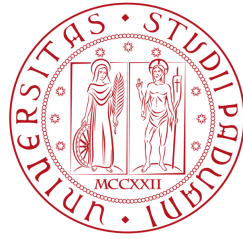


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A Model of Glucose Production During Exercise in Normal and Type 1 Diabetic Subjects

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*“Prendete la vita con leggerezza,
che leggerezza non è superficialità,
ma planare sulle cose dall’alto,
non avere macigni sul cuore.”*

Contents

Contents	5
List of Figures	7
1 Introduction	13
1.1 Background	13
1.2 Aim and outline of the thesis	15
2 Glucose metabolism and physical activity	17
2.1 The glucose-insulin regulatory system	17
2.2 Diabetes	21
2.3 Physical activity	22
3 Experimental Protocol	25
3.1 Subjects	25
3.2 Clamp study	26
3.3 Experimental design	28
3.3.1 Screen visits	29
3.4 Estimates of plasma glucose, insulin, glucagon and glucose fluxes . . .	34
4 Models	41
4.1 EGP during a meal	41
4.2 Model identification in the pre-exercise phase	43
4.2.1 Single-visit identification	43
4.2.2 Three-visits multi-identification	46
4.3 Models of EGP incorporating exercise	50
5 Results	55
5.1 ND	55
5.2 T1D	62

6 Conclusion	65
Bibliography	69

List of Figures

2.1	Daily glyceimic pattern	18
2.2	Daily insulin pattern	19
2.3	Maintenance of blood glucose levels by glucagon and insulin	20
2.4	Traslocation of GLUT4 glucose trasporters	24
3.1	Anthropometric characteristics of healthy subjects: mean and standard deviation (SD).	26
3.2	Anthropometric characteristics of subjects with type 1 diabetes: mean and standard deviation (SD).	26
3.3	Glucose levels and rate of insulin infusion in the three visits.	29
3.4	Study Visits structure.	30
3.5	V1: euglycemic clamp and low insulin level.	32
3.6	V2: euglycemic clamp and high insulin level.	33
3.7	V3: hyperglycemic clamp and low insulin level.	33
3.8	Average glucose concentration in ND and T1D subjects in the three visits.	35
3.9	Average insulin concentration in ND and T1D subjects in the three visits.	35
3.10	Average glucagon concentration in ND and T1D subjects in the three visits.	36
3.11	Average tracer-to-tracee ratio in ND and T1D subjects in the three visits.	37
3.12	Average glucose disappearance in ND and T1D subjects in the three visits.	38
3.13	Average glucose production in ND and T1D subjects in the three visits.	39
4.1	Insulin action model.	43
4.2	Estimates of k_2 in the three visits.	46
4.3	Insulin action model.	48

4.4	Physical activity signal modeled as a square wave.	50
4.5	Model 2: instantaneous action of physical activity on k_2	51
4.6	Model 5: delayed action of physical activity on k_2	52
4.7	Physical activity signal modeled as a ramp.	53
5.1	Epinephrine signal.	58
5.2	Model 8	60
5.3	Model 8: average data vs model prediction, and weighted residuals. . .	61
5.4	Model 8 with one subject excluded: average data vs model prediction, and weighted residuals.	62
5.5	Model 8, T1D subjects: average data vs model prediction, and weighted residuals.	64

Abstract

English

It is well established that a regular physical activity offers important benefits in both healthy and subjects with diabetes. For the latter, however, the best conditions in terms of intensity, duration and insulin therapy to reach a good glucose control are not clear yet. Despite the numerous studies conducted in this area, none of them allows to accurately describe the effects of exercise on endogenous glucose production. The quantification of these effects can be extremely important, especially for diabetic individuals, since this information could be incorporated in the control algorithms of open-and closed loop (artificial pancreas), thus improving the therapy of this disease.

The goal of this work is to develop a mathematical model which is able to describe the effects of physical activity on endogenous glucose production in different experimental conditions in both healthy and type 1 diabetic subjects. To address this task, data of 12 individuals (6 healthy and 6 with type 1 diabetes) were examined. They performed three visits in random order in different glycemc and insulinemic levels using the clamp technique: the first visit was in euglycemia and low insulin, the second visit was in euglycemia and high insulin, while the third visit was in hyperglycemia and low insulin. In each visit, a session of 60 minutes of moderate physical exercise was performed. The glucose fluxes (production and disposal) were estimated through glucose tracer infusions using the tracer-to-tracee clamp technique.

The starting point of this work, has been a model which is already known in literature that provides a good prediction for EGP profile that occurs after food ingestion (Dalla Man et al. [7]). This model, which does not include exercise, has been modified and adapted to our experimental conditions in order to incorporate the effect of physical activity and, therefore, to provide a good prediction of EGP.

The best model selected, assumes a delayed effect of exercise on insulin sensitivity, a direct effect of glucose, a delayed effect of insulin and a direct effect of glucagon on EGP. Despite the satisfactory results obtained with ND subjects, further investigation is necessary, especially for subjects with diabetes.

Italiano

È scientificamente provato che una regolare attività fisica comporti importanti benefici sia in soggetti sani che diabetici. In questi ultimi però non sono ancora state definite le condizioni ottimali in termini di intensità, durata e terapia insulinica per un buon controllo del glucosio. Nonostante i numerosi studi condotti in quest'ambito, gli effetti dell'esercizio fisico sulla produzione endogena di glucosio, in diverse condizioni sperimentali, non sono ancora stati esaminati. La quantificazione di questi effetti può risultare estremamente importante soprattutto per i soggetti diabetici dato che tale informazione potrebbe in futuro essere incorporata in algoritmi di controllo di sistemi open e closed-loop (pancreas artificiale).

Lo scopo di questa tesi è quello di sviluppare un modello che descriva gli effetti dell'attività fisica sulla produzione endogena di glucosio in diverse condizioni sperimentali sia in soggetti sani che diabetici di tipo 1. Per lo sviluppo sono stati esaminati i dati di 12 soggetti (6 sani e 6 diabetici) i quali hanno effettuato 3 visite in ordine casuale con livelli glicemici e insulinemici differenti utilizzando la tecnica del clamp: la prima visita è stata condotta in normoglicemia e bassa insulina, la seconda visita in normoglicemia e alta insulina, mentre la terza visita in iperglicemia e bassa insulina. In ognuna di esse è stata prevista una sessione di esercizio fisico moderato della durata di 60 minuti. I flussi di glucosio disponibili (produzione ed utilizzazione) sono stati ottenuti utilizzando le infusioni di un tracciante del glucosio secondo la tecnica del clamp del rapporto tracciante-tracciato.

Il punto di partenza di questa tesi è stato lo studio di un modello già presente in letteratura che fornisce una buona predizione della produzione endogena di glucosio susseguente ad un pasto (Dalla Man et al. [7]). Questo modello, che non considera l'esercizio, è stato successivamente modificato e adattato alle nostre condizioni sperimentali al fine di incorporare l'effetto dell'attività fisica e fornire quindi una buona descrizione di EGP.

Il modello risultato migliore incorpora un effetto ritardato dell'esercizio sulla sensibilità insulinica, un effetto immediato del glucosio, un effetto ritardato dell'insulina, e un effetto immediato del glucagone su EGP. Nonostante i buoni risultati ottenuti per i soggetti sani, ulteriori studi sono necessari per la validazione del modello selezionato, soprattutto nel caso dei soggetti diabetici.

Glossary

T1D	Type 1 diabetes
ND	Non-diabetic subject
PA	Physical activity
BMI	Body mass index
FFA	Free fatty acid
EGP	Endogenous glucose production
Rd	Rate of glucose disposal
TTR	Tracer-to-tracee ratio
CV	Coefficient of variation
AICc	Corrected Akaike information criterion

Chapter 1

Introduction

1.1 Background

Glucose is the most abundant monosaccharide in nature and the main source of energy for living organisms. In our body many tissues can also use fat or protein as an energy source but others, such as the brain and red blood cells, can only use glucose. In healthy subjects glucose level is maintained in a narrow range (70 - 110 mg/dL) thanks a complex control system which includes organs such as liver, pancreas and kidneys. The liver is the main organ involved in glucose production, while kidneys are responsible in a minor part. As regard the pancreas, it secretes two important hormones that act in an opposite sense: insulin and glucagon. Insulin is produced by β -cells in response to high levels of plasma glucose and it promotes glucose utilization and inhibits the endogenous glucose production. Glucagon is produced by α -cells in response to a fall in plasma glucose below the hypoglycemic threshold (70 mg/dL). It stimulates glucose production promoting glycogenolysis and gluconeogenesis, with a consequently increase of glucose concentration.

Impairment of the glucose regulatory system can lead to a several metabolic disorders such as glucose intolerance and, at worst, to diabetes. Hypoglycemia is the condition in which glucose level is lower than its normal range, while the opposite condition is called hyperglycemia. The first one is dangerous in the short term because it can cause important complications to the dependent-glucose organs, in particular in the brain for which glucose is the predominant energy source. Therefore, if it is not treated in time, can lead to hypoglycemic coma. The second one is dangerous in the long term and the main complications are: limb loss, blindness, ischemic heart disease and renal disease. In presence of chronic hyperglycemia we

talk about diabetes. Diabetes is estimated to currently affect 415 million of people in the world, number that is supposed to increase, and according to the World Health Organization it caused an estimated 1.6 million deaths in 2016. There are two type of diabetes: type 1 and type 2. The type 2 is the most common form of diabetes and it affects 90% of cases and it is characterized by high blood glucose and insulin resistance. The type 1 of diabetes is characterized by no insulin production and therefore affected individuals need exogenous insulin injections to compensate the lack of secretion from the pancreas.

It is well established that physical activity promotes a healthy lifestyle, with significant benefits for both healthy and diabetic subjects. Physical exercise, in fact, reduces blood pressure, controls the level of blood sugar, positively modulates cholesterol in the blood, helps to prevent metabolic, cardiovascular, neoplastic diseases. A lot of studies have been conducted in order to understand glucose metabolism during physical activity in both healthy and diabetics subjects. It is well known that exercise increases glucose utilization (R_d) and, therefore, EGP must increase to meet the increased metabolic demands of the muscle to minimize risks of hypoglycemia [6]. It has been observed that in healthy subjects, in the postprandial state (2 hours after a mixed meal), glucose concentration falls during moderate-intensity exercise due to an increase of its demands; this change is facilitated by falling insulin and rising glucagon and catecholamine levels. The result is an almost eightfold rise in rates of EGP and an increase by 75% of insulin sensitivity (the ability of insulin to promote glucose uptake and inhibit EGP) [34].

Physical exercise can contribute to improve the quality of life even for diabetic subjects, but the optimal operating conditions are still unclear. In particular, physical activity can lower blood glucose in both the short and long term with the risk of hypoglycaemia which represents the main barrier to exercise for these individuals [2]. Hypoglycemia can occur during, immediately after, or many hours after physical activity and this requires that the patient has both an adequate knowledge of its metabolic and hormonal responses to exercise as well as well-tuned self-management skills [1]. Another study was conducted to determine the effects of exercise (75min of moderate-intensity exercise) on postprandial glucose metabolism and insulin action in type 1 diabetic subjects with the triple tracer technique [25]. T1D subjects appeared to be insulin resistant in the postprandial state and particularly during exercise since both plasma insulin and glucose concentrations were higher than in healthy subjects. The raising of EGP during exercise occurs also in T1D diabetic

individuals: in healthy subjects this was facilitated by falling insulin and glucose levels and rising glucagon concentrations. The equally rapid increase in EGP in T1D during exercise despite higher glucose levels and lower glucagon implies that robust exercise induced hepatic responsivity despite adverse hormonal and substrate milieu, but it is necessary further investigation.

Factors that could contribute to the raising of EGP could be related to the increases in net hepatic glycogenolysis and/or increases in gluconeogenesis. In [28] it has been discussed the role of these two processes during physical activity: the major findings are that in healthy subjects the rate of glucose production increased in proportion to the intensity of exercise, which can be entirely attributed to increases in net hepatic glycogenolysis. In contrast, diabetic subjects exhibit increased rates of glucose production both at rest and during exercise, which can be entirely accounted for by increased gluconeogenesis.

1.2 Aim and outline of the thesis

Although numerous studies have been conducted in order to improve the understanding of glucose metabolism during exercise in both healthy and diabetic subjects [15, 26, 34], none of them has allowed to quantify the effects of exercise on endogenous glucose production. It is known that EGP increases during exercise but the way in which it increases and if there are some differences between healthy and diabetic subjects is not clear yet. This represents an important knowledge gap, especially in type 1 diabetes, because this information could be incorporated into open-and closed loop (artificial pancreas) control algorithms, and therefore helping the treatment of the disease.

The aim of this thesis is to develop a model which is able to describe the effects of moderate physical activity on endogenous glucose production in both healthy and type 1 diabetic subjects in different experimental conditions.

The thesis is articulated as follows:

Chapter 2 presents a brief overview on glucose homeostasis and diabetes disease. Moreover, exercise effects on glucose metabolism are described in order to better understand the results of the thesis.

Chapter 3 provides the reader a brief description about the protocol and the clamp study realized.

Chapter 4 presents some mathematical models developed to describe endogenous glucose production during physical activity.

Chapter 5 presents the results of the models proposed in the previous chapter.

Chapter 6 provides to the reader some conclusions of this work and proposals for future research.

Chapter 2

Glucose metabolism and physical activity

This chapter introduces the reader to the functioning of the glucose regulatory system and, in particular, it describes the role of the main organs involved such as the liver, kidneys and pancreas. In addition, the diabetes disease is briefly described, especially type 1 diabetes, and the effects of physical activity on glucose metabolism.

2.1 The glucose-insulin regulatory system

In healthy subjects, blood glucose concentration is controlled and kept close to its normal range ($70\text{-}110 \frac{\text{mg}}{\text{dL}}$, $3.9\text{-}6.7 \frac{\mu\text{mol}}{\text{mL}}$) thanks to a complex glucose-insulin system which includes organs, metabolic and nervous controls that act to maintain blood sugar into this range within 24 hours after a perturbation.

The main organs involved in the endogenous glucose production are the liver and kidneys. The liver provides glucose for all the tissues either by gluconeogenesis or by glycogenolysis. The first process involves the formation of glucose-6-phosphate from precursors such as lactate, glycerol, and amino acids with its subsequent hydrolysis to glucose, while glycogenolysis involves the breakdown of glycogen to glucose-6-phosphate and its subsequent hydrolysis to free glucose. Moreover the liver is the only organ that contains appreciable glycogen and glucose-6-phosphate, and therefore the only organ that can directly release glucose as result of glycogenolysis.

During a meal the food is absorbed in its different parts: carbohydrates, proteins, fats, vitamins and other nutrients. The carbohydrates consumed turn into blood sugar which is absorbed by the gastrointestinal wall; one part goes to the brain and the other part is stored in the liver as glycogen through a process called glycogenesis. Hence the liver both stores and produces glucose depending upon the body's need. In this way, the liver can release glucose during periods of food deprivation

by glycogenolysis [5].

Glucose does not require any digestive processing and its absorption is therefore rapid: blood sugar usually starts to rise 10 – 15 minutes after a meal and reaches its peak after an hour. However, these are just some approximate features because it depends on several factors, such as the type of food consumed.

