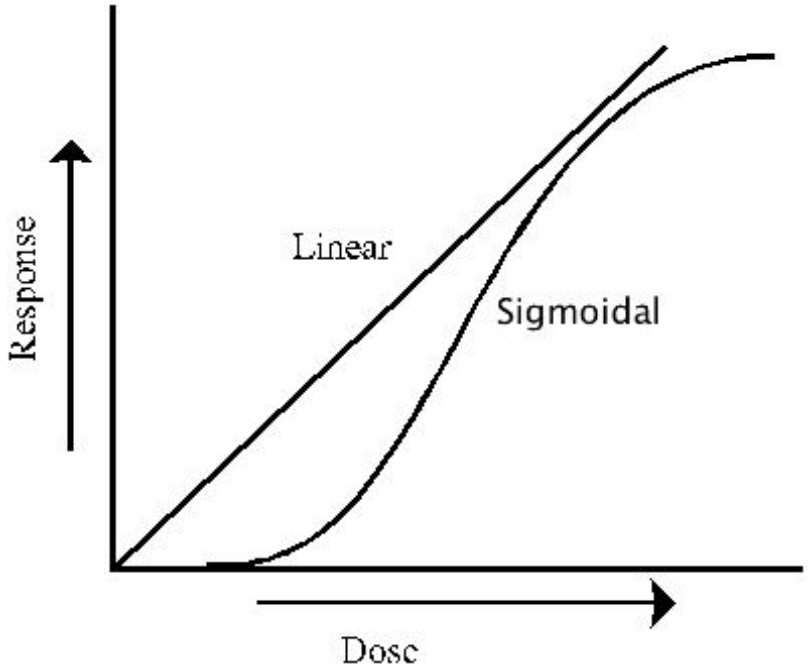


Figure 6.1: Dose-Response Graphs: in this experiments it's assumed the linear one.

relationship between dose and response is a graph X-Y, where the response, Y, is the effect of Pramlintide. If the Pramlintide dose X is increased, the Y increases, i.e. increases the action of the drug. Usually a drug has a *sigmoidal* behavior or parabolic, in our case, due the lack of data, a dose-response, starting with a linear model, will be taken (Fig.6.1).

6.1 Extraction of the response

Being the response linear, I obtain the relationship dose-response calculating two note points of the graph: the first one is for zero dose (placebo), the second one is at 30μg dose (the known case already studied) (Fig.6.2).

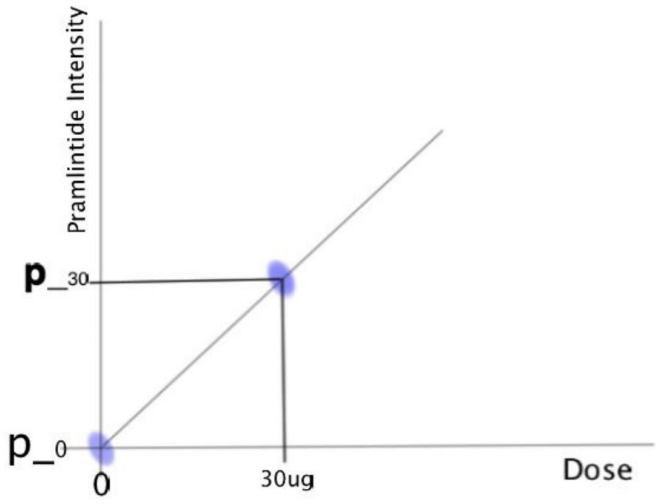


Figure 6.2: The two known work points: placebo at dose=0, and Pramlintide already studied at dose=30μg.

From Placebo (first work point) to a Pramlintide normal treatment (second work point) the Pramlintide lowers the Ra's peak of 33% (1.36 mg/kg/min=7.57μmol/kg/min) and induces a Ra delay of about one hour (according with the amylin effects). But these are only qualitative results and I need more precise informations to evaluate the difference between the two points to estimate a proportional law for two generic points. So the effect of Pramlintide is all included in the experiment identification parametric vector p, e.g. all the information of dose=0 is included in the p_0 values. From Fig.5.1 it's shown the parametric differences between the two known work points. It can be used the same difference to evaluate the effect between other two generic work points, knowing the proportionate relationship (concept of linear dose-response model for Pramlintide in this case).

6.1.1 Simulation in silico with several doses of Pramlintide

The following experiments use different Pramlintide dosages with the previous hypothesis of linear relationship in dose-response. All the simulations are implemented in GIM, with a morning breakfast (8.00 am) of 50g of glucose. The simulation will be for: 10 ,20 ,30 ,40, 60 and 90μg dose represented from Fig.6.3 to 6.8. Another information is given by Fig.6.9, i.e. the CVGA grids for each dosage.

From the plots It's clear that from 0 to 30 μg dosage, the Pramlintide intensity increases with the Pramlintide's effects, as expected. But from the dose of 30μg onwards, more increasing hasn't any effects on the body, in fact for dose 40,60 and 90 μg the plots are basically the same, with not remarkable differences, the cvga show that the cluster shifts from A zone to C, indicating a lowering of BG in the therapy. This is because 30μg dosage is probably the most effective dose and afterward, no effects are evident, in other words 30μg is a threshold for the Pramlintide effects on human body. This makes me think that the actual dose-response shape is parabolic with 30 as a upper threshold (Fig.6.10).