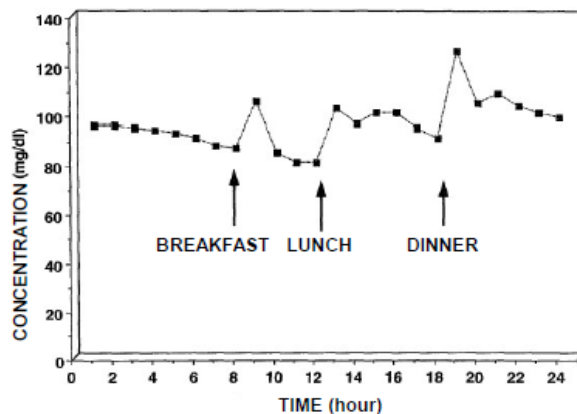


Figure 2.1: Daily glycemic pattern

Until recently it was thought that glucose production occurred almost entirely in the liver and that the human kidney played a minor role in glucose homeostasis [3, 37]. Most recent studies [27, 36] demonstrated that in postabsorptive state the kidney simultaneously takes up and releases appreciable amounts of glucose. The kidney's contributions to maintaining glucose homeostasis include production of glucose via gluconeogenesis, uptake of glucose from the circulation to satisfy their energy needs, and reabsorption of glucose at the level of the proximal tubule.

The pancreas is another important organ involved in the regulation of glucose homeostasis. In particular the islets of Langerhans, a specific region of the pancreas, contain endocrine cells among which alpha and beta cells. When blood glucose level is high, beta-cells secrete insulin which is released into the bloodstream by the portal vein and then reaches the liver where about 50% is degraded. The remaining part is released into the bloodstream through the hepatic vein and then can reach tissues where it carries out its functions. The most important are:

- to stimulate the storage of glucose as glycogen in the liver, muscle and fat cells;
- to promote glucose utilization by insulin-dependent tissues (muscle and adipose tissue);

- to inhibit the endogenous glucose production (EGP).

Insulin is primarily secreted in response to elevated glucose levels, such as those occurring after a meal. It enables the insulin-dependent uptake of glucose into muscle and adipose tissue and is the only known hormone lowering blood glucose [22].

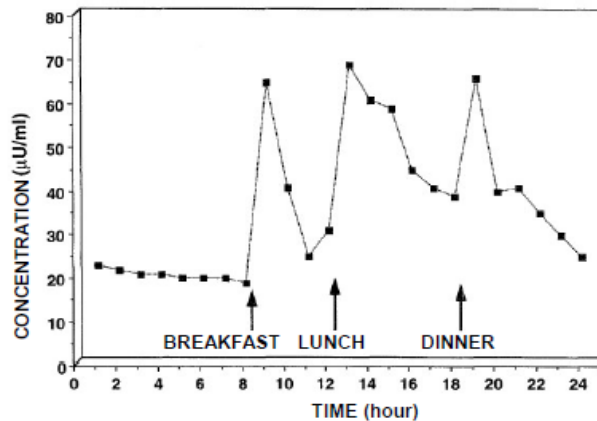


Figure 2.2: Daily insulin pattern

This hormone allows the blood glucose to be transported from the blood into the cells thanks some transmembrane proteins. Currently, there are five established functional facilitative glucose transporter isoforms (GLUT1-4 and GLUTX1), with GLUT5 being a fructose transporter. The GLUT4 isoform is the major insulin-responsive transporter that is predominantly restricted to striated muscle and adipose tissue. These transporter proteins are sequestered into specialized storage vesicles that remain within the cell's interior under basal conditions. As postprandial glucose level rises, the subsequent increase in circulating insulin activates intracellular signaling cascades that ultimately result in the translocation of the GLUT4 storage compartments to the plasma membrane. This process is reversible so that when circulating insulin levels decline, GLUT4 transporters are removed from the plasma membrane by endocytosis and are recycled back to their intracellular storage compartments [39].

On the other hand, alpha cells are responsible for glucagon secretion which plays the primary role in counter-regulation to hypoglycemia [22]. Glucagon effects are the opposite of the effects induced by insulin: it is released in response to low blood glucose levels and to events where the body needs additional glucose, such as in response to physical activity. It acts on the liver in several ways:

- stimulating the conversion of stored glycogen in the liver to glucose, which can be released into the bloodstream (glycogenesis).

- promoting the production of glucose from amino acid molecules (gluconeogenesis).
- reducing glucose consumption by the liver so that as much glucose as possible can be secreted into the bloodstream to maintain blood glucose levels.

Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels stable. Glucagon acts to prevent hypoglycemia (glucose under 50mg/dL) by promoting endogenous glucose production, while insulin acts to prevent hyperglycemia (glucose up to 200mg/dL) by promoting storage and utilization of glucose.

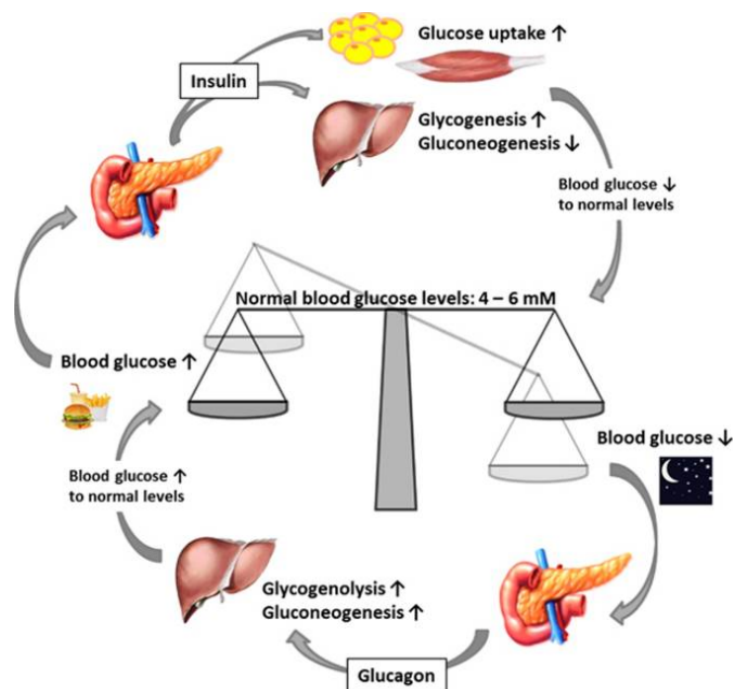


Figure 2.3: Maintenance of blood glucose levels by glucagon and insulin. When blood glucose levels are low, the pancreas secretes glucagon, which increases endogenous blood glucose levels through glycogenolysis. After a meal, when exogenous blood glucose levels are high, insulin is released to trigger glucose uptake into insulin-dependent muscle and adipose tissues as well as to promote glycogenesis [31].

Also catecholamines, a family of neurotransmitter and hormones, act to regulate blood glucose level. They include epinephrine, dopamine, and norepinephrine. Epinephrine, also called adrenaline, is produced by the adrenal glands and certain neurons and one of their function is to prevent hypoglycaemia. It sends signals to the liver and kidneys to produce more glucose, keeps tissues, such as muscle, from using as much glucose from the bloodstream, and works to reduce insulin secretion. The norepinephrine is similar to epinephrine since it acts by promoting glycogenolysis, gluconeogenesis, reducing insulin and increasing glucagon secretion.

2.2 Diabetes

As regard diabetic subjects, there are important differences in the regulation of glucose homeostasis. There are two type of diabetes: type 1 diabetes is a chronic autoimmune disease in which no insulin is produced, while with type 2 diabetes the body either resists the effects of insulin or does not produce enough insulin to maintain normal glucose levels. In particular, type 1 diabetes is caused by the production of antibodies that attack the β -cells inside the pancreas responsible for the insulin secretion. In this way wherever blood glucose is high there is no natural mechanism which can reduce its level and therefore a condition of hyperglycemia occurs. Hyperglycemia has no immediate damaging consequence on the organism, but if this state persists for long time it becomes critical and can lead to a variety of serious complications, such as nerve damage, eye problems, kidney damage, cardiovascular damage, feet and legs problems. In order to avoid these complications diabetes therapy attempts to keep blood glucose level within the euglycemic range. The therapy consists in dietary management, physical activity and the administration of exogenous insulin with subcutaneous injections before meals which is able to mimic the endogenous insulin secretion by β - cells.

An optimal control of diabetes requires frequent self-monitoring of blood glucose level and the consequent adaptation of the exogenous insulin doses. But for diabetic subjects avoiding high blood sugar is not enough. A danger is also represented by low blood sugar (under $70 \frac{mg}{dL}$) which can lead to a condition of hypoglycemia which is critical into the short-term. It mostly occurs in the interval between meals or overnight and is also usually associated with fasting, physical activity or stress. It represents a serious risk since if the blood glucose level continues to drop, the brain does not get enough glucose and stops functioning. Hypoglycemia is a condition generally perceived by the subject, especially when glucose falls below $50 \frac{mg}{dL}$. This condition causes the release of a series of hormones that, after the appearance of a general sense of weakness due to the suffering of the central nervous system, stimulate the body to react. Treatment of hypoglycaemia is by eating foods or taking sugar. If a person is not able to take food, an injection of glucagon may help. If not treated in time, hypoglycaemia can lead to hypoglycaemic coma , which usually appears when the concentration of glucose in the blood falls below $20 \frac{mg}{dL}$.

2.3 Physical activity

It is well established that performing a regular physical activity promotes a healthy lifestyle, with significant benefits. At any age, regular physical activity, even moderate, contributes to improving the quality of life as it positively affects both the state of health (helping to prevent and alleviate many of the chronic diseases) and the degree of personal satisfaction (helping to develop social relationships and helping psychological well-being). Physical exercise, in fact, reduces blood pressure, controls the level of blood sugar, positively modulates cholesterol in the blood, helps to prevent metabolic, cardiovascular, neoplastic diseases. It also reduces the symptoms of anxiety, stress, depression and reduces the risk of several types of cancer [23].

During physical activity, whole-body oxygen consumption may increase by as much as 20-fold, and even greater increases may occur in the working muscles. Carbohydrates and lipids are generally considered the most important substrates during exercise. Although amino acids are used during exercise as well, their quantitative role in the energy provision to exercising muscle is limited under most circumstances [24].

Substrate metabolism during exercise depends on its intensity and duration, together with the training status of the exercising individual. Muscle and liver glycogen become more important to energy provision with increasing exercise intensity, while the relative contribution of plasma free fatty acids (FFA) decrease. In addition, intramuscular triglycerides (IMTG) play a role in muscle metabolism during moderate to intense exercise, but probably not at very low intensities [11]. When exercise is prolonged (> 60 min), muscle glycogen stores eventually become depleted, and, consequently, the contribution of plasma substrates to total energy expenditure must increase if power output is to be sustained [32].

In general, glucose utilization increases during exercise and therefore endogenous glucose production must increase to meet the increased metabolic demands of the muscle to minimize risks of hypoglycemia [2]. Liver glycogenolysis accounts for approximately 75% of glucose output at the onset of exercise. Since liver glycogen stores after an overnight fast are approximately 75 - 100 g, after 40 min of strenuous exercise as much as 20 - 25% of pre-exercise liver glycogen will have been mobilized. As liver glycogen becomes increasingly depleted, the rate of glycogenolysis will fall, and gluconeogenesis will contribute up to 50% of total glucose output. Subsequently, a decline in plasma insulin levels is probably the most important factor stimulating glucose production by the liver and is likely due to epinephrine which suppresses insulin secretion. A lot of studies have been conducted to under-

stand the role of catecholamines during exercise. Epinephrine and norepinephrine in fact, are responsible for many adaptations both at rest and during exercise and they are the main hormones whose concentrations increase markedly during physical activity (many researchers reported 1.5 to > 20 times basal concentrations depending on exercise characteristics as duration and intensity) [41]. Moreover, several studies have shown that adrenaline and noradrenaline are involved in cardiovascular and respiratory adjustments and in substrate mobilization and utilization. Many studies [13, 18, 21] reported that for a given duration the circulating noradrenaline concentrations increases exponentially with the intensity of exercise. Most findings then reported a higher adrenaline response to exercise in endurance-trained compared with untrained subjects in response to intense exercise at the same relative intensity as all-out exercise. This phenomenon is referred to as the “sports adrenal medulla” and for some authors it can partly explain the higher physical performance observed in trained compared to untrained subjects. Many studies have also focused on gender effects on catecholamine response to exercise to verify if significant differences in catecholamine responses could be partly responsible for the different performances observed between trained men and women. However, the fact that there are differences in catecholamine response between men and women does not achieve unanimity since there are studies which do not report gender differences and studies which observe significantly higher catecholamine concentration in men than in women. Another important consideration is the intensity of exercise which is known to have a greater effect on catecholamine response. Moreover, parameters such as age, nutritional and emotional state have been found to influence catecholamine concentrations.

Glucagon also plays an important role during physical activity and its concentrations is correlated significantly with norepinephrine and epinephrine concentrations during prolonged and with epinephrine during graded exercise. Although increments in catecholamines were similar, the glucagon secretion was larger during prolonged than during graded exercise. While increments in catecholamines might explain increased glucagon secretion during graded exercise, they cannot account completely for the rise of glucagon during prolonged exercise [14].

Similarly to insulin, a single bout of exercise increases the rate of glucose uptake into the contracting skeletal muscles, a process that is regulated by the translocation of GLUT4 glucose transporters to the plasma membrane and transverse tubules [16]. Despite the fact that the result is an increased content of GLUT4 in the plasma membrane, insulin and exercise act through two different mechanisms. Some studies suggest there are different intracellular “pools” of GLUT4, one stimulated by

insulin and one stimulated by exercise which perhaps explains why humans with insulin resistance can increase muscle glucose transport in response to an acute bout of exercise [17]. Insulin signaling involves the rapid phosphorylation of the insulin receptor, insulin receptor substrate-1/2 (IRS-1/2) on tyrosine residues, and the activation of phosphatidylinositol 3-kinase (PI3-K) [10,20]. Muscle contraction is initiated by a necessary release of calcium to permit cross bridge formation. Intracellular calcium activates PKC serine kinases which have been hypothesized to stimulate GLUT4 recruitment by unknown mechanisms [Figure: 2.4].

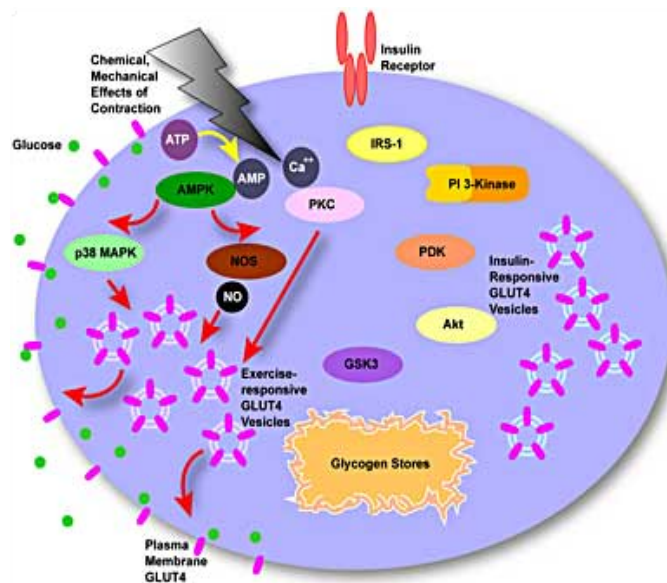


Figure 2.4: Mechanisms involved in the stimulation of glucose transport by exercise. Muscle contractile activity induces a recruitment of a separate pool of intracellular GLUT4 to the plasma membrane and a subsequent increase in glucose transport [40].

Physical exercise can contribute to improve the quality of life even for diabetic subjects, but the optimal conditions in which operate in terms of intensity, duration and insulin therapy are still unclear. In particular, physical activity can lower blood glucose in both the short and long term with the risk of hypoglycaemia which represents the main barrier to exercise for these individuals [2]. In fact, it is well known that physical activity in T1D patients influences glucose concentrations not only during exercise, but also several hours after, leading to late evening and nocturnal hypoglycemia [19]. The glycemic response depends largely on the type, intensity and duration of the activity, as well as the circulating insulin and glucose counter-regulatory hormone concentrations [29].