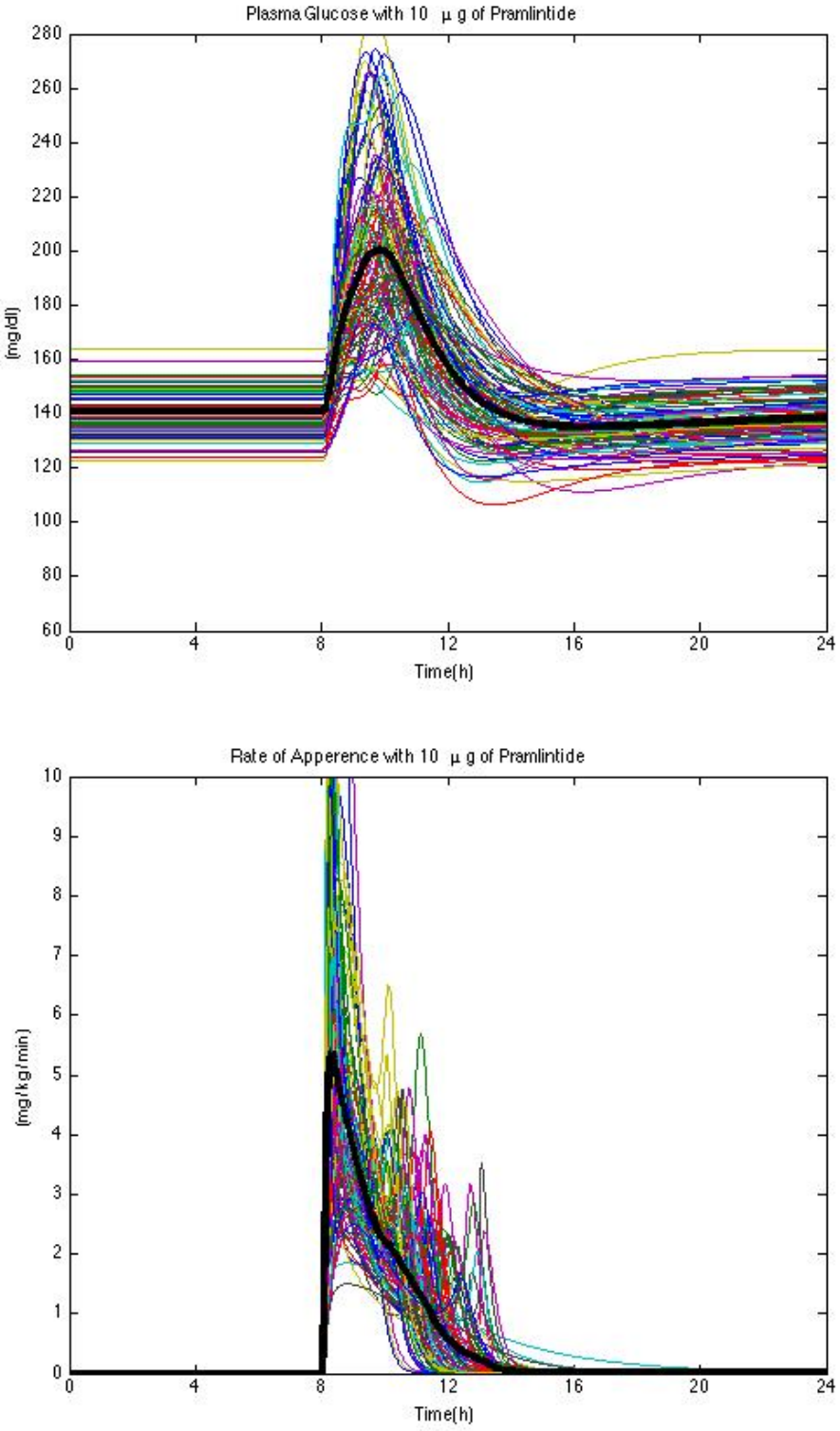


Figure 6.3: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=10μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=10μg). The bold path is the mean of the Ra paths.

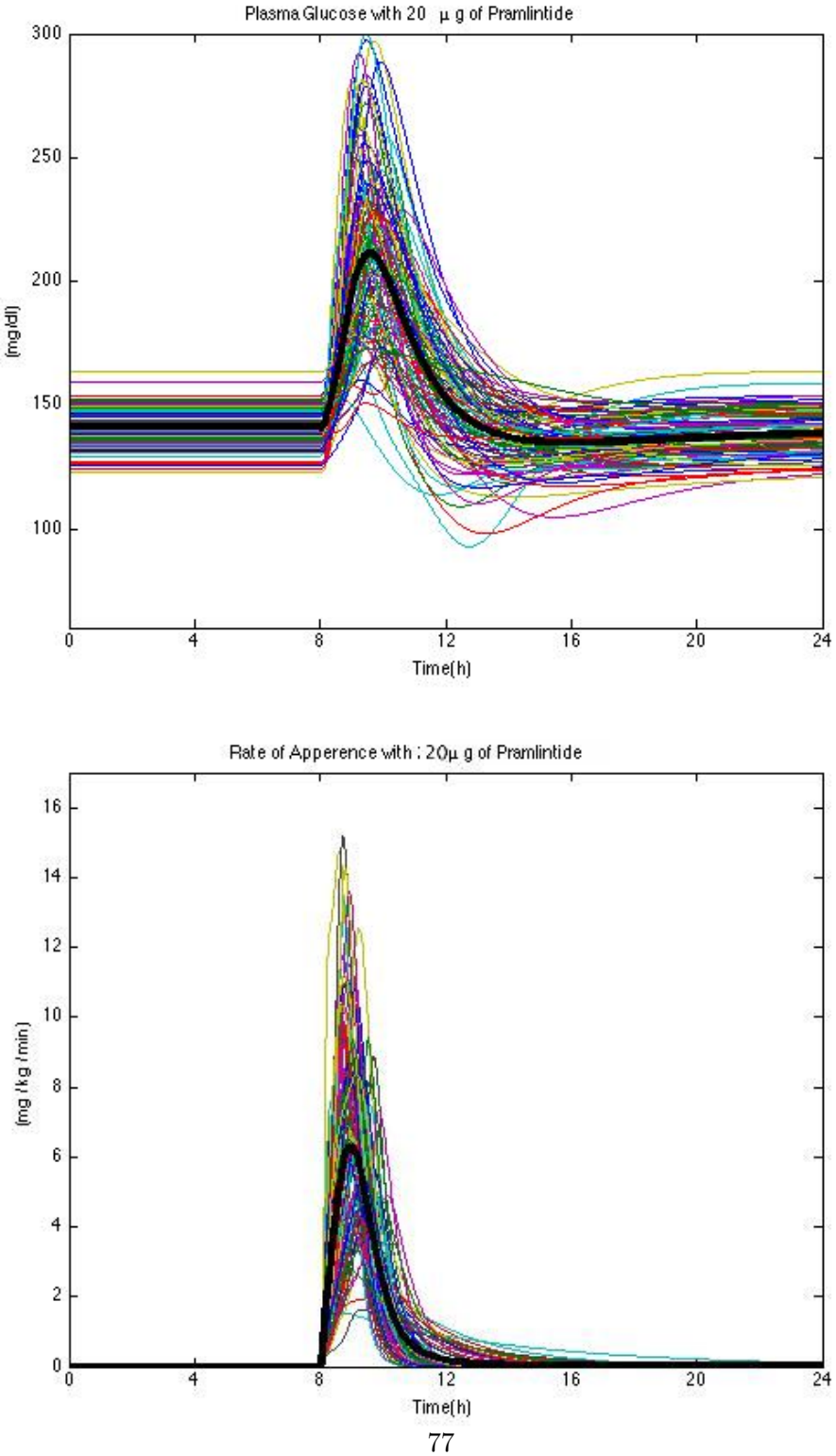
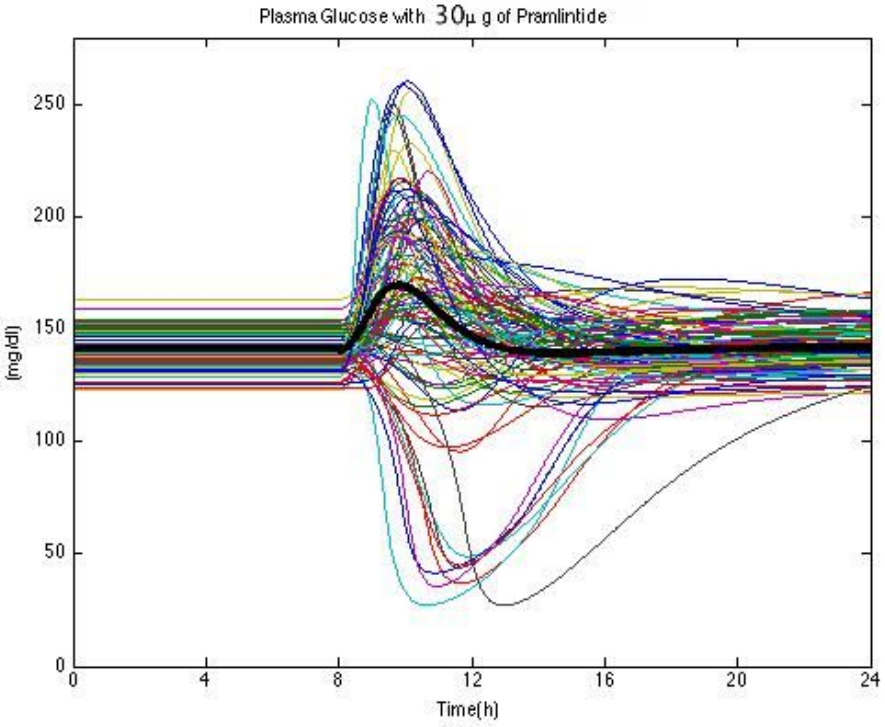


Figure 6.4: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=20μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=20μg). The bold path is the mean of the Ra paths.



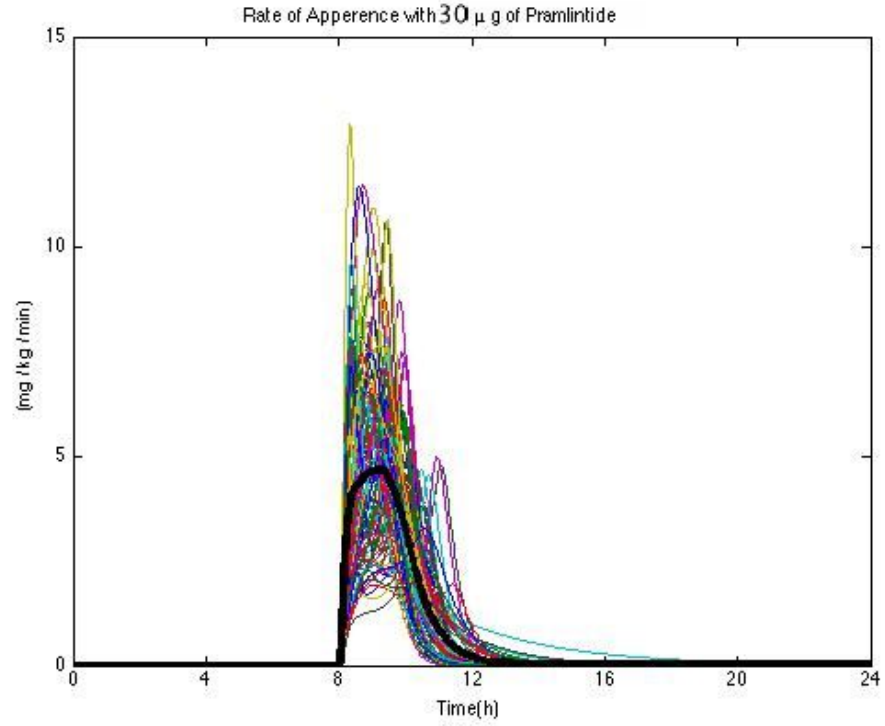
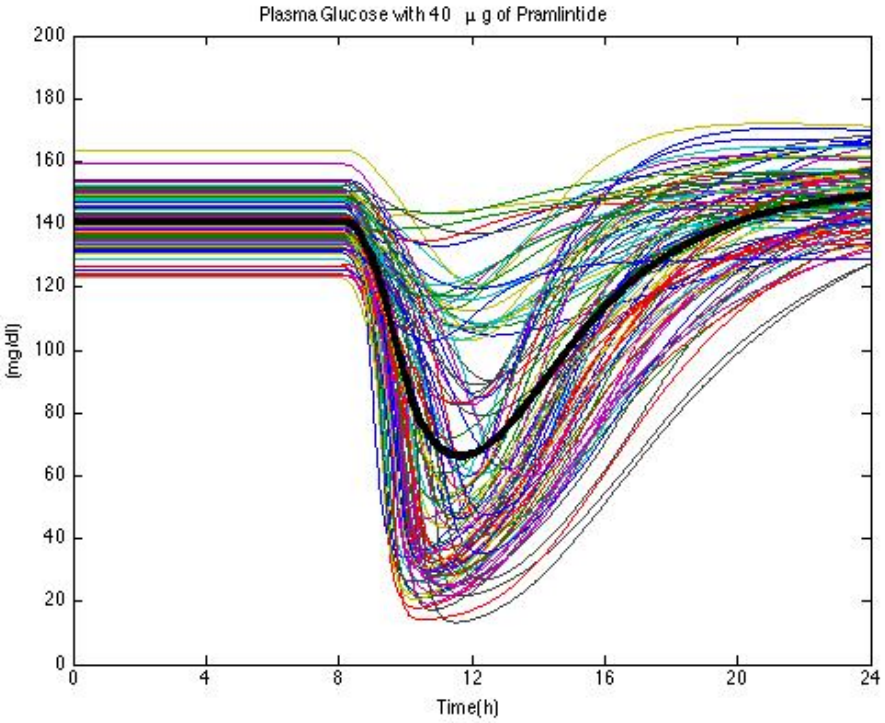


Figure 6.5: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=30μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=30μg). The bold path is the mean of the Ra paths.