Chapter 3

Experimental Protocol

In this chapter it is briefly described the glucose clamp technique and how it has been realized for the study. It is also presented the experimental protocol used for the estimation of glucose fluxes during physical activity. Finally, concentration profiles of glucose, insulin, glucagon and glucose fluxes are shown.

3.1 Subjects

In this thesis we want to develop a model which can describe the effects of physical activity on the endogenous glucose production. The data comes from a study conducted at Mayo Clinic Rochester Minnesota on 12 subjects, 6 ND and 6 T1D, with age between 18 and 65 years, and body mass index (BMI) of 19-40 $\frac{kg}{m^2}$.

The inclusion criteria for both ND and T1D participants are:

- Age 18-65 years;
- BMI of 19-40 $\frac{kg}{m^2}$;
- Creatinine $\leq 1.5 \frac{mg}{dL}$;
- Normotensive; hypertension controlled on meds;
- No acute disease;
- No history of current substance abuse;
- Negative pregnancy test in premenopausal woman;
- No history of macrovascular disease;

For only T1D diabetic subjects:

- HbA1c \leq 11%;
- On insulin pump or multiple daily injection therapies;
- No history of diabetic microvascular complications except for stable background retinopathy.

	Age	Height (m)	Fat free mass (kg)	Weight (kg)	BMI (kg/m ²)	VO2max (mL/kg/min)
MEAN	28.17	1.75	54.96	72.62	23.61	36.58
SD	10.38	0.08	10.80	7.87	2.32	7.02

Figure 3.1: Anthropometric characteristics of healthy subjects: mean and standard deviation (SD).

	Age	Height (m)	Fat free mass (kg)	Weight (kg)	BMI (kg/m ²)	VO2max (mL/kg/min)	Diabetes' dur.	HbA1C% \leq 11%
MEAN	28.66	1.76	56.21	87.82	28.29	28.72	16	7.83
SD	7.12	0.06	7.94	15.56	4.79	10.38	10.60	1.60

Figure 3.2: Anthropometric characteristics of subjects with type 1 diabetes: mean and standard deviation (SD).

3.2 Clamp study

This study is based on the clamp technique which is, in general, a method for quantifying insulin secretion and resistance [8]. In this case, the clamp technique was realized to avoid confusing effects of glucose and insulin in the regulation of the dynamics occurring during the experiment.

There are two types of clamp:

- **hyperglycemic clamp:** the goal is to raise the plasma glucose concentration to a fixed hyperglycemic plateau. The desired plateau is maintained by adjustment of a variable glucose infusion, based on the negative feedback principle. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of insulin secretion and glucose metabolism [8].

- **euglycemic clamp:** the plasma insulin concentration is raised and kept constant to a new plateau by a prime-continuous infusion of insulin. The plasma glucose concentration is held constant at basal levels by a variable glucose infusion. At steady-state, the glucose infusion rate equals glucose uptake by tissues. Basically, the test measures the amount of glucose needed to compensate for the increased insulin levels.

As we will see in section (3.3), each subject performed three visits in different conditions of glucose and insulin. In particular, in the first and second visit is realized the euglycemic clamp while in the third visit is realized the hyperglycemic clamp.

In this study three types of infusion are used to realize the glucose clamp:

1. Insulin infusion using a syringe pump through a peripheral vein. The rates of insulin infusion are chosen in order to replace the basal insulin in T1D subjects or to maintain a high physiological insulin level.
2. Intravenous glucose infusion: is used to raise the glucose level in plasma to the desired plateau. The maintenance dose is frequently adjusted so that if the actual glucose concentration is higher than the goal, the infusion is decreased and vice versa. The glucose infusion rate is called GIR and represents the amount of glucose needed to compensate for the increased insulin levels. If a high glucose rate is required it means that the subject is insulin-sensitive; on the other hand, very low glucose rate indicates insulin resistance (this is not always true since it depends by the experimental conditions). Hence, under this steady-state conditions of glycemia, GIR equals glucose uptake by all tissues.
3. Tracer infusion: it is necessary to estimate glucose fluxes. A metabolic tracer is, by definition, a substance used to follow the biological transformation of an endogenous substrate (tracee). Tracer properties are:
 - **Principle of indistinguishability:** glucose and tracer must be separately measurable. Usually it means that glucose is isotopically labelled.
 - **Kinetic equivalence:** from a kinetic standpoint, the tracer must be indistinguishable from glucose.
 - **Quantitatively negligible:** the quantities of tracer administered have to be small since they have not to perturb the system [30].

The tracer used for the estimation of EGP was a stable isotope of glucose: [6,6 -²H₂]glucose. It was administered together with an exogenous glucose infusion (GIR). The rate of this infusion is called G_{inf} and is used to reduce TTR oscillation.

The rate of infusion of [6,6 -²H₂]glucose is obtained by sampling glucose before the start of the experiment in one visit only, since it can be assumed that glucose kinetics does not change between visits.

The use of the glucose tracer is fundamental for the estimation of glucose fluxes: the idea is to use “intelligent” infusions of this tracer aiming to estimate in a “model-independent” manner the EGP. The estimates generally depend on the chosen model in terms of model order and parameters values. This dependence is minimized by tracer infusions which try to mimic the expected pattern of EGP, which leads to minimize TTR time variations (tracer-to-tracee ratio). This technique is called tracer-to-tracee clamp [7].

3.3 Experimental design

Each subject performed 3 visits in different glycemic and insulin levels with a session of moderate physical activity. The three visits are chosen in order to reproduce the various conditions that can occur in real life, and in each one is used the clamp technique:

1. **Euglycemic and low insulin clamp:** this kind of visit was chosen to evaluate the effects of physical activity in standard conditions.
2. **Euglycemic and high insulin clamp:** this kind of visit was chosen in order to evaluate the effects of physical activity on a condition characterized by high insulin level. With this visit we can evaluate glucose response, such as glucose utilization and suppression of EGP due to high insulin levels.
3. **Hyperglycemic and low insulin clamp:** this kind of visit was chosen in order to evaluate the effects of exercise on a condition characterized by high glucose values. With this visit we can evaluate glucose response, such as glucose utilization and suppression of EGP due to high glucose levels. Moreover, this visit can be very important for T1D subjects since it reproduces what happens in real life.

The three visits are in random order, which ensure that any differences between ND and T1D subjects results are only due to differences in treatment. Furthermore, these visits reproduce what can happen in real life: for a hyperglycemia condition,

a T1D subject may also decide to perform physical activity (third visit), or he/she may decide to inject a bolus of insulin first (second visit).

The rates of insulin infusion to ensure a clamp study are chosen in order to respect the real insulin levels: for the first and third visit (low insulin) a rate of 0.25 mU/Kg/min is fixed. This value is chosen to replace the basal insulin in T1D subjects. For the second visit (high insulin) the infusion rate is fixed to 0.75 mU/Kg/min . This value was chosen in order to suppress endogenous insulin secretion still respecting the insulin physiological range.

The figure below will provide the reader a schematic representation of the conditions realized for each visit for both healthy and diabetic subjects.

	VISIT 1 Euglycemia Low insulin	VISIT 2 Euglycemia High insulin	VISIT 3 Hyperglycemia Low insulin
Glucose concentration (mg/dL)	100	100	180
Insulin infusion (mU/kg/min)	0.25	0.75	0.25

Figure 3.3: Glucose levels and rate of insulin infusion in the three visits.

3.3.1 Screen visits

All subjects involved in this experiment gave their informed consent to participate in the study. They reported to the Mayo CRTU in the morning after an overnight fast where a physical examination (height, weight, waist-hip measurement, vital signs) was performed. A negative pregnancy test was performed by all woman of child-bearing potential. Moreover, screening tests as CBC, electrolyte panel, liver function (AST, ALT), urinalysis were done, as well as the Paffenbarger Activity questionnaire to confirm habitual physical activity. A DXA scan was done to measure body composition, percent body fat and fat free mass where the last one was used to determine dose of infusions in the visits. T1D subjects also performed HbA1c tested. To determine the intensity of exercise during the visits, each individual performed a graded exercise test on bike where $VO_2\text{max}$ and heart rate response to maximum exercise were evaluated. $VO_2\text{max}$ represents the maximum volume of oxygen that an athlete can use and based on this value and on the watts produced to reach it, it is possible to define a measure of the aerobic fitness for each subject.

To monitor glucose levels in T1D subjects a fingerstick blood glucose was used before and after the exercise test. If during the study were observed some hypoglycemic symptoms, additional blood glucose readings were done. The use of caffeinated drinks was asked to be limited and other dietary preferences during in-patient study visits were discussed with the dietician.

As previously said, the study includes three visits for each subject with a session of moderate physical activity. The figure below provides the reader with an elementary description of the visit with the three types of infusion used to realize the clamp and then a more detailed description of each visit (V1, V2, V3) is provided.

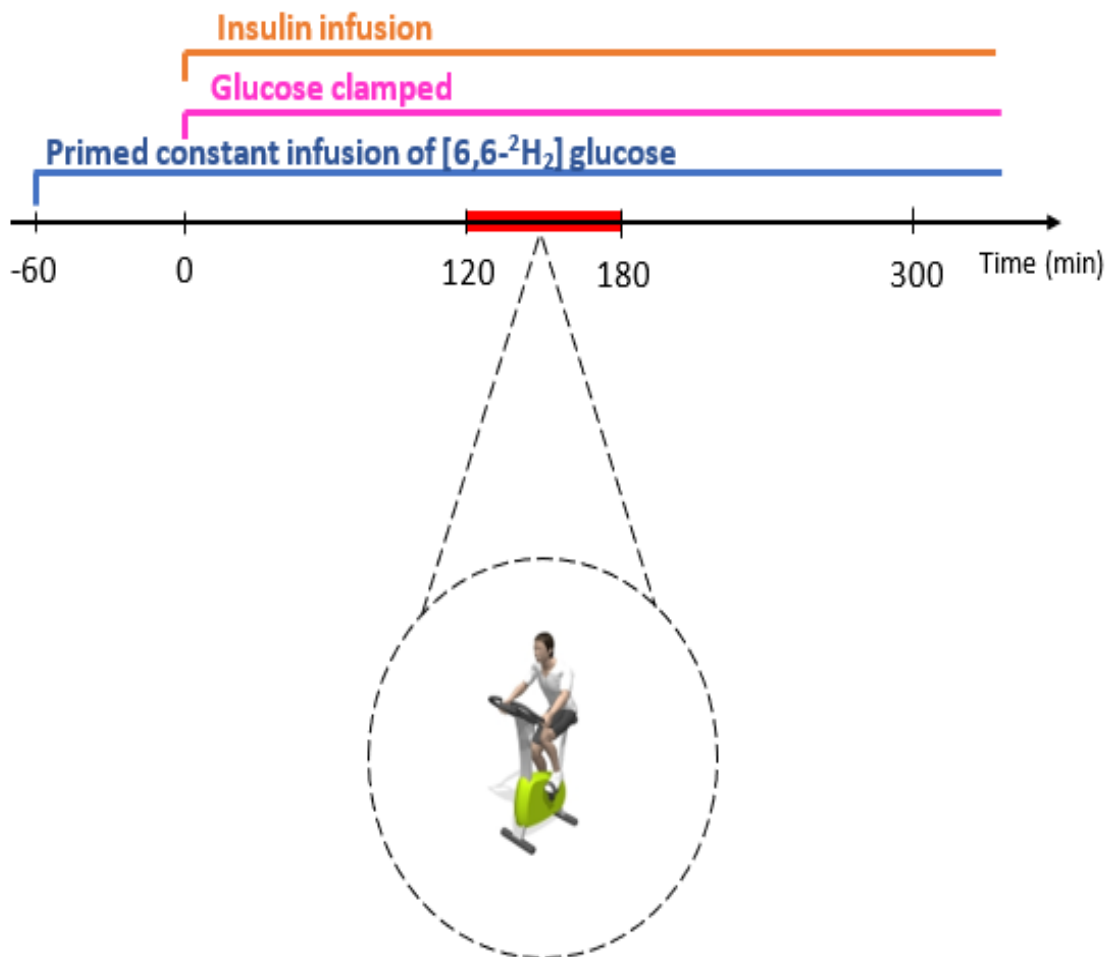


Figure 3.4: Study Visits structure.

V1: EUGLYCEMIC AND LOW INSULIN CLAMP

All subjects were admitted to the CRTU at 6 PM of the day prior the study and at ~1700 of the same day were provided with a 10 kcal/kg meal which included 20% of protein, 50% of carbohydrate and 30% of fat. T1D subjects administered their customary bolus dose of insulin according to their insulin/carbohydrate ratio with the evening meal. No additional food is eaten until the end of the study the next day.

For those who are on MDI (Multiple Daily Injection) program, they will not administer their basal insulin dose that evening. In T1D subjects two intravenous cannulae have been inserted: one was used to infuse insulin and the other to monitor blood glucose overnight. To maintain normoglycemia, insulin infusion started at ~2100 and continued throughout the night and the insulin pump was discontinued for those with the insulin pump.

In case of stuff problems or preference of the subject not to be admitted the evening before the study, then it was asked to report to the CRTU at 5 am for an outpatient study day instead. In this case any instruction to follow the evening before is given. As regard T1D subjects, the physician will discuss with the patient about evening dose of insulin and overnight management of glucose. In the morning of study, a venous catheter was inserted either in a retrograde fashion into a hand vein or antegrade fashion in the arm for periodic blood draws in both T1D and healthy subjects; then the hand is placed in a heated plexiglass box whose temperature is kept at 55C. The IV line used for overnight blood withdrawal in T1D subjects may be saline locked. In the morning, up to 2 intravenous cannulae may be placed in a forearm vein for tracer infusions using aseptic technique in healthy subjects and in subjects with type 1 diabetes.

As we can see in [Figure: 3.4], at -60 minutes (~0700 hour) an infusion of the tracer [6,6 -²H₂] glucose (33 μmol/kg prime and 0.33 μmol/kg/min continuous) was started and continued until the end of study. At time zero (approximately 0800 hour), an infusion of insulin and glucose was started until the end of the study: insulin was infused with a rate of $0.25 \frac{mU}{Kg*min}$ while glucose containing [6,6 -²H₂] glucose is infused in amounts sufficient to clamp glucose at 5.5 mmol/L (100 mg/dl). Moreover, the basal infusion of tracer is changed beginning at time zero in a pattern that mimics the anticipated changes in glucose production in order to minimize the changes in plasma glucose enrichment and tracer-to-tracee ratio TTR.

Once euglycemia is reached, the patient performed 60 minutes on a cycle ergometer at 65% of VO₂max.

During the visit blood samples of glucose, insulin, glucagon, lactate, and $[6,6\text{-}^2\text{H}_2]$ glucose were sampled at timed intervals. Only in this visit $[6,6\text{-}^2\text{H}_2]$ glucose was sampled from $T-60$ to $T0$ for the estimation of glucose kinetics parameters since it can be assumed that glucose kinetics does not change between visits.

The visit ended at ~ 1300 and all infusions and blood draws have been stopped.

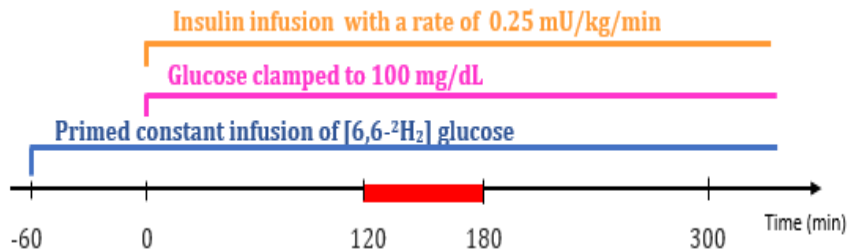


Figure 3.5: V1: euglycemic clamp and low insulin level.

V2: EUGLYCEMIC AND HIGH INSULIN CLAMP

In this visit, as in V1, subjects were admitted to the CRTU at 6 PM of the day prior the study. In case of stuff problems or preference of the subject not to be admitted the evening before the study, then it was asked to report to the CRTU at 5 am for an outpatient study day instead and any instruction to follow the evening before is given. All the guidelines and tests of visit 1 were repeated.

At -60 minutes (0700 hour) an infusion of the tracer $[6,6\text{-}^2\text{H}_2]$ glucose ($33\ \mu\text{mol}/\text{kg}$ prime and $0.33\ \mu\text{mol}/\text{kg}/\text{min}$ continuous) was started and continued until the end of study. At time zero (approximately 0800 hour), an infusion of insulin and glucose was started until the end of the study: insulin was infused with a rate of $0.75\ \frac{\text{mU}}{\text{Kg}\cdot\text{min}}$ while glucose containing $[6,6\text{-}^2\text{H}_2]$ glucose is infused in amounts sufficient to clamp glucose at $5.5\ \text{mmol}/\text{L}$ ($100\ \text{mg}/\text{dl}$). Moreover, the basal infusion of tracer is changed beginning at time zero in a pattern that mimics the anticipated changes in glucose production in order to minimize the changes in plasma glucose enrichment and tracer-to-tracee ratio TTR. From $T120$ to $T180$ the patient performed 60 minutes on a cycle ergometer at 65% of VO_2max .

During the visit blood samples of glucose, insulin, glucagon, lactate, and $[6,6\text{-}^2\text{H}_2]$ glucose have been sampled at timed intervals.

The visit ended at 1300 and all infusions and blood draws have been stopped.

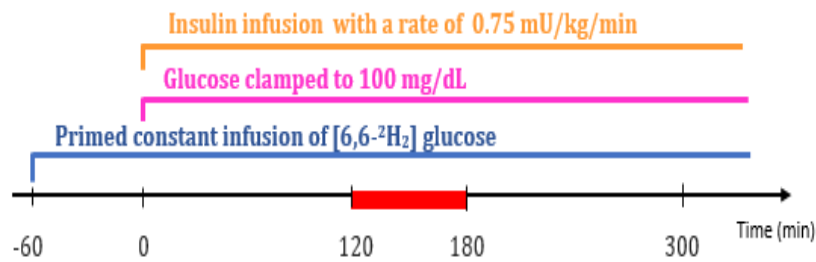


Figure 3.6: V2: euglycemic clamp and high insulin level.

V3: HYPERGLYCEMIC AND LOW INSULIN CLAMP

For this visit the only difference with V1 was glucose clamped at 180 mg/dl (10 mM) to maintain hyperglycemia. The visit ended at 1300 and all infusions and blood draws have been stopped. A meal is provided and T1D take their customary dose of insulin based on insulin/carbohydrate ratio, then subjects are dismissed from CRTU.

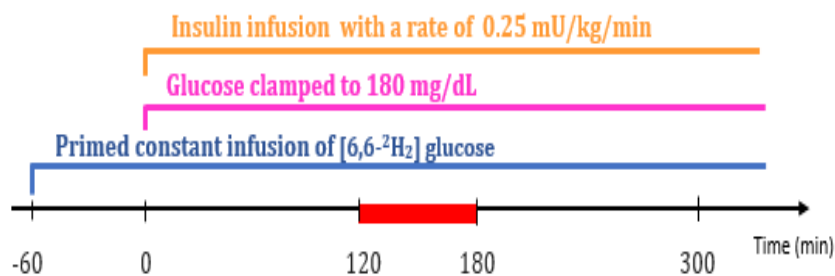


Figure 3.7: V3: hyperglycemic clamp and low insulin level.

For each visit all blood samples are immediately placed on ice, centrifuged at 40C, separated and stored at -800C until analyses.

3.4 Estimates of plasma glucose, insulin, glucagon and glucose fluxes

The following section reports the concentrations of plasma glucose, insulin, glucagon and glucose fluxes estimated during the clamp study with a single tracer approach. For each visit, the average concentrations are reported. Each visit will be referred as follows:

- V1 : euglycemia and low insulin
- V2: euglycemia and high insulin
- V3 : hyperglycemia and low insulin

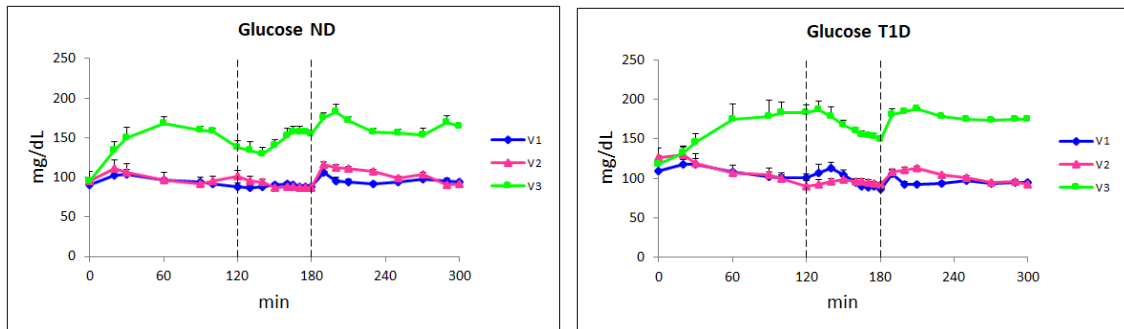
Plasma glucose

In [Figure: 3.8] the comparison of glucose concentrations between ND and T1D is reported, and, as we can see, glucose is generally well clamped. The easiest visit is the V1 where glucose is clamped at 100 mg/dL to maintain euglycemia and insulin is proximal to its basal value ($0.25\ \mu\text{U/mL}$). As it is shown in [Figure: 3.8(a)], for ND subjects glucose clamp is quickly reached in V1 and it is maintained until the end of the study with minimal oscillations. Even in V2 glucose clamp is quickly reached but some more oscillations are presented after exercise. Although in V3 it was more difficult to maintain the glucose in the desired range, the oscillations are minimized and the results are still satisfactory. In this visit, plasma glucose concentration is higher than V1 and V2, by design.

As regard T1D subjects [Figure: 3.8(b)], glucose at time T0 is higher with respect healthy individuals and they show more oscillations. Even for these subjects, the third visit was the most complicated. In general, T1D individuals have slightly higher glucose concentration values, especially in V3.

Plasma insulin

For what concerns plasma insulin concentration, the first and third visit have the same insulin infusion rate (0.25 mU/kg/min), but for ND individuals insulin level in V3 is higher than in V1. This is due to the fact that no somatostatin is infused, thus, for ND, additional endogenous insulin is secreted in V3 in response to hyperglycemia. This does not occur in T1D subjects since they do not produce insulin



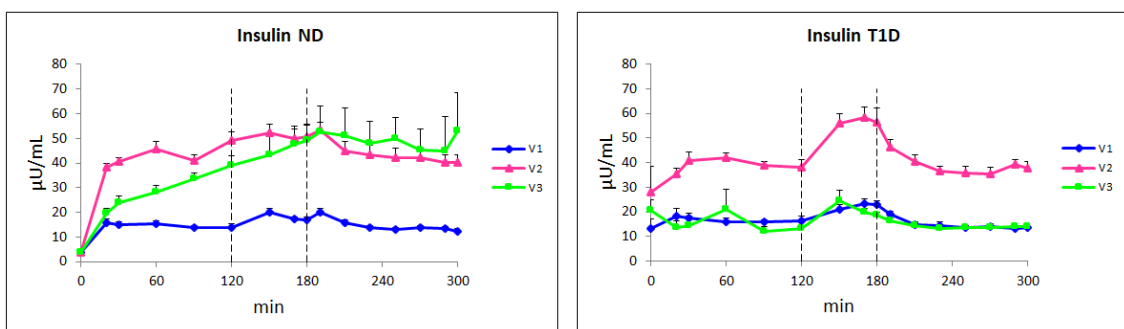
(a) Mean of glucose in ND subjects

(b) Mean of glucose in T1D subjects

Figure 3.8: Average glucose concentration in ND (left) and T1D (right) subjects in the three visits. Vertical bars represent the standard error.

by themselves. In V2 insulin infusion rate is 0.75 mU/kg/min and its concentration does not exceed $70 \mu\text{U/mL}$. This rate of infusion was chosen specifically to be high, but physiologically reasonable, and it ought suppress endogenous insulin secretion. Moreover insulin at T_0 is almost equal in the three visits (around $4 \mu\text{U/mL}$) for ND individuals while in T1D there is more variability. Another difference between the two groups is that insulin rises during physical activity and this can be seen especially in T1D individuals and in particular in V2. This increase may seem rather unexpected since insulin is infused at constant rate and T1D subjects do not produce endogenous insulin, but this may be due to a reduction of all body insulin clearance or a reduction of the insulin distribution volume.

In general, insulin slightly increase during exercise, despite the constant infusion.



(a) Mean insulin in ND subjects

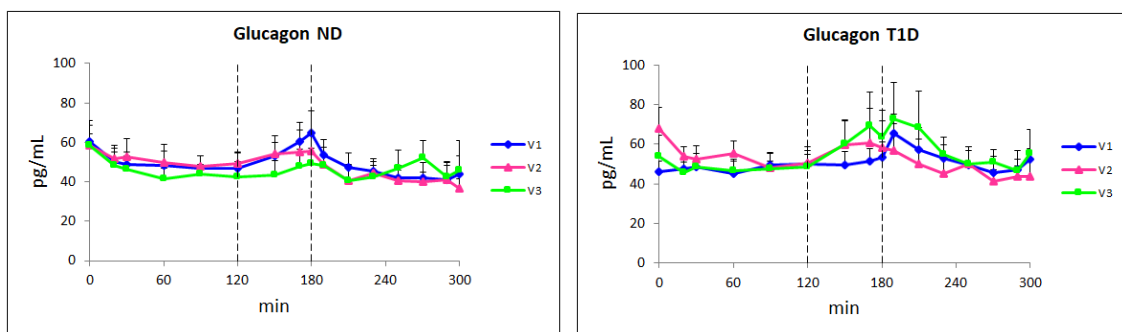
(b) Mean insulin in T1D subjects

Figure 3.9: Average insulin concentration in ND (left) and T1D (right) subjects in the three visits. Vertical bars represent the standard error.

Glucagon

In this study glucagon is not controlled, but we can see that its level is quite flat before the beginning of exercise in all visits in both ND and T1D subjects. The average concentration is in the range 40-80 pg/mL in both groups with a slight increment during exercise especially in V1 for ND and V3 for T1D.

In general, glucagon rises during exercise for both ND and T1D individuals, even if not always markedly, while it decreases in the recovery phase in all visits. Moreover, glucagon concentrations are not significantly different between ND and T1D.



(a) Mean glucagon in ND subjects

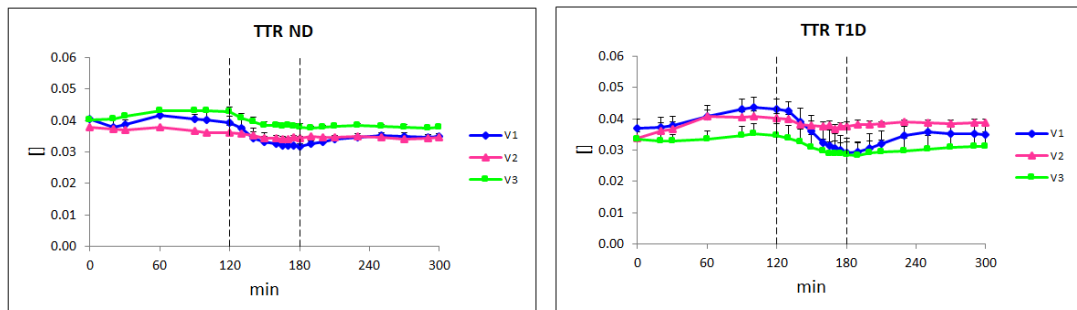
(b) Mean glucagon in T1D subjects

Figure 3.10: Average glucagon concentration in ND (left) and T1D (right) subjects in the three visits. Vertical bars represent the standard error.

Glucose fluxes

For the estimation of glucose fluxes a single tracer approach was used. The tracer must have a unique property that allows its detection, but at the same time be chemically identical to the tracee. Its very important that its chemical concentration is insignificant in comparison to that of the tracee in order to not altered the system.

Let's define the tracer-to-tracee ratio (TTR) as the ratio between the concentration of the glucose tracer ($[6,6\text{-}^2\text{H}_2]$ glucose) with respect to the total glucose concentration. The idea is the adoption of an “intelligent” infusion of tracer in order to minimize changes in the TTR and this means modifying tracer infusion rate to reproduce the anticipated EGP pattern. As we can see in [Figure: 3.11], realizing a perfect clamp for TTR is almost impossible, but minimizing its fluctuations is fundamental because the larger the oscillation in the signal are, the more uncertain is the estimation of glucose fluxes. When this condition is reached, at least approximately, $dTTR/dt$ is close to zero and a flat TTR is maintained throughout the system. Therefore, EGP, close to the ratio between the rate of infusion and the TTR, is minimally influenced by the choice of the model. [7]



(a) Mean of TTR in ND subjects

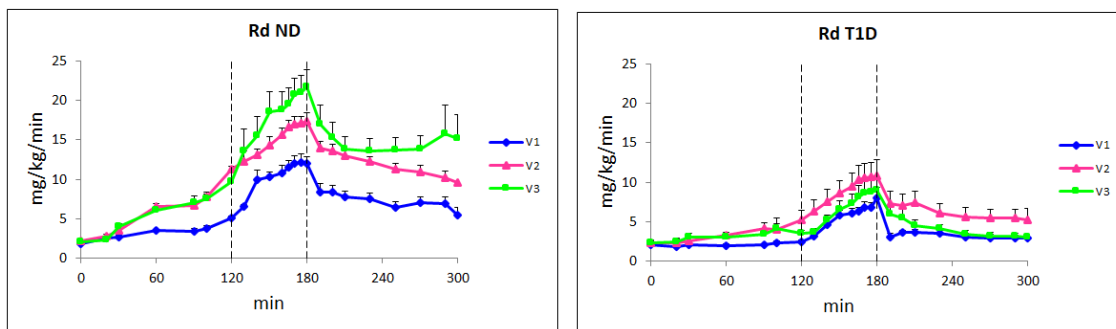
(b) Mean of TTR in T1D subjects

Figure 3.11: Average tracer-to-tracee ratio in ND (left) and T1D (right) subjects in the three visits. The vertical bars represent the standard error.

Rd

As regard the rate of glucose disappearance R_d , the first consideration we can make is that it is always higher in ND with respect to T1D, especially during physical activity, proving extra-hepatic insulin resistance of T1D individuals. At time T_0 both groups present values around 2 mg/kg/min in all visits and then R_d starts to increase in V2 and V3 while remains quite flat in V1. In ND subjects R_d does not increase immediately in V2 and V3 but it starts to rise after 30 minutes due to the time needed by insulin to exert its action. As expected, R_d increases between T_{120} and T_{180} in both groups, and it is higher in ND subjects with values around 11 mg/kg/min in V1, 16 mg/kg/min in V2, and 22 mg/kg/min in V3, against 6 mg/kg/min in V1, 10 mg/kg/min in V2, 9 mg/kg/min in V3 in T1D subjects. During the recovery, the average of R_d decreases in both groups and in [Figure: 3.12]) we can see that it reaches values higher than the two hours before the start of the exercise.