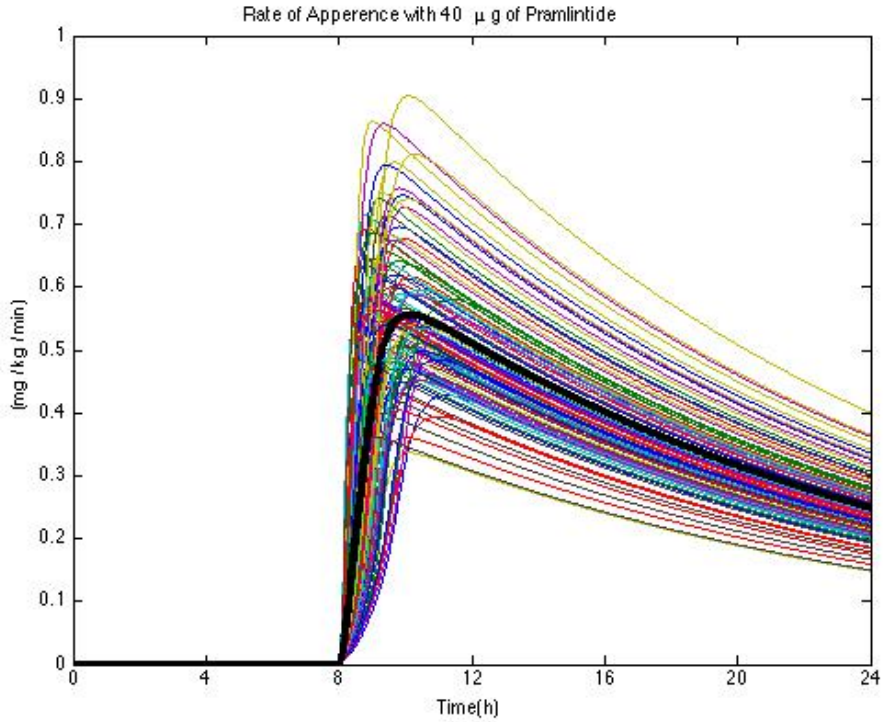
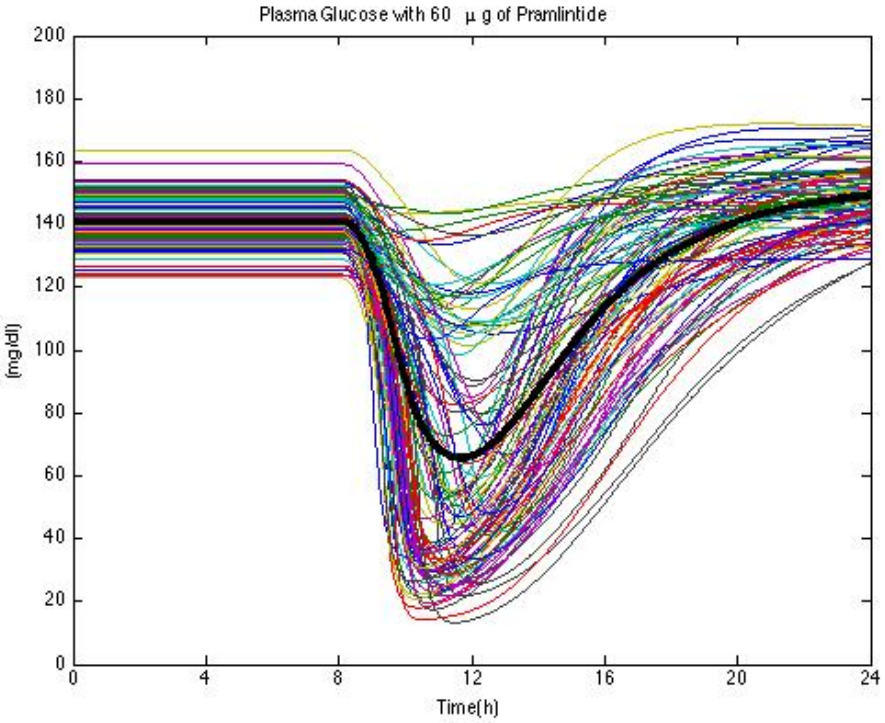


Figure 6.6: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=40μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=40μg). The bold path is the mean of the Ra paths.



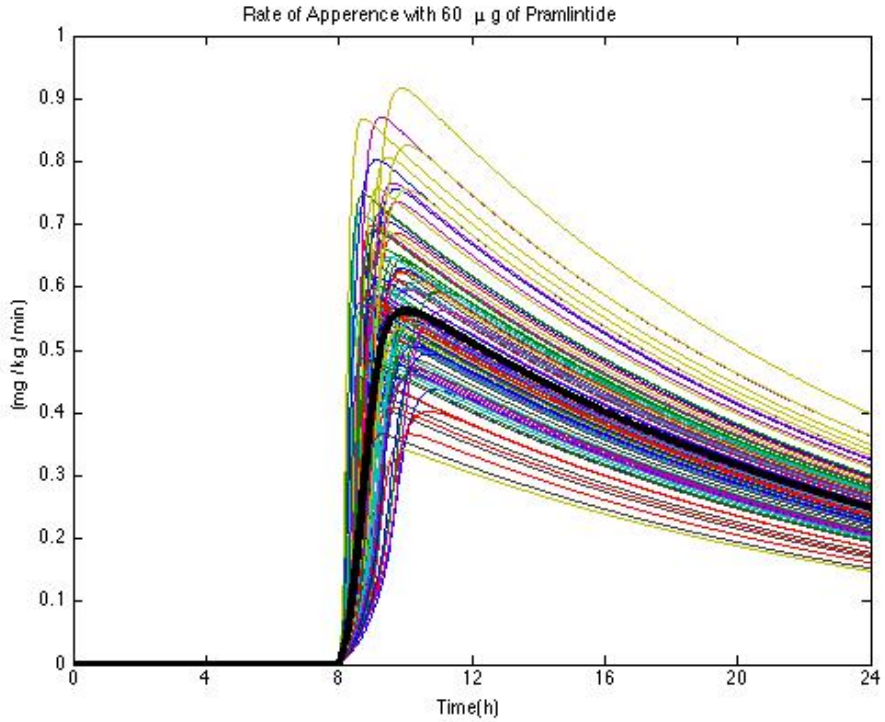
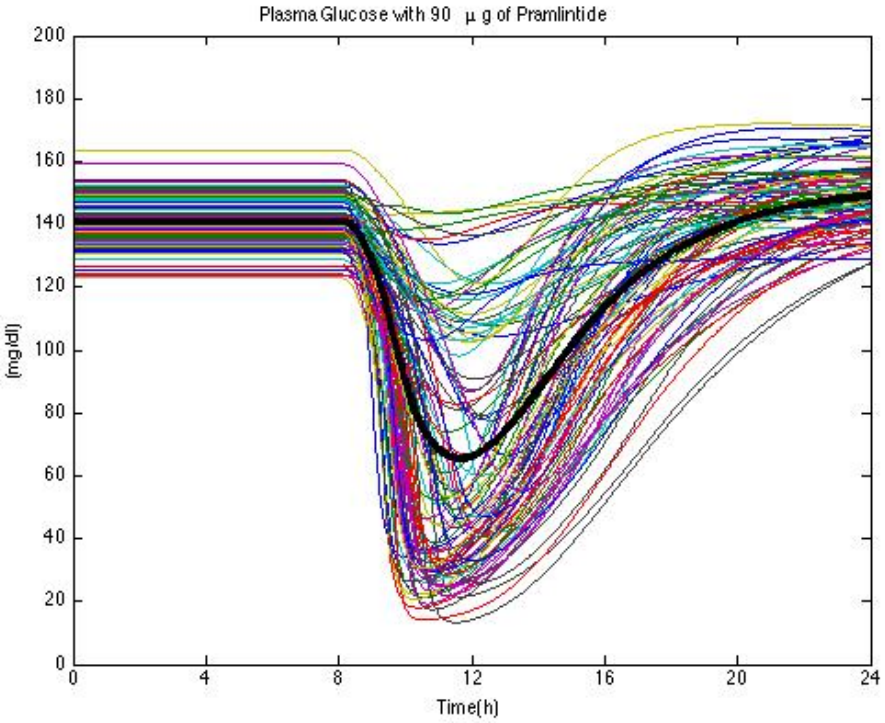


Figure 6.7: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=60μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=60μg). The bold path is the mean of the Ra paths.



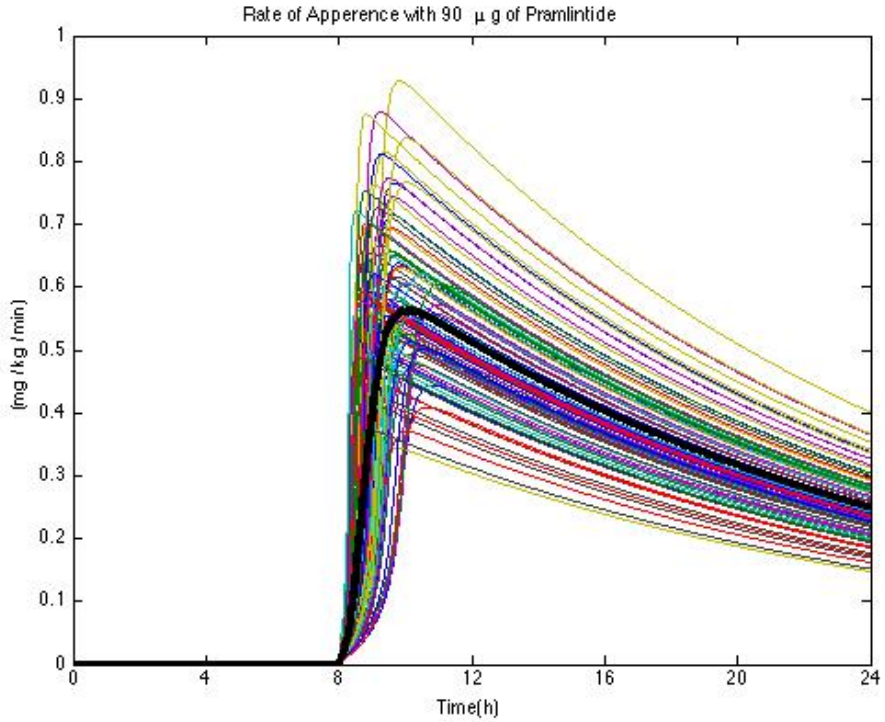


Figure 6.8: Upper: Blood Glucose patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=90μg). The bold path is the mean of the BG paths. Lower: Rate of appearance patterns for 100 in silico T1DM subjects with Pramlintide addition (dose=90μg). The bold path is the mean of the Ra paths.

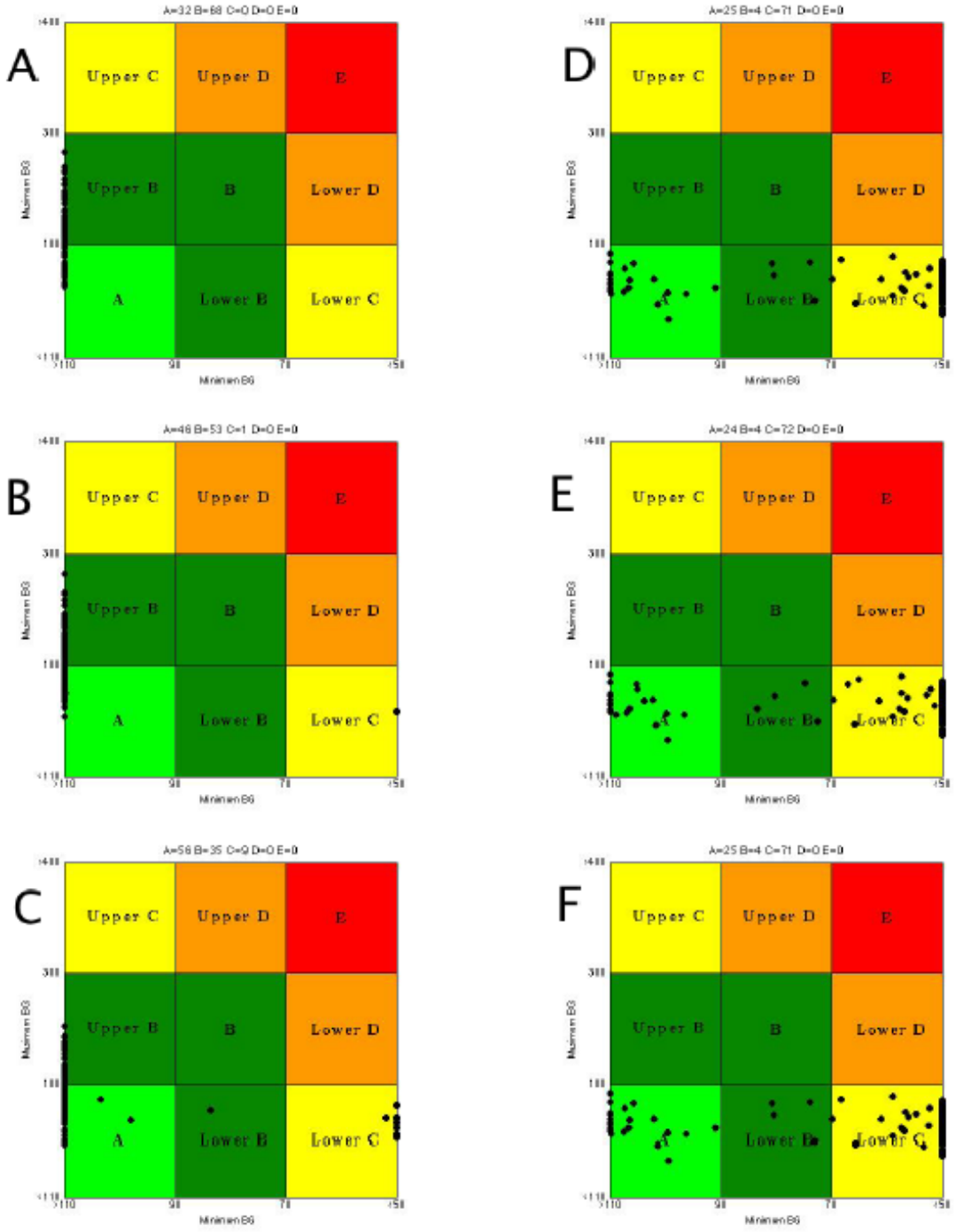
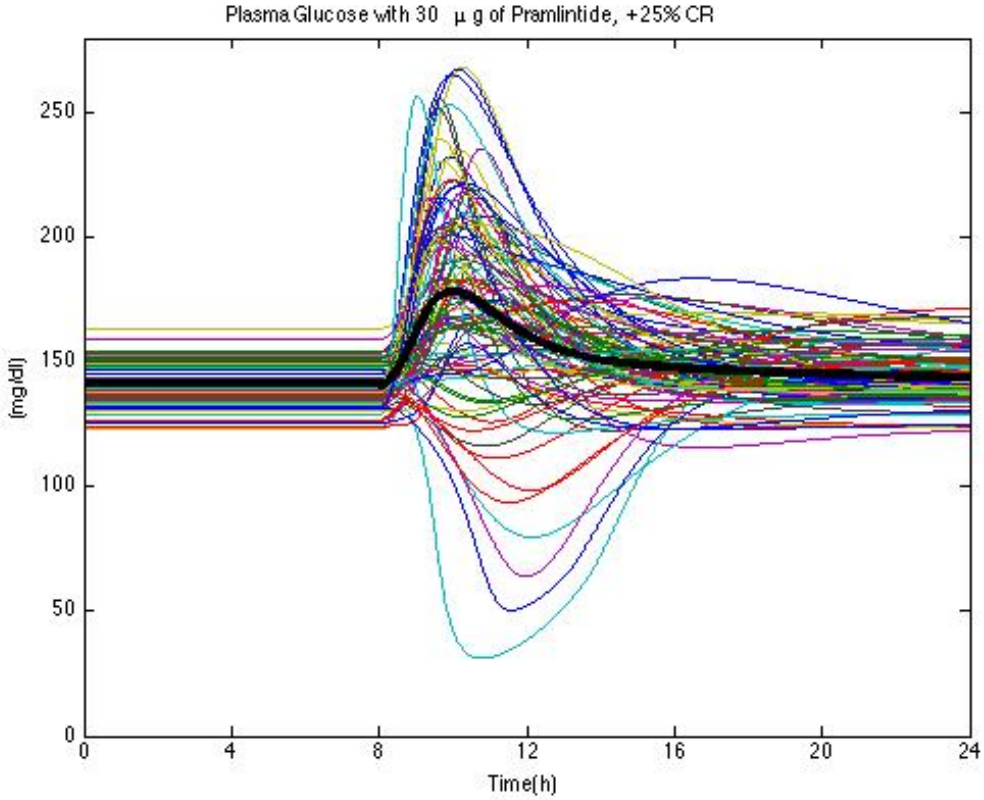