In general, R_d is significantly lower in V1 than V2 and V3 for ND subjects, due to higher insulin levels in the these two visits. For T1D instead, R_d in V1 is markedly lower than V2, but only slightly lower than V3. In summary, R_d is higher whenever insulin is elevated: in V2 and V3 for ND, and only in V2 for T1D.



(a) Mean of R_d in ND subjects

(b) Mean of R_d in T1D subjects

Figure 3.12: Average glucose disappearance in ND (left) and T1D (right) subjects in the three visits. The vertical bars represent the standard error.

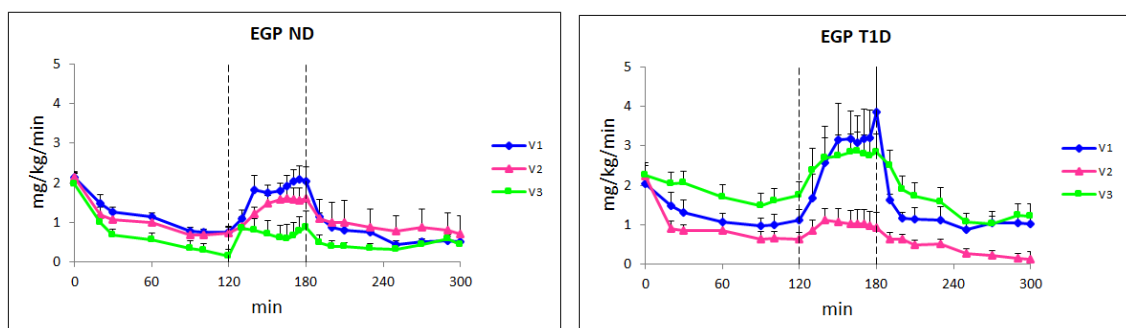
EGP

Finally Figure: 3.13 shows the average of estimated EGP. Throughout the study, EGP is higher in T1D with respect to ND in V1 and V3, while it is lower in V2. At time T_0 EGP is around 2 mg/kg/min and then decreases slightly until T_{120} in both groups due to insulin and/or glucose increment as expected by design. This drop is shown in particular in V3 for ND subjects.

During physical activity EGP increases in both groups: in ND individuals the flux increases from 0.8 mg/kg/min at T_{120} to 2 mg/kg/min at T_{180} in V1, from 0.7 mg/kg/min at T_{120} to 1.6 mg/kg/min at T_{180} in V2, from 0.1 mg/kg/min at T_{120} to 0.9 mg/kg/min at T_{180} in V3. In T1D individuals the flux rises from 1.1 mg/kg/min at T_{120} to 4 mg/kg/min at T_{180} , from 0.6 mg/kg/min at T_{120} to 0.9 mg/kg/min at T_{180} in V2, from 1.8 mg/kg/min at T_{120} to 2.8 mg/kg/min at T_{180} in V3. In the last two hours of the study, EGP decreases in both groups: in ND subjects the flux seems to return to the values of the pre-exercise while in T1D subjects we can observe that the flux reaches values lower with respect to the pre-exercise in V2 and V3 and in particular this occurs after T_{230} in both visits.

In summary, as expected, EGP rises during exercise and decreases during the recovery in both groups. In [Figure: 3.13(a)] we can see that the higher response of EGP is in V1, which is reasonable as in V2 there is high insulin level and in V3 there are high levels of both insulin and glucose. As regard T1D subjects, the rise of EGP is more pronounced in V1 and V3. In the interval from T_{120} to T_{180} the higher response is always in V1, but in contrast with ND, the increment is marked also in V3 due to low insulin level in the circulation.

At the end of the exercise EGP is immediately suppressed, especially in V1 in both groups.



(a) Mean of EGP in ND subjects

(b) Mean of EGP in T1D subjects

Figure 3.13: Average glucose production in ND (left) and T1D (right) subjects in the three visits. The vertical bars represent the standard error.

Chapter 4

Models

In this chapter, the models developed to describe the endogenous glucose production during physical activity are presented. We started by considering the *model 6* discussed in *Dalla Man et al.* [7] which provides a good prediction of EGP profiles of 20 subjects obtained with a triple-tracer meal protocol. This model provides an accurate assessment of the pattern of endogenous glucose production that occurs after food ingestion. Since this model was not developed to consider the effects of physical activity, some changes were necessary to adapt it to our experimental conditions. The strategy adopted was to identify the models proposed during the pre-exercise phase in order to evaluate the results in steady-state conditions and then identify the models introducing the effects of physical activity.

Several models have been developed but in this section only the most relevant are presented.

4.1 EGP during a meal

In this study we started by considering the *model 6* of EGP proposed by [7] tested on model-independent EGP data of 20 subjects obtained with a triple-tracer meal protocol. The EGP estimates were obtained in a virtually model-independent manner by using the TTR clamp technique. This model is able to distinguish glucose and insulin contributions on the suppression of EGP, and therefore glucose effectiveness and hepatic insulin sensitivity can be estimated. In particular, are considered three control signals on the EGP suppression: the first term is X^L which represents the delayed insulin action. From the physiological point of view, the delayed insulin action can be interpreted as a signal surrogating the suppression of FFA level, which

leads to the suppression of EGP. The second term is X^{Der} which accounts for glucose derivative control on the fast suppression of EGP. The third control signal is proportional to above-basal glucose concentration through a parameter k_G [7].

Model equations are:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X^L(t) - X^{Der}(t) \quad EGP(0) = EGP_b \quad (4.1)$$

with $X^{Der}(t)$ defined as:

$$X^{Der}(t) = \begin{cases} k_{GR} \cdot \frac{dG(t)}{dt} & \text{if } \frac{dG(t)}{dt} \geq 0 \\ 0 & \text{if } \frac{dG(t)}{dt} < 0 \end{cases} \quad (4.2)$$

where:

- EGP_b ($mg/kg/min$) is basal EGP;
- $G(t)$ (mg/dL) is plasma glucose concentration;
- G_b (mg/dL) is basal glucose;
- k_G ($dL/kg/min$) is the constant rate governing the magnitude of insulin secretion in response to the glucose deviation from the basal;
- k_{GR} (dL/kg) is the constant rate accounting for glucose derivative control on the fast suppression of EGP.

Delayed insulin kinetics is described by a two-compartment model, with the following dynamic equations:

$$\begin{cases} \dot{X}_L(t) = -k_1 \cdot X^L(t) + k_1 \cdot X_1(t) & X^L(0) = 0 \\ \dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] & X_1(0) = 0 \end{cases} \quad (4.3)$$

where:

- $X_1(t)$ ($mg/kg/min$) is delayed insulin action with respect to plasma insulin concentration (deviation from basal);

- $I(t)$ ($\mu U/mL$) is plasma insulin concentration;
- I_b ($\mu U/mL$) is basal insulin;
- k_1 (min^{-1}) is a rate constant describing the dynamics of insulin action on glucose production. It quantifies the delay between plasma insulin and delayed insulin action;
- k_2 ($mg * mL / (\mu U * kg * min)$) is insulin sensitivity, a parameter governing the magnitude of insulin action.

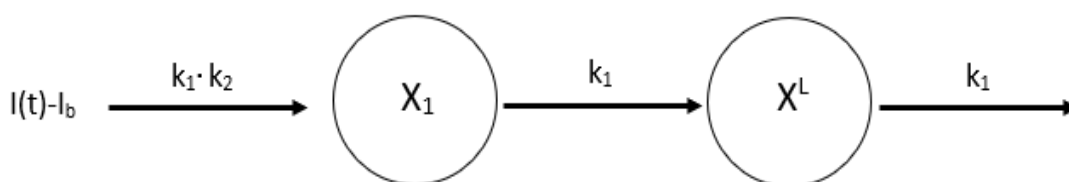


Figure 4.1: Insulin action model.

In summary, the model proposed assumes that EGP suppression is linearly dependent on plasma glucose concentration, delayed insulin action, and glucose derivative.

Glucose and insulin concentrations are the model forcing functions assumed to be known without error.

4.2 Model identification in the pre-exercise phase

In this section, models used for the identification in the pre-exercise phase are presented. First, we analysed each visit alone and, then, we moved to multi-identification of all three visits for each subject. In this step is very important to obtain a correct estimation of the parameters in order to incorporate this information in the model of glucose production during exercise that will be described in the next section (4.3).

4.2.1 Single-visit identification

The first step was to consider the model above under steady-state conditions, and therefore during the first 120 minutes. We started with a single-visit identification and not considering $X^{Der}(t)$ action on EGP in order to highlight, if any, the main differences.

So the model is:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X^L(t) \quad EGP(0) = EGP_b \quad (4.4)$$

with insulin action described as in (4.3):

$$\begin{cases} \dot{X}^L(t) = -k_1 \cdot X^L(t) + k_1 \cdot X_1(t) & X^L(0) = 0 \\ \dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] & X_1(0) = 0 \end{cases} \quad (4.5)$$

The model is *a priori* uniquely identifiable, and was identified using the non-linear least squares estimation, with SD constant but unknown. Model performances were compared on the basis of several criteria: precision of the estimated parameters (expressed as coefficient of variation CV), models ability to describe the data (expressed as the residual sum of squares RSS) , model parsimony (Corrected Akaike information criterion AICc) and physiological plausibility.

The objective function contains the weighted residuals until T=120 minutes obtained from the fit of glucose production:

$$R(p) = \sum_{i=1}^N \frac{(y_i - s(\hat{p}, t_i))^2}{V(s(\hat{p}, t_i), y_i, \hat{v})} \quad (4.6)$$

where:

p is the vector of parameters

$R(p)$ is the objective function

N number of data points

y_i is the i -th datum in the data set

$s(p, t_i)$ is the model value corresponding to y_i at time i

v is the variance parameter in the data set

$V(s(\hat{p}, t_i), y_i, \hat{v})$ is the variance model for y_i

Three parameters k_1 (min^{-1}), k_2 ($mg * mL / (\mu U * kg * min)$) and k_G ($dL / kg / min$) have been estimated for each visit. In particular, k_G was well estimated only in V3 (CV $\sim 30\%$), while it was estimated with poor precision (high CV) in V1 and V2. It is important to note that k_G in this model can be interpreted as an overall measure of

the ability of glucose to inhibit glucose production both directly and indirectly (i.e., by stimulating insulin secretion). Although it is known that glucose concentration per se has an effect on glucose production [38], we have to take into account that V1 and V2 are characterized by a condition of euglycemia and, therefore, only in V3 (condition of hyperglycemia) we can probably quantify and appreciate this effect. Individual values of this parameter are reported in Table 4.1 with coefficient of variation indicated in parentheses.

Table 4.1: Estimates of k_G in the three visits. Number in parenthesis indicate coefficient of variation (CV%)

Subjects	V1	V2	V3
1	$1 \cdot 10^{-5}$ (> 500)	$1 \cdot 10^{-5}$ (> 500)	0.020 (22)
2	$1 \cdot 10^{-5}$ (> 500)	0.006 (99)	0.015 (4)
3	0.027 (13)	0.001 (> 500)	0.017 (39)
4	0.007 (80)	0.027 (32)	0.025 (22)
5	$1 \cdot 10^{-5}$ (> 500)	0.013 (23)	0.041 (59)
6	$1 \cdot 10^{-5}$ (> 500)	$1 \cdot 10^{-5}$ (> 500)	0.007 (54)
mean	0.006 (> 500)	0.008 (> 500)	0.021 (33)
SD	0.011	0.011	0.011

As regard the estimates of k_2 , they are quite different in the three visits: as we can see in [Figure: 4.2], k_2 is higher in V1 and V3 with mean 0.14 and 0.20 ($mg * mL / (\mu U * kg * min)$), respectively, while it has lower values in V2 for all subjects with a mean of 0.03 ($mg * mL / (\mu U * kg * min)$).

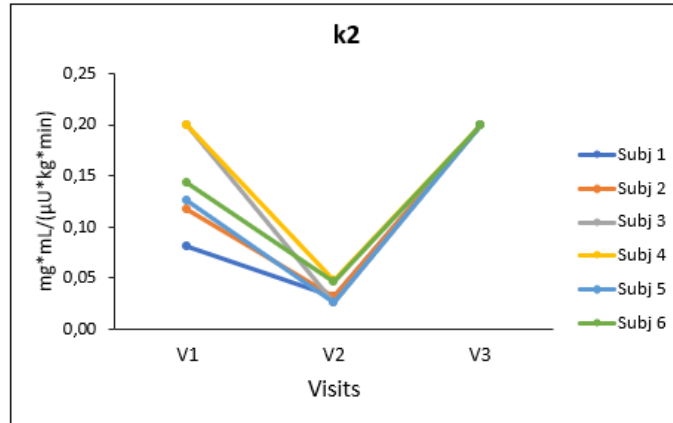


Figure 4.2: Estimates of k_2 in the three visits.

Due to these considerations, we then proceeded with an identification which considered for each subject the three visits together. In addition, we decided to exploit *a priori* information for k_G and to estimate k_2 per each one of the three visits considered.

4.2.2 Three-visits multi-identification

As discussed above, we then proceeded with a multi-identification of all three visits, using *a priori* information for k_G and estimating k_2 differently for each visit. The objective function (4.6) was modified in order to consider the weighted residuals of the whole three visits, and other terms accounting for the Bayesian information were included.

$$\begin{aligned}
 R(p) = & \sum_{j=1}^J \sum_{i=1}^{N_j} \left(\frac{(y_{i,j} - s(\hat{p}, t_{i,j}))^2}{V_{i,j}(s(\hat{p}, t_{i,j}), y_{i,j}, \hat{v}_j)} \right) + \sqrt{\log[V_{i,j}(s(\hat{p}, t_{i,j}), y_{i,j}, \hat{v}_j)]} \\
 & + \sum_{k=1}^{N_b} \left(\sqrt{\frac{(p_k - m_{p,k})^2}{\sigma_{p,k}^2}} + \sqrt{\log(\sigma_{p,k}^2)} \right)
 \end{aligned} \tag{4.7}$$

The notation is:

$R(p)$ is the objective function

p is the vector of parameters

J is the number of data set and therefore the number of visits

N_j is the number of data points in the j -th data set

$y_{i,j}$ is the i -th datum in the j -th data set

$s(\hat{p}, t_{i,j})$ is the model value corresponding to $y_{i,j}$ at time $t_{i,j}$

v_j is the variance parameter in the j-th data set

$V_{i,j}(s(\hat{p}, t_{i,j}), y_{i,j}, \hat{v}_j)$ is the variance model for y_i

N_b is the number of Bayesian parameters

$\sigma_{p,k}$ is the standard deviation of parameter p_k in that population

Different models have been developed to describe endogenous glucose production until $T=120$. In this section we will present the following models:

- **Model 1:** Model of EGP with a double delay in insulin action.
- **Model 2:** Model of EGP with a single delay in insulin action.
- **Model 3:** Model of EGP which includes the action of X^{Der} .
- **Model 4:** Model of EGP which includes the action of glucagon.

Model 1: is the model presented in section (4.1) but without the action of X^{Der} . Insulin action on the suppression of EGP has a double delay and its dynamic equations are the same of (4.3), which are reported below:

$$\begin{cases} \dot{X}_L(t) = -k_1 \cdot X^L(t) + k_1 \cdot X_1(t) & X^L(0) = 0 \\ \dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] & X_1(0) = 0 \end{cases} \quad (4.8)$$

Hence, the parameters to be estimated are k_1 , k_2-V1 , k_2-V2 , k_2-V3 , and k_G .