Figure 6.9: CVGA grids for treatments at various dosages of PRamlintide for 100 T1DM subjects set. A: cvga grid, using 10μg dose. B: cvga grid, using 20μg dose. C: cvga grid, using 30μg dose. D: cvga grid, using 40μg dose. E: cvga grid, using 60μg dose. F: cvga grid: using 90μg dose.

6.1.2 Carbo Ratio assessment

The most effective result there is for 30μg in which the Pramlintide planes the post-prandial excursion, i.e. no evident BG variability is seen after the meal (Fig.6.5). It is evident that some subjects go in hypoglycemia area after meal. Despite the fact it's not the aim of this thesis the system control, something could be said about. The CR (Carbo Ratio), i.e. the grams of carbohydrate that are approximately covered by 1 unit of insulin, is subject dependent. In this kind of simulation the CR represents the only point that could be tuned to reduce the insulin given, according to the clinical trials, where the insulin given is about 30% less. An in silico try has been done when the CR is increased for everyone of 25%, and the results are in (Fig.6.10), in which less hypoglycemic episodes are observed.



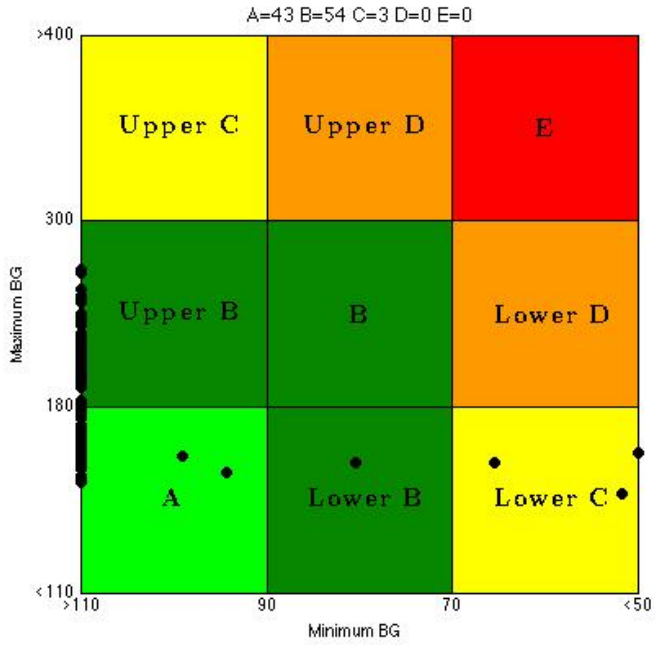


Figure 6.10: Experiments in silico with 100 T1DM subject treated with 30μg of Pramlintide. Here there is also the addition of an increase of CR of 25% to get a better BG control. Upper: Blood Glucose patterns, with mean bolded. Lower: CVGA plot.

Chapter 7

Conclusions and Prospectives

Even though in Europe this new drug is not commercialized, USA seems to be more optimistic about it. In fact it's clear from the tests developed in this thesis that Pramlintide addition to a normal treatment (placebo) lowers the blood glucose after a meal. Not only helps to deal with hyperglycemia health issues, but reduces the blood glucose variability, which could lead to more dangerous problems. Pramlintide acts like the hormone amylin, which is absent in T1DM, slowing the gastric emptying. The gastric contribute to blood glucose control, seems to be more important day after day, where not only the amylin is involved, but also other hormones, as GLP etc.. Slowing the gastric emptying, it's not the only action of Pramlintide. It Reduces the secretion of glucagon, leading the system to have less endogenous glucose. Despite the fact the main action of Pramlintide is to slow the gastric emptying, the second effect should not be neglected, and could be a future aim.

The identification of the action model of the pharmacodynamics of this drug has been made with a good model, the best in literature, which considers the nonlinearity of the problem. A point should be made: the b value tends to one, not only for the average, but for all the subject's identifications. This is true, because it means that a great slowing in gastric emptying happens and it is visible from the k_{empt} plot, in which it starts from its maximum value k_{max} and decrease quickly to k_{min} as shown in Fig.7.1. Physiologically talking this is fact is not possible. So a question arises, is this model reliable? Yes it is, even if it has this lack of coherence with physiology (b goes to one and c is small). In fact the identification for the 15 subjects made, is reliable and with a good precision. About the identification a note should be pointed out: the standard deviation of the error is not known. In the glucose models identification the CV is usually put constraint at 2%, here in Rate of Appearance it's not possible to do that.The Ra derives from a complex extraction using a triple-tracer approach to assess the postprandial glucose metabolism fluxes, as Ra[47]. Also to affect

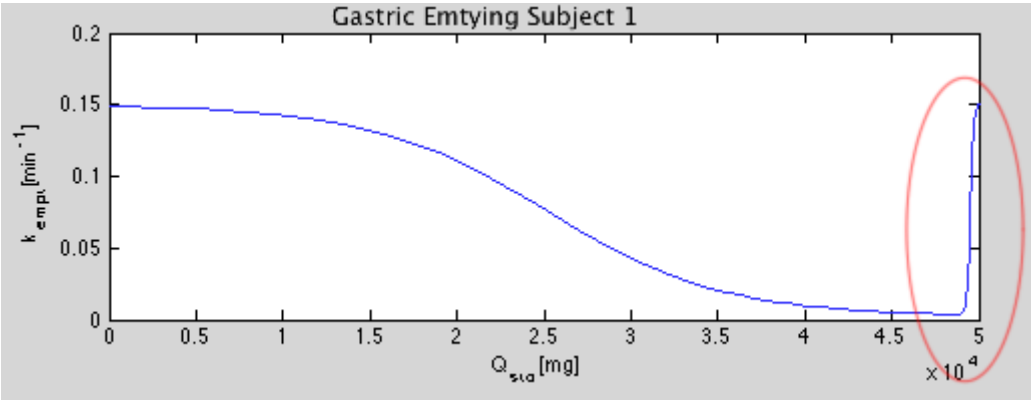


Figure 7.1: Gastric Emptying for the average subject treated with Pramlintide (30μg); the red circle points the physiological incoherence of the model.

the identification occur all the errors deriving from the complex measures [47]. After all good results are obtained. Moreover, a hint could be, to evaluate the inter variability of the 15 subjects calculating the covariance matrix, which it wouldn't be diagonal and extract a stochastic function for insertion in the silico experiments.

After the identification of the action model, an inclusion in silico has been done. Here is important to underscore the open-loop control. In this control is more easy to assess the Pramlintide effect, which is the actual purpose of this thesis. Besides using high dosage of drug it takes the system to have some subjects in hypoglycemia. To try to correct them a first step should be that one to correct for each subject the CR or to use a closed loop control. It has been done for simplicity an automatic increase of CR for everyone of +25%, and the number of subjects that are in hypoglycemia, are decreased (only in open-loop).

Using the Pramlintide at 30μg dose, as expected from clinical trials, is the best choice. In fact trying all the doses, 30μg seems to be the most effective therapy among them, in terms of Pramlintide effects on human body of a type 1 diabetic. From 30μg onwards no more effect is given by Pramlintide. In general Drugs have a usual sigmoidal dose-response model (Pramlintide seems to not be an exception) in fact after the threshold value of amount of medicine, the body has all the drug's receptors full, and more amount given, would mean that the overdose is in stand-by to enter into the target receptors and so it is inactive. Pramlintide follows this phenomenon and in its case the threshold is 30μg, as expected (Fig.7.2).

Furthermore the usage of other dosages of Pramlintide doesn't bring the system in a bad control, as shown in CVGA grids: none of the CVGA grids show a failure in control, showing the fact that ,Pramlintide injected in a good-controlled system doesn't alter the

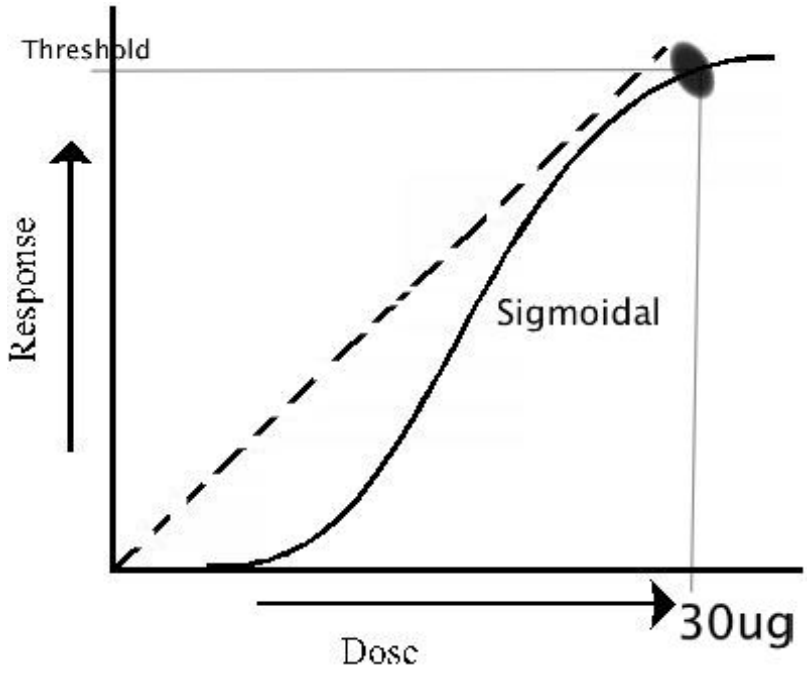


Figure 7.2: Dose-Response Graphs: in this experiments it's assumed the linear, but a threshold is evident for 30μg of Pramlintide.

system. Speaking about systems point of view, Pramlintide action of lowering the output (Ra) and BG variability, it could be seen as a low-pass filter, considering Pramlintide as an agent which get rid of all the high speed components, i.e. the high frequency components. So the controllers should prefer a configuration with Pramlintide to study and assess a more easy BG control.