Model 2: in this model we considered the insulin action with a single delay. The reason was to try to provide a better estimation of EGP in those subjects in which the fit model was overestimating the data. The equation of the model becomes:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X_1(t) \quad EGP(0) = EGP_b \quad (4.9)$$

The model of insulin action is shown in [Figure: 4.3], with dynamic equation:

$$\dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] \quad X_1(0) = 0 \quad (4.10)$$

Hence, the parameters to be estimated are k_1 , k_2-V1 , k_2-V2 , k_2-V3 , and k_G .

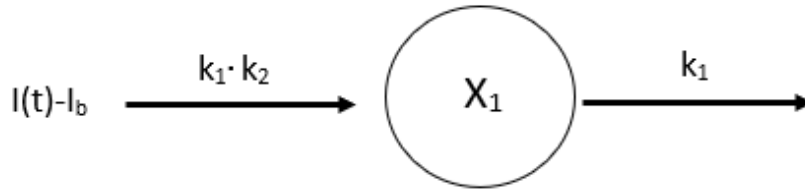


Figure 4.3: Insulin action model.

Model 3: it includes the action of insulin X^{Der} proportional to the glucose rate of change as presented in section (4.1), but with a single delay in insulin action.

Model equation is:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X_1(t) - X^{Der}(t) \quad EGP(0) = EGP_b \quad (4.11)$$

with:

$$\dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] \quad X_1(0) = 0 \quad (4.12)$$

The parameters to be estimated are k_1 , k_2-V1 , k_2-V2 , k_2-V3 , k_G and k_{GR} .

Model 4: it includes the action of glucagon in glucose production. Like in **Model 2**, we considered only one delay in insulin action.

Model equation is:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X^L(t) + k_{Gluc} \cdot (Gluc(t) - Gluc_b) \quad EGP(0) = EGP_b \quad (4.13)$$

with insulin equation as in (4.12).

The notation is:

- k_{Gluc} ($mg * mL / (pg * kg * min)$) is the constant rate governing the magnitude of glucagon in glucose production.
- $Gluc$ (pg/mL) is glucagon concentration.
- $Gluc_b$ (pg/mL) is basal glucagon.

Hence the parameters to be estimated are k_1 , k_2-V1 , k_2-V2 , k_2-V3 , k_G and k_{Gluc} .

RESULTS

Below are reported the results for ND subjects.

As summarized in Table 4.2 all the four models provided similar RSS, AICc and SD. As we can see, **Model 2** has slightly lower RSS and AICc with respect to **Model 1**, and both models provide precise parameters estimates ($< 50\%$). Due to these considerations we decided to exclude **Model 1** and always consider simpler models with a single delay in insulin action. **Model 3** and **4** provide slightly lower RSS with respect to **Model 2** essentially due to the higher number of parameters and their AICc is only modestly higher. However, **Model 3** provides very poor precision for the parameter k_{GR} ($CV > 100\%$ in 4 subjects). Although the inclusion of this derivative control in Model 6 of [7] significantly improved the ability to fit EGP data, in this case it does not seem to bring any improvement and it is probably due to the fact that glucose is clamped. Consequently, we decided to exclude this model. As regard **Model 4**, it provides poor precision for the parameter k_{Gluc} ($CV > 100\%$ in 4 subjects) and it is probably due to the fact that glucagon is quite flat, especially before the beginning of exercise. However, this model is physiologically more appealing since it includes the action of glucagon and this is the reason why we did not exclude it. Hence we selected **Model 2** and **4** as the best models to predict EGP data during the pre-exercise.

Table 4.2: Comparison of EGP model until $T=120$

	CV, %	RSS	AICc	SD	No. of Parameters
Model 1	19	1.43	20.70	0.26	5
Model 2	22	1.31	18.61	0.25	5
Model 3	> 500	1.19	20.59	0.24	6
Model 4	> 500	1.24	21.51	0.24	6

4.3 Models of EGP incorporating exercise

In this section, models including the effect of physical activity in the EGP models described in section 4.2.2 are presented.

In particular, the following models exploit both the parameters estimated (k_1 , k_2 -V1, k_2 -V2, k_2 -V3, k_G) and the covariance matrix of the identification in pre-exercise as initial *a priori* information.

During exercise, blood flow rises rapidly due to an increase of its demand by the active skeletal muscle, and to an increase of the body temperature. The blood flow rate increases by as much as 20 times the resting state, reaching the steady level within 10-150 seconds of exercise [4, 12]. For this reason, we considered physical activity as a forcing function, modeled as a square wave with unitary amplitude. As we can see in [Figure: 4.4], the signal is equal to 0 till $T120$, then it increases to 1 till $T121$ with a step function, and, then, it is kept constant till $T180$. At time $T181$ the signal is equal to 0 and it is kept constant till the end of the study ($T300$).

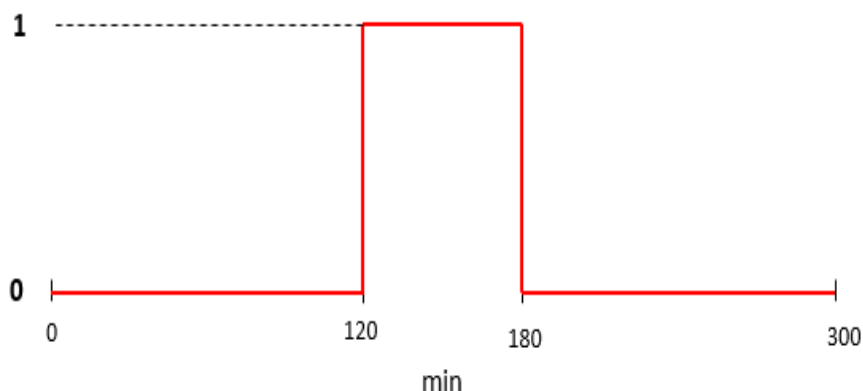


Figure 4.4: Physical activity signal modeled as a square wave.

To describe the effects of physical activity on glucose effectiveness (k_G) and insulin sensitivity (k_2), new parameters are included:

- $p5$ is the effect of exercise on k_G .
- $p3$ (min^{-1}) represents the delayed effect of exercise on k_G .
- $p6$ is the effect of exercise on k_2 .
- $p4$ (min^{-1}) represents the delayed effect of exercise on k_2 .

The effect of PA on k_G and k_2 have been considered both instantaneous and delayed.

Below 9 models that include the effects of physical activity on EGP are presented . Model from 1 to 7 refer to the model of equation (4.9) with a single delay in insulin action, which is reported below.

$$\begin{cases} EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X_1(t) & EGP(0) = EGP_b \\ \dot{X}_1(t) = -k_1 \cdot [X_1(t) - k_2 \cdot (I(t) - I_b)] & X_1(0) = 0 \end{cases} \quad (4.14)$$

Model 1: it considers the instantaneous effect of PA on k_G only. It is described as follows:

$$k_G = k_G \cdot (1 - p5 \cdot PA(t)) \quad (4.15)$$

Model 2: it considers the instantaneous effect of PA on k_2 only. It is described as follows:

$$k_2 = k_2 \cdot (1 - p6 \cdot PA(t)) \quad (4.16)$$

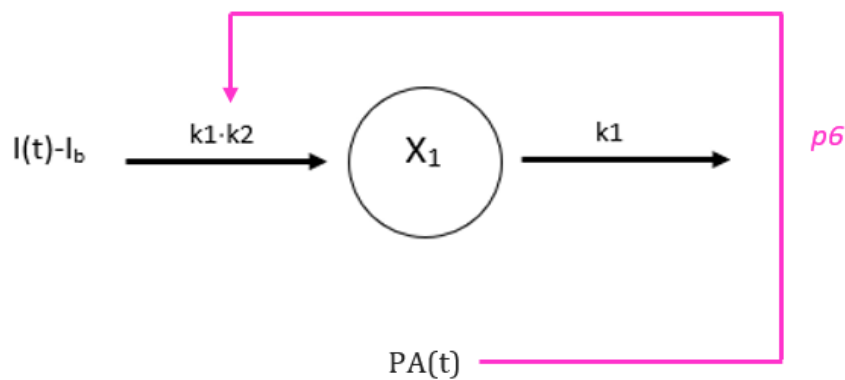


Figure 4.5: Model 2: instantaneous action of physical activity on k_2 .

Model 3: it considers the instantaneous effects of PA on both k_G and k_2 :

$$k_G = k_G \cdot (1 - p5 \cdot PA(t)) \quad (4.17)$$

$$k_2 = k_2 \cdot (1 - p6 \cdot PA(t)) \quad (4.18)$$

Model 4: it considers the delayed effect of PA on k_G only. The dynamic equation describing the delayed action of exercise is:

$$PA'(t) = -p3 \cdot PA' + p3 \cdot PA(t) \quad (4.19)$$

The action on k_G is described by:

$$k_G = k_G \cdot (1 - p5 \cdot PA'(t)) \quad (4.20)$$

Model 5: it considers the delayed effect of PA on k_2 only. The dynamic equation describing the delayed action of exercise is:

$$PA'(t) = -p4 \cdot PA' + p4 \cdot PA(t) \quad (4.21)$$

The action on k_2 is described by:

$$k_2 = k_2 \cdot (1 - p6 \cdot PA'(t)) \quad (4.22)$$

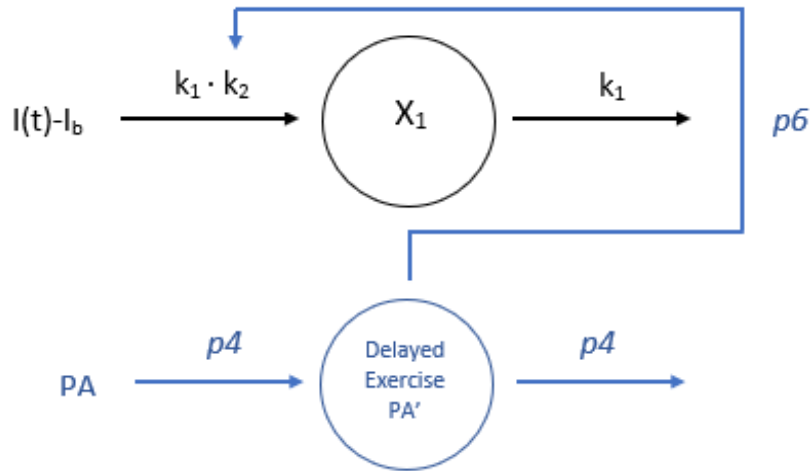


Figure 4.6: Model 5: delayed action of physical activity on k_2 .

Model 6: it considers the instantaneous effect of PA on k_G , and a delayed effect of PA on k_2 :

$$k_G = k_G \cdot (1 - p5 \cdot PA(t)) \quad (4.23)$$

$$PA'(t) = -p4 \cdot PA' + p4 \cdot PA(t) \quad (4.24)$$

$$k_2 = k_2 \cdot (1 - p6 \cdot PA'(t)) \quad (4.25)$$

Model 7: PA signal is modeled as a ramp between $T120$ and $T180$ as in [Figure: 4.7] (in the previous models PA is modeled with a square wave signal of unitary amplitud).

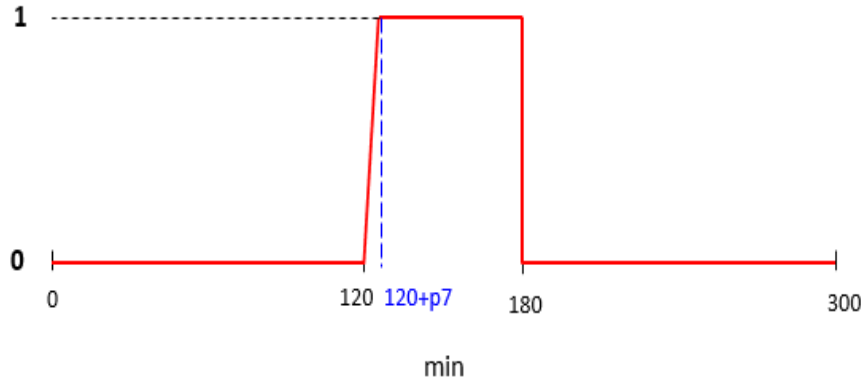


Figure 4.7: Physical activity signal modeled as a ramp.

For this model another parameter $p7$ (*min*) is estimated: it describes the time required to rise from 0 to 1 after $T120$.

Model 8: it considers the action of glucagon on EGP as in (4.13).

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X_1(t) + k_{Gluc} \cdot [Gluc(t) - Gluc_b] \quad EGP(0) = EGP_b \quad (4.26)$$

Also for this model we considered the delayed effect of PA on k_2 and no effects of PA on k_G .

$$PA'(t) = -p4 \cdot PA' + p4 \cdot PA(t) \quad (4.27)$$

$$k_2 = k_2 \cdot (1 - p6 \cdot PA'(t)) \quad (4.28)$$

Model 9: from the literature it is known that catecholamines are responsible for many actions both at rest and during exercise [41]. In this model we included the effect of epinephrine on EGP as follows:

$$EGP(t) = EGP_b - k_G \cdot [G(t) - G_b] - X^L(t) + k_{Epi} \cdot (Epi(t) - Epi_b) \quad EGP(0) = EGP_b \quad (4.29)$$

The notation is:

- k_{Epi} ($mg * mL / (pg * kg * min)$) is the constant rate governing the magnitude of epinephrine action on EGP.

- $Epi(t)$ (pg/mL) is epinephrine concentration.
- Epi_b (pg/mL) is basal epinephrine.

Chapter 5

Results

This chapter describes the results of the identified models of the previous section. The best model selected for ND, was then tested also on T1D individuals. Classical criteria typically used in model selection were adopted: the model's ability to describe the data (RSS), the precision of the parameter estimates (CV), the physiological plausibility of the estimated values and model parsimony (AICc).

5.1 ND

As regards **Model 1** (instantaneous effect of PA on k_G), it does not provide a good prediction of EGP during and after exercise, especially in V1 and V2, where the predicted profile of EGP stays quite flat in almost all subjects during exercise and does not rise as it should. The bad performances of this model in V1 and V2 are not surprising, since these two visits were performed in euglycemia with a quite flat glucose level, and therefore a greater effect of PA on k_G is not expected. The parameter p_5 is estimated differently for the three visits and with a poor precision ($CV > 100\%$) for all subjects.

Similarly, **Model 4** (delayed effect of PA on k_G) does not provide a good fit of the data. Like **Model 1**, it provides imprecise estimates for p_5 and also p_3 is estimated with poor precisions ($CV > 500\%$).

On the other hand, **Model 2** (instantaneous effect of PA on k_2) provides good precisions ($CV < 50\%$) for all parameters in all subjects and a reasonable good prediction of the EGP profile. This made us suppose that physical activity affects insulin sensitivity more than glucose effectiveness in these experimental conditions, but we have also to take into account that glucose is clamped and this makes the

PA effects on k_G more difficult to quantify.

In **Model 5** the delayed effect of PA on k_2 is considered. The introduction of this delay improves the performance of the model in terms of its ability to fit the data, while the precisions are slightly worse due to the increase in the number of parameters (p_4 is estimated differently for each visit). The improvement in the estimated EGP profile can be seen especially during the post-exercise and in particular in those subjects where p_4 assumes a low value ($< 0.02 \text{ min}^{-1}$).