7.0.3 Future

In 2005, the Food and Drug Administration (FDA) has approved Symlin (pramlintide acetate), an injectable medicine to control blood sugar for adults with type 1 and type 2 diabetes. Symlin is to be used in addition to insulin therapy in patients who cannot achieve adequate control of their blood sugars on intensive insulin therapy alone. Symlin will be the only therapy for the treatment of type 1 diabetes other than insulin. Patients with type 2 diabetes already have several other types of oral therapies available. The safety and efficacy of Symlin were studied in approximately 5000 patients. Overall Symlin therapy was associated, in patients with both types of diabetes, with improvements in the control of blood glucose and with weight loss. So-called "tight" control of blood sugar is desirable

in all patients with diabetes in order to reduce risks for long-term adverse consequences of the disease, including blindness, kidney disease, and vascular disease. Symlin is to be used only in combination with insulin to help lower blood sugar during the 3 hours after meals. Symlin will have a Medication Guide (FDA-approved patient labeling) and a Risk Minimization Action Plan (RiskMAP) due to three areas of concern. First, the principle risk associated with Symlin therapy is hypoglycemia, and this risk is greatest in patients with type 1 diabetes and in patients with gastroparesis (motility problems of the stomach-a long-term complication of diabetes). Second, the potential for medication errors, specifically mixing of Symlin with insulin in the same syringe, which can alter the activity of the insulin, is addressed in the Medication Guide and in physician labeling. Finally, the potential for off-label use in patients where the benefit/risk profile has not been characterized or demonstrated is also a concern and will be monitored by the sponsor. The Medication Guide informs patients that Symlin should only be used if they are already using their insulin as prescribed, but still need better blood sugar control; will follow their doctor's instructions exactly; will follow-up with their doctor often; will test their blood sugar levels before and after every meal, and at bedtime; and understand how to adjust Symlin and insulin doses.

A whiteness to the fact that Pramlintide is a good drug, with a good potential, is the recent (July, 2012) investment of Bristol-Myers Squibb, which agreed to buy Amylin Pharmaceuticals, the maker of promising Pramlintide, in a complicated deal that is valued at about 7 billion dollars. To help finance the transaction, Bristol-Myers teamed up with AstraZeneca, which payed about 3.4 billion dollars in cash and will share in the profits from Amylin's sales. To be more specific the deal is not only for Pramlintide copyrights, in fact other couple of drugs and some projects are developed by Amylin Pharmaceuticals (Pramlintide is the main bargain after all).

Pramlintide/Metreleptin

Another kind of treatment with Pramlintide may be with others drugs like Metreleptin, i.e. the synthetic analog of human Leptin. Indeed the neurohormonal control of body weight involves a complex interplay between long-term adiposity signals (e.g., leptin), and short-term satiation signals (e.g., amylin). In diet-induced obese (DIO) rodents, amylin/leptin combination treatment led to marked, synergistic, fat-specific weight loss. To evaluate the weight-lowering effect of combined amylin/leptin agonism (with pramlintide/metreleptin) in human obesity, a 24-week, randomized, double-blind, active-drug-controlled, proof-of-concept study was conducted in obese or overweight subjects. Combination treatment with pramlintide/metreleptin led to significantly greater weight loss from enrollment to week 20. The greater reduction in body weight was significant as early as week 4, and weight loss continued throughout the study, without evidence of a plateau. The most common adverse events with pramlintide/metreleptin were injection site events and nausea, which

were mostly mild to moderate and decreased over time. These results support further development of pramlintide/metreleptin as a novel, integrated neurohormonal approach to obesity pharmacotherapy[44].

T2DM Pramlintide Treated

The Pramlintide is having a good success in the treatment of type 2 diabetics treatments. Most of the normal treatments are a combination of: Insulin, Insulin Secretagougues and Insulin Sensitizer. All these treatments are often followed by weight gain, it's note the fact that more insulin is given more you gain weight (but it's not clear which is the cause and which the effect). The body mass increased is obvious not good for a type2. Moreover the amylin activity is deficient. From an important clinical trial, in which the Pramlintide is given to type2, in addition to their normal treatment some interesting results are expressed: less insulin is given and A1c final achievement. The less insulin given equals less weight gain, which is good, but the A1c is not correlated with the Pramlintide dose because we will get the similar A1c without it. But the important thing is: more weight loss is evident in patient with big BMI, e.g. obesity class III. Furthermore the combination of Insulin, Pramlintide and Metformin (drug which reduces BG level acting on intestinal absorption and liver production) brings the body to a more weight loss[45].

Future

The possible future developments is to include the Pramlintide in the artificial pancreas devices. The issues linked with this is to calibrate the controls, the engine and a strategy to use another way of injection, which takes a synchronization with insulin control. The artificial pancreas is getting more sophisticated and more user-friendly, for instance a project is being developed by University of Virginia, in which a Android application is made to control your pancreas fluxes directly from your smartphone: a street lighter image on the screen tells you if you are in hypo. (red light) if you are in a sufficient range (yellow light) or in a good range (green light). An addition of Pramlintide complicates the whole system but the green light would be more frequent of course. Also we need to analyze if there is some correlation between Pramlintide action and insulin sensitivity, if there is, lots of controls might change. How to not cite a new and charming prospective of type 1 treatment studied by Southwestern Medical Center of Texas University. They say that if we block the glucagon action the lack of insulin which causes the diabetes is not more a disease! They have genetically modified some mice getting rid of their glucagon's receptors. The mice respond the same way if the mice are with or without insulin, i.e. they don't develop diabetes. They in fact affirm that insulin is important for the grown of the body but in adult age the insulin function is only to control the glucagon action! This open to

a set of study of blocking the glucagon action.

Pramlintide in Europe

Amylin Pharmaceuticals, via a subsidiary called Amylin Europe Ltd., submitted Marketing Authorization Applications for SYMLIN(TM) (Pramlintide acetate) to the European Agency for the Evaluation of Medicinal Products (EMEA) under the centralized European procedure for the authorization of medicinal products in May 2001, but so far we don't know anything about it. Besides in USA, San Diego exactly, in October 2007 Amylin Pharmaceuticals, Inc. announced that the U.S. Food and Drug Administration (FDA) has approved the SymlinPen 120 and the SymlinPen 60 pen-injector devices for administering Symlin (Pramlintide acetate) injection. These new pre-filled pen-injector devices feature simple, fixed dosing to improve mealtime glucose control. *'SymlinPen 120 and SymlinPen 60 offer patients improved convenience and accuracy,'*, said Daniel M. Bradbury, President and CEO, Amylin Pharmaceuticals. *'For people with diabetes using mealtime insulin, the addition of Symlin can enhance glucose control with the potential for weight loss.'*. SymlinPen 60 features fixed dosing to deliver 15, 30, 45, or 60 micrograms per dose. SymlinPen 120 features fixed dosing to deliver 60 or 120 micrograms per dose. Both pen-injector devices can be conveniently stored at room temperature not to exceed 86 degrees F (30 degrees C) after first use. The pens are available to patients from December 2007.

Some Pramlintide engineer projects are nowadays developing in Italy, between the collaboration of University of Pavia and Unifersity of Padova, in which I'm working on and belong to.

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