In summary, **Models 1, 2** and **4** were excluded, together with **Models 3** and **6**. The latter two models consider the instantaneous and delayed effects of PA on both k_G and k_2 , respectively. They would be the most appropriate models, but they were excluded because **Model 3** collapses in **Model 2** for 5 subjects, and **Model 6** collapses in **Model 5** for 4 subjects since p_5 does not bring any additional information to the models. For these reasons we decided to consider only a delayed effect of PA on k_2 and no effects of PA on k_G .

Regarding **Model 7**, the only difference with **Model 5** is that PA is considered as a ramp modeled through a parameter p_7 which determines the time required to rise from 0 to 1 after $T120$. In contrast with the square wave model, in which the time needed to reach the target range of exercise was fixed to 1 minute, in model 7 we estimate this time which is represented by p_7 . However, the results are not satisfactory: p_7 is estimated with good precision (low CV) for only three subjects with values 34.94 min, 0.16 min, 0.12 min respectively. The latter two values are too low and therefore no changes are observed in the EGP fit. The only evident change in the data fit is observed for the subject whose $p_7 = 34.94$ min, which is an unrealistic large value. The fit of EGP for this subject is somewhat better in V1, while in the other visits it remains the same. In addition, also p_4 estimates are worse than **Model 5**, so we decided to exclude **Model 7**.

Model 8 adds the glucagon effects on EGP. The parameter k_{Gluc} , which governs the magnitude of glucagon action on glucose production, is precisely estimated in four subjects ($CV < 50\%$). In these subjects it assumes the values of 0.031, 0.009, 0.029, 0.009 $\text{mg} * \text{mL} / (\text{pg} * \text{kg} * \text{min})$ respectively. The addition of glucagon slightly improved model performances, in particular during the recovery in V1 and V2. The greatest effect was seen in those subjects where the prediction was higher than the EGP data during the post-exercise, since we obtained a lower fit with a consequent better adherence of the prediction to the data.

In section (3.4) we noted that glucagon slightly increases during exercise in ND subjects, and it fell below the basal value after exercise in V1 and V2 (glucose slightly increased). This probably contributes to the observed reduction of glucose produc-

tion during the recovery. In V3 no particular changes have been observed.

Like **Model 5**, it considers a delayed effect of PA on k_2 that, as previously said, it markedly improves the data fit whenever it assumes values lower than 0.02 min^{-1} . The parameter $p4$ is not estimated with good precision for all subjects, and it is worth noting that in all these cases it assumes large values ($> 0.2 \text{ min}^{-1}$). This could suggest that in those individuals the instantaneous effects of PA would be more appropriate.

Model 8 provides lower AICc in those subjects for which glucagon effect is well estimated. Considering the slight improvement of this model and the fact that it is more physiologically complete, we prefer it with respect to **Model 5**.

Table 5.1 reports the comparison between **Model 5** and **Model 8** in terms of RSS and $AICc$ for each ND subject. Subjects for which k_{Gluc} is estimated with good precisions are Subj 1, 2, 3, 5.

Table 5.1: Comparison of model 5 and 8

	RSS_{model5}	$AICc_{model5}$	RSS_{model8}	$AICc_{model8}$
Subj 1	4.60	131.85	2.85	101.92
Subj 2	2.93	100.88	2.49	92.93
Subj 3	5.17	130.74	4.94	130.82
Subj 4	5.32	141.92	5.32	144.96
Subj 5	5.48	143.98	5.38	145.63
Subj 6	15.65	216.40	15.72	219.68
mean	6.52	144.29	6.12	139.271
SD	4.57	38.54	4.87	45.12

Finally, **Model 9** includes epinephrine effect on EGP. For this model some preliminary considerations are necessary. Epinephrine was sampled only at time $T-60$, $T180$, $T300$ and therefore we can consider only three values of concentration of this hormone for each subject (in many cases only one or two samples are available since the concentration value was too low and, therefore, not detectable). For this reason, we modeled epinephrine signal as in [Figure: 5.1]. The value of epinephrine sampled at time -60 is kept constant till $T120$. Then the signal is modeled with an exponential growth till $T180$ and, then, with an exponentially decreasing function till $T300$.

The parameter k_{Epi} , which governs the magnitude of epinephrine action on glucose

production, is estimated with good precisions in four subjects. For these subjects, the estimated values for k_{Epi} are 0.004, 0.007, 0.004, 0.007 $mg * mL / (pg * kg * min)$, respectively. Although it is known that epinephrine plays an important role in glucose production, **Model 9** does not provide particular improvements in the fit of EGP

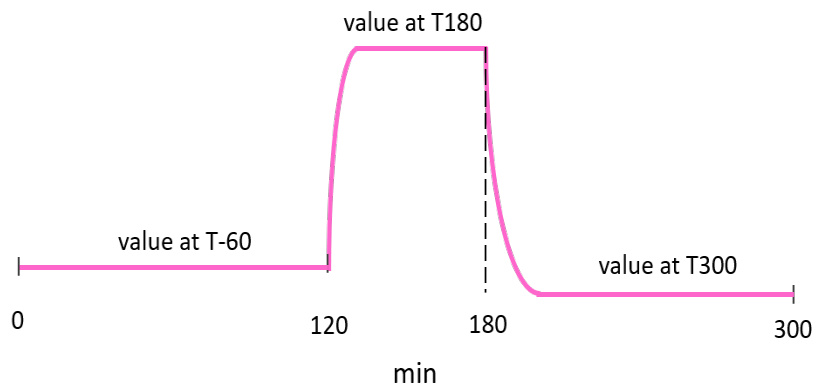


Figure 5.1: Epinephrine signal.

data. However, we have to consider that we have very few samples of epinephrine, so we can not guarantee a correct estimation of its signal and, therefore, a correct quantification of its action on EGP. Moreover, this model provides less precise estimates for $p4$ and $p6$ compared to **Model 8**. For these reasons we excluded **Model 9**, even if the incorporation of this catecholamine could be appealing.

The table below reports the comparison between **Model 8** and **Model 9** in terms of RSS and $AICc$ for each ND subject.

Table 5.2: Comparison of model 8 and 9

	RSS_{model8}	$AICc_{model8}$	RSS_{model9}	$AICc_{model9}$
Subj 1	2.85	101.92	4.22	128.95
Subj 2	2.49	92.93	2.94	103.88
Subj 3	4.94	130.82	4.05	118.43
Subj 4	5.32	144.96	5.32	144.94
Subj 5	5.38	145.63	4.97	140.17
Subj 6	15.72	219.68	14.88	215.86
mean	6.12	139.271	6.06	142.04
SD	4.87	45.12	4.40	39.10

In summary, **Model 1**, **3**, **4**, **6**, and **7** were all excluded due to bad fit and poor precision (high CV) of parameter estimates. **Model 2** was excluded because **Model 5** provided a better fit of the data. Of the remaining models, **Models 5**, **8** and **9**, **Models 8** and **9** are physiologically appealing since they consider glucagon and epinephrine action, respectively. Furthermore, they both provide lower RSS and AICc with respect to **Model 5** and, therefore, it was excluded. Because of the few epinephrine samples and the consequent difficulty in estimating its profile, we selected **Model 8** as the best able to predict EGP pattern during physical activity for ND subjects.

The table below summerizes the results obtained, while Table 5.4 reports the estimated parameters from **Model 8**.

Table 5.3: Comparison of EGP models

	RSS	AICc	SD	No. of Parameters
Model 1	30.88	237.22	0.64	8
Model 2	9.71	150.01	0.34	8
Model 3	9.56	152.31	0.34	9
Model 4	40.75	240.48	0.68	9
Model 5	6.52	144.29	0.30	11
Model 6	4.85	134.84	0.27	12
Model 7	6.31	141.51	0.29	12
Model 8	6.12	139.27	0.28	12
Model 9	6.06	142.04	0.29	12

In Figure: 5.2 **Model 8** is shown. It includes: glucose action on EGP proportional to k_G ; insulin inhibition of EGP; glucagon stimulation of EGP proportional to k_{Gluc} ; effect of PA on k_2 .

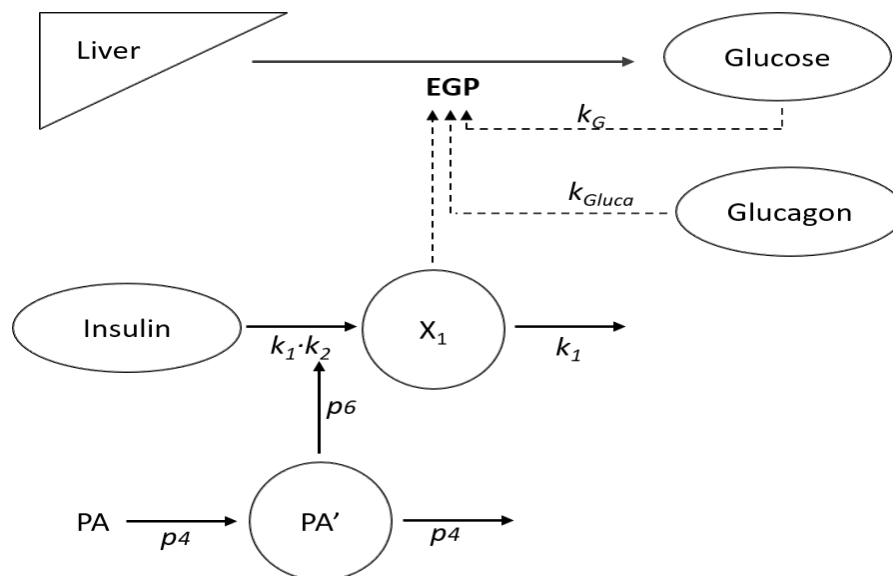


Figure 5.2: **Model 8** which includes glucose, glucagone and insulin action on EGP, with a delayed effect of exercise on insulin sensitivity.

Table 5.4: Parameter estimates of **Model 8**. Numbers in parenthesis indicate coefficient of variation (**) for $CV > 100\%$).

	k_1	k_2			k_G	k_{Gluc}	p_6			p_4		
	min^{-1}	$\frac{mg \cdot mL}{\mu U \cdot kg \cdot min}$			$\frac{dL}{kg \cdot min}$	$\frac{mg \cdot mL}{pg \cdot kg \cdot min}$	[adim]			min^{-1}		
		V1	V2	V3			V1	V2	V3	V1	V2	V3
1	0.08 (22)	0.09 (6)	0.03 (5)	0.02 (21)	0.01 (14)	0.03 (15)	0.92 (7)	0.67 (35)	0.21 (63)	0.72 (0)	0.02 (49)	0.95 (**)
2	0.06 (15)	0.12 (5)	0.03 (4)	0.06 (6)	0.01 (10)	0.01 (27)	1.48 (4)	0.62 (12)	0.64 (62)	0.32 (62)	0.08 (46)	0.01 (85)
3	0.05 (27)	0.08 (15)	0.02 (21)	0.01 (56)	0.01 (13)	0.03 (24)	1.01 (18)	0.96 (18)	0.10 (**)	0.70 (6)	0.60 (**)	0.21 (**)
4	0.02 (23)	0.12 (10)	0.05 (8)	0.03 (13)	0.02 (10)	$3 \cdot 10^{-6}$ (**)	0.58 (25)	0.73 (16)	1.46 (15)	0.21 (**)	0.88 (15)	0.19 (74)
5	0.04 (20)	0.12 (7)	0.02 (10)	0.05 (11)	0.01 (16)	0.01 (30)	1.04 (10)	1.08 (11)	2.21 (40)	0.93 (**)	0.97 (0)	0.01 (55)
6	0.08 (22)	0.15 (9)	0.04 (7)	0.02 (19)	0.01 (17)	$2 \cdot 10^{-5}$ (**)	0.77 (17)	6.61 (57)	6.24 (25)	0.06 (50)	$4 \cdot 10^{-3}$ (69)	0.01 (29)
mean	0.05 (21)	0.11 (9)	0.03 (9)	0.03 (21)	0.01 (13)	0.01 (**)	0.97 (13)	1.78 (25)	1.81 (**)	0.49 (95)	0.43 (**)	0.23 (**)
SD	0.02	0.02	0.01	0.02	0.003	0.01	0.31	2.37	2.31	0.34	0.45	0.37

In [Figure: 5.3] average data vs prediction and average weighted residuals of **Model 8** are reported. The section between the two dashed lines ($T120$ and $T180$) represents the exercise period.

As we can see, V3 does not provide a good fit in the recovery phase. This is mainly due to one subject who presented some experimental drawbacks during the third visit. For this subject none of the models provided a good prediction of EGP profile during and after PA in V3. Average data vs prediction and average weighted residual of **Model 8** without this subject are reported in [Figure: 5.4]. EGP prediction is somewhat better in V3, while in the other two visits there are not significant changes.

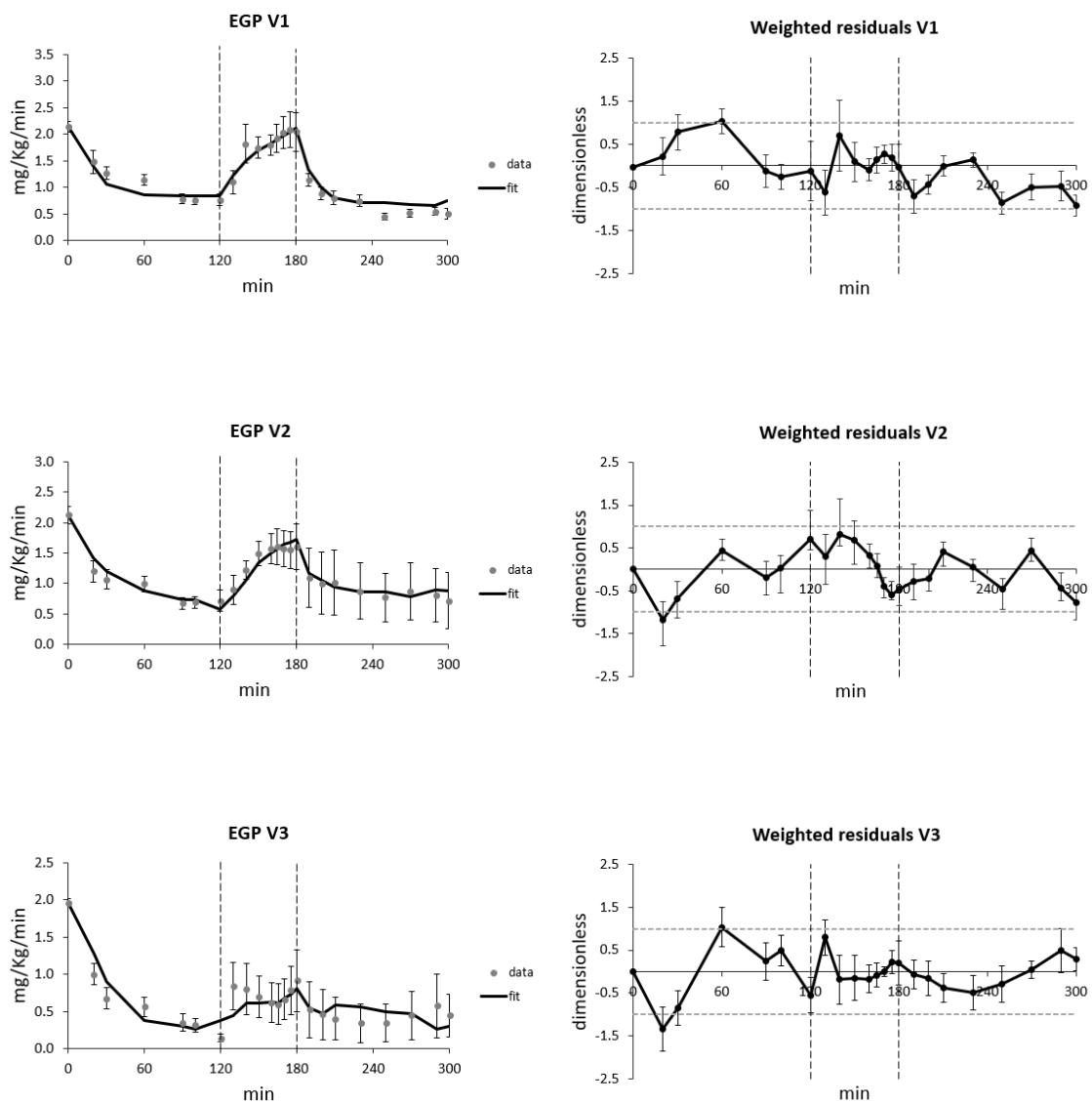


Figure 5.3: Model 8 in ND subjects: average data vs model prediction (left) and weighted residuals (right) of the three visits. Vertical bars, SE.

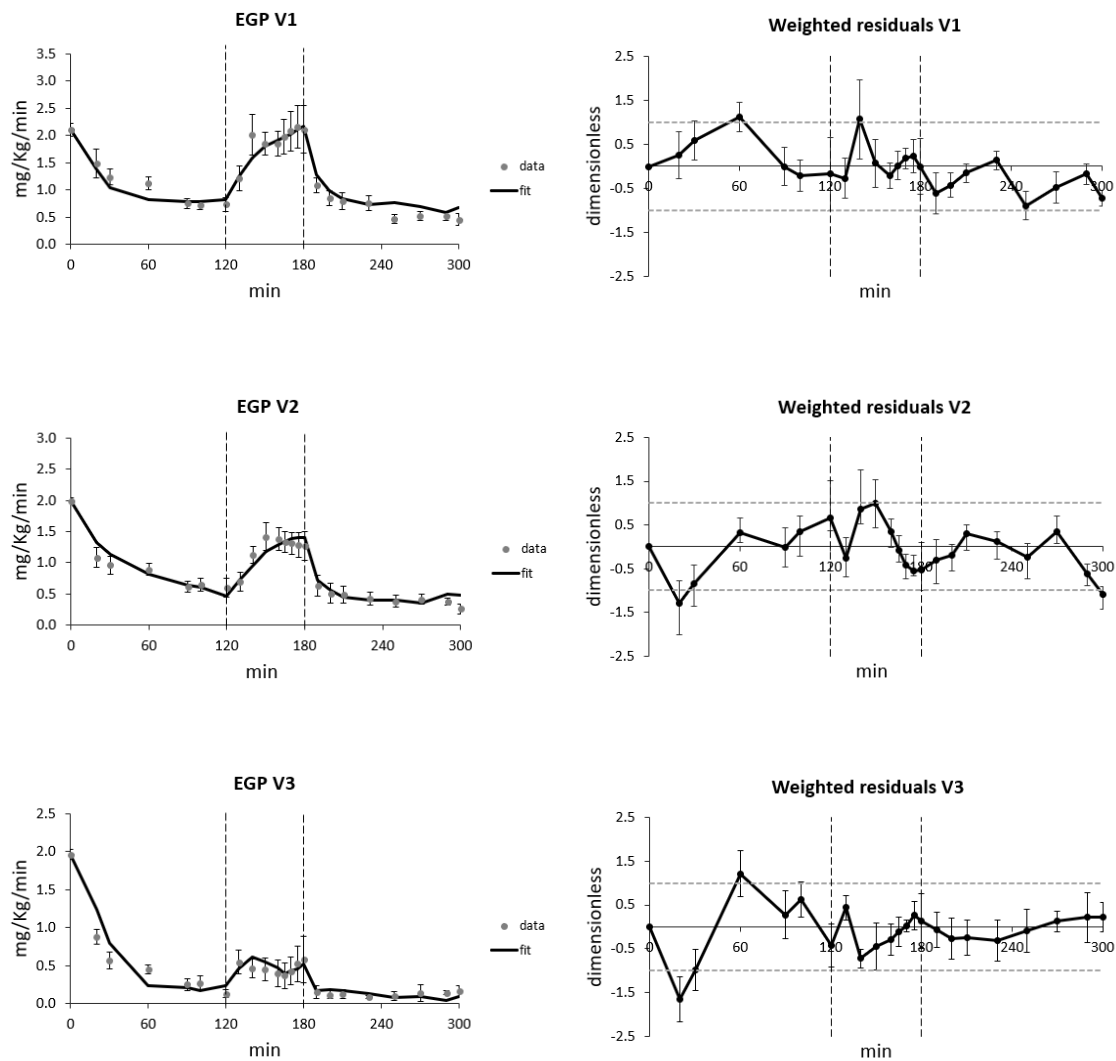


Figure 5.4: Model 8 in ND subjects with subject 6 excluded: average data vs model prediction (left) and weighted residuals (right) of the three visits. Vertical bars, SE.

5.2 T1D

The best model selected for ND subjects has been tested also in T1D subjects. As discussed in section (3.3), we considered 6 type 1 diabetic subjects that performed the same three visits as healthy subjects, in the same experimental conditions. It is worth noting that, in V3, insulin concentration is lower in T1D with respect to ND since they do not secrete endogenous insulin in response to high glucose level. For this reason, this visit is not comparable between ND and T1D. Moreover, for ND subjects, we considered insulin concentration at time T_0 as the basal insulin, since this value did not differ significantly from the concentration at $T-60$. In contrast, for T1D subjects, the insulin level at T_0 usually differs from the value at $T-60$, and therefore we decided to estimate the basal insulin for these individuals with the

consequent increase in the number of parameters of the model.

The performance of **Model 8** tested on T1D are less satisfactory compared with ND subjects. The parameter k_{Gluc} is precisely estimated in four subjects with values 0.033, 0.016, 0.010, 0.04 $mg * mL / (pg * kg * min)$, respectively, but generally, the precisions of all the estimated parameters are poorer than those obtained with ND. As we can see in [Figure: 5.5], we obtained a good prediction of EGP profile during the pre-exercise. It is worth noting that, in all visits, the prediction at time $T0$ is not well fitted, probably due to the uncertain initial conditions of insulin, as previously discussed. However, for what concerns the recovery, the fit is higher than EGP data in all visits, which suggests that there may be some other effects that could play an important role, especially during this phase. During exercise instead, an increment of the EGP prediction is observed in all visits, even if the fit is slightly lower than EGP data in V1 and V3.

With **Model 8**, we are considering a direct effect of glucose, a delayed effect of insulin, a delayed effect of PA on insulin sensitivity and a direct effect of glucagon on glucose production which turned out to be sufficient for a good estimation of EGP profile in ND subjects, but these results point out that further studies are necessary to improve the prediction of EGP profile in T1D subjects.

The following figure reports the average data against the model prediction, and the weighted residuals of **Model 8** tested on T1D individuals (only four T1D subjects performed V1) .

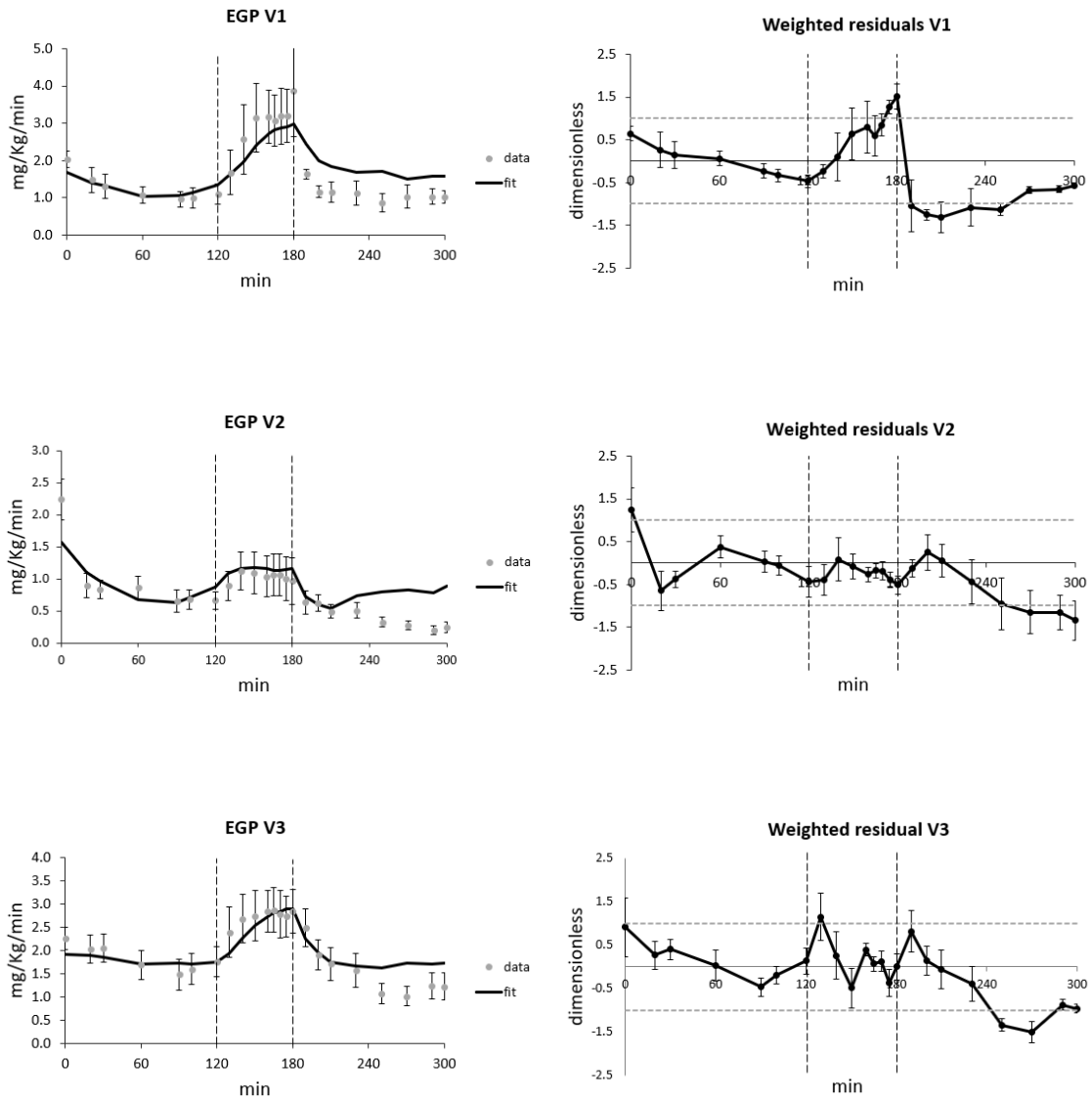


Figure 5.5: Model 8 in T1D subjects: average data vs model prediction (left) and weighted residuals (right) of the three visits. Vertical bars, SE.

Chapter 6

Conclusion

In healthy subjects, glucose level is controlled and kept close to its normal range ($70 - 110 \frac{mg}{dL}$) thanks to a complex glucose-insulin system that aims to maintain blood sugar into this range after perturbations, represented, for example, by food ingestion or physical activity. The latter, is an important component of our daily life, since it positively affects both the state of health and the degree of personal satisfaction.

Although it is known that even subjects with diabetes can benefit from a regular physical activity, the optimal conditions in terms of intensity, duration and insulin therapy are still unclear. In particular, it is well known that physical activity in T1D patients influences glucose concentrations not only during exercise but also several hours after, leading to late evening and nocturnal hypoglycemia, which represents a critical problem to exercise for these individuals [2, 19].

Physical activity, in general, increases glucose utilization (R_d) and, therefore, endogenous glucose production (EGP) must increase to meet the increased metabolic demands of the muscle to prevent hypoglycemia, in both healthy and diabetic subjects [2, 25, 34].

The aim of this thesis was the development of a mathematical model which can accurately predict the EGP profile during physical activity in different experimental conditions in subjects with and without type 1 diabetes. For this purpose, data of 12 subjects (6 healthy and 6 with type 1 diabetes) who performed three visits in different glycemic and insulin levels with a moderate-intensity exercise session of 60 minutes have been used. The three visits are: V1 in euglycemia and low insulin, V2 in euglycemia and high insulin, V3 in hyperglycemia and low insulin, and they were performed in random order.

In order to provide a model able to predict the EGP pattern during physical activity, a number of models of increasing complexity incorporating the effects of exercise were developed. The model selection was tackled by using standard criteria as models ability to describe the data, precision of the parameter estimates, physiological plausibility of the estimated values and model parsimony. We started by considering only the healthy subjects: direct effects of exercise were initially considered on glucose effectiveness (k_G) and insulin sensitivity (k_2) alone. While it is known that glucose level affects the EGP, by adding the exercise effects on k_G we did not observe any improvement in the EGP prediction during exercise (especially in V1 and V2), since these effects were always estimated with very poor precisions in all subjects ($CV > 100\%$). The results did not change even considering a delayed effect of PA on k_G . Consequently, we decided to exclude this effect from the model. On the other hand, the inclusion of exercise effect on insulin sensitivity markedly improved EGP prediction, and the performances improved even more when we considered a delayed effect of exercise on k_2 . Moreover, the improvement was more evident whenever the delayed effect of PA on k_2 was elevated ($p4 < 0.02 \text{ min}^{-1}$). This brought us to think that physical activity may affect insulin sensitivity more than glucose effectiveness, but we have to take into account that in this study glucose is clamped and this makes the PA effects on k_G more difficult to quantify. The best model selected (**Model 8**), includes a delayed PA effect on insulin sensitivity, a delayed effect of insulin, a direct effect of glucose, and a direct effect of glucagon on EGP. The results in terms of data fit, precision of the estimates and model parsimony for ND subjects were satisfactory. The addition of glucagon slightly improved the model performance, in particular during the recovery in V1 and V2. The greatest effect was seen in those subjects where the prediction was higher than EGP data during the post-exercise, since we obtained a lower fit with a consequent better adherence of the prediction to the data. A model which includes epinephrine effects was also tested. Despite it is well known that epinephrine plays an important role in glucose production [41], this model did not show particular improvements than the model with glucagon. This is likely due to the very few samples of available epinephrine, since we had only three samples of this hormone per visit for each subject (in many cases only one or two samples were available since the concentration value of this catecholamine was too low and therefore not detectable). However, in these conditions, we can not guarantee a correct estimation of epinephrine signal and, therefore, a correct quantification of its effect on EGP. For this reason, we excluded this model.

The model selected for ND subjects was also tested in T1D subjects. The fit obtained is able to well predict EGP profile during the pre-exercise phase in all visits, while performances are less satisfactory during and after exercise compared with ND subjects. Furthermore, the precisions of all the estimated parameters were poorer (higher CV) with respect to those obtained with ND. One reason could be the increase in the number of parameters since with T1D individuals we estimated also the basal insulin. The reason was that, in contrast with ND individuals, insulin value at time T_0 is often different than the value at $T-60$. This could also lead to an inaccurate prediction at time 0 of EGP [Figure: 5.5]. Furthermore, the prediction remains above the EGP data during the recovery phase for all the visits, suggesting that other effects may play a role on EGP for T1D subjects.

In conclusion, although **Model 8** will require further validation, especially for Type 1 diabetic individuals, the present thesis suggests that it likely provides an accurate assessment of the pattern of EGP during physical activity for healthy individuals. Future works could also better investigate the role of epinephrine on EGP during exercise in both healthy and T1D subjects since for this study the available samples were not enough. However, these data show a large increase of catecholamine concentration during exercise which supports the hypothesis that they could give additional information to the model, but more studies are needed.

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