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FACOLTÀ DI INGEGNERIA

Master's degree in Biomedical Engineering

IDENTIFICATION OF HIDDEN PATTERNS IN CLINICAL DATABASE THROUGH DATA MINING TECHNIQUES FOR THE STUDY OF DIABETES PATHOPHYSIOLOGY

Supervisor:

Dr. Micaela Morettini

Co-supervisors:

Prof. Laura Burattini

Dr. Andrea Tura

Master thesis of:

Ludovica Ilari

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Abstract

This study aimed to identify hidden patterns for the study of diabetes pathophysiology through data mining techniques in a database constituted by features that quantify specific metabolic processes in a population of women with a previous history of gestational diabetes and thus, particularly prone to progress toward type 2 diabetes (T2DM). Interest was reserved to insulin clearance process as relevant feature for the prediction of T2DM. In particular, a systematic review has been conducted to examine the epidemiological determinants, phenomena and processes categories affecting insulin clearance.

Insulin clearance (L/min) was defined as “the disappearance of insulin from the bloodstream in the entire organism”. In fact, it accounts for the degradation of insulin by liver, kidney and other tissues diminishing the plasma insulin about 50% of its secretion. Other terms related to insulin clearance are insulin degradation or insulin extraction. Together with insulin action, insulin secretion and glucose effectiveness, insulin clearance is involved in the regulation of blood glucose level into the plasma called glucose homeostasis.

After its secretion from the beta cells of the pancreas, insulin reaches the liver through the portal vein where it is cleared (first pass); the remained insulin enters the systemic circulation through the hepatic vein and it reaches the insulin sensitive tissues performing its action. After insulin action, insulin is degraded by the insulin sensitive tissues including skeletal muscle, kidneys and liver (after recirculation) by the cooperative action of CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), IDE (Insulin-Degrading Enzyme) and other proteases.

Insulin clearance is composed of two independent processes: hepatic clearance and extrahepatic clearance. Hepatic clearance accounts for 80% of insulin clearance and it includes the removal of a portion of secreted insulin by the liver during the first pass through the hepatic portal vein (50 %) and later through the hepatic artery (after recirculation). Extrahepatic clearance accounts for 20 % of insulin clearance includes the removal of a portion of secreted insulin by other organs, mainly by kidney (15 %) and muscle (5%).

The measurement of insulin clearance in humans was performed through several methods applying some modelling-based techniques or some indexes like the molar ratio between the C-peptide and insulin or the AUC ratio between the C-peptide and insulin, both in the fasting state and after oral or intravenous glucose challenges.

The determinants of insulin clearance were divided into the epidemiological ones which included sex, age and ethnicity and the determinants accounting for the phenomena and processes category that were the biological/cell physiological/chemical phenomena, the circulatory physiological phenomena, the urinary physiological phenomena, the anthropometric parameter, the genetic phenomena, the carbohydrate metabolism, the fat metabolism, the pathological process (like T2DM), the chemical and drug categories and the behaviour category.

After the examination of the determinants of insulin clearance as a complex not completely known mechanism, this study analysed the influence of insulin clearance on the prediction of T2DM.

Considering this aspect, this study identified hidden patterns in 2 databases having or not insulin clearance as feature through feature scoring methods. For the analysis, a population of women with a history of gestational diabetes (pGDM) progressing or not to T2DM (PROG or NONPROG, respectively) was involved. The features considered were age (age), body mass index (BMI), first phase mean of insulin clearance (CL-mean_0-10), second phase mean of insulin clearance (CL-mean_10-180), insulin sensitivity (S_I), basal insulin effect of glucose effectiveness (BIE) and glucose effect at zero insulin of glucose effectiveness (GEZI) and finally, disposition index (Disp Index). Moreover, rate of glucose disappearance before and after insulin injection (KG (1) and KG (2) respectively), first phase insulin secretion (F1c), basal secretion rate (BSR) and distribution volume of glucose (DIST VOL) were included in the analysis.

The logistic regression feature scoring showed insulin clearance at the 8^{th} and 9^{th} position of the ranking (score = 0.483 for CL-media_0-10 and score = 0.434 for CL-media_10-180) while the decision tree feature scoring showed insulin clearance at the 3^{rd} and 6^{th} position (score = 0.205 for CL-media_10-180 and score = 0.174 for CL-media_0-10).

For the logistic regression analysis, CL-media_0-10 and CL-media_10-180 were found more informative for the prediction of the PROG or NONPROG with respect to KG (2) (score = 0.127); for the decision tree analysis, CL-media_10-180 resulted more informative with respect to KG (1) (score = 0.191) and S_I (score = 0.177) while CL-media_0-10 resulted less informative with respect to KG (1) and S_I.

In conclusion, being insulin clearance involved in the top ten features in the logistic regression as well as in the decision tree, it resulted a relevant feature for the prediction of type 2 diabetes in a population of women with a history of gestational diabetes. The information obtained from the resultant pattern could be of interest for the diabetes pathophysiology.

Introduction

Diabetes is a chronic disease characterized by the presence of high levels of glucose in the blood (hyperglycemia) that occurs when the pancreas does not produce enough insulin or when the body is not able to effectively use the produced insulin. The first case occurs in type 1 diabetes (T1DM), the second case happens in type 2 diabetes (T2DM). Tissue resistance to the action of insulin (insulin resistance), altered insulin secretion by the pancreas and altered insulin clearance are the three main processes underlying the development of T2DM. T2DM is a widespread disease that is estimated to affect approximately 693 million people worldwide by 2045 [1] and is associated with severe long-term complications (such as amputations, chronic kidney disease and blindness) and premature death. Due to the high prevalence and costs associated with the management of T2DM and related complications, early identification of T2DM development risk factors may be a key element in public health policy [2]. The diagnosis of T2DM occurs when typical signs and symptoms occur and this shows a lack of knowledge of the pathology and of tools that identify the alterations in the underlying processes rather than their biochemical consequences (high blood sugar) [2]. Of these processes, insulin clearance is the least known [3]. Significant advances in technology have led to a significant production of data, which can play a key role in the study of the pathophysiology of T2DM. It is possible to find genetic data and clinical information, contained within the electronic health records, alongside the large amount of data that is generated by wearable devices, which provide information on physical activity and consumed calories. In this context, the application of data mining techniques that combine machine learning, pattern recognition, statistics, database theory and data visualization can be used to automatically extract from them concepts, interrelations of concepts and models. These techniques have been extensively explored in recent years for the prevention and management of T2DM [4]–[12] but also showing possible criticalities. In fact, very often the analysis with these techniques on large amounts of heterogeneous data leads to identify spurious correlations [13], indicating that the creation of appropriate databases, with selected groups of subjects and characteristics, it is an aspect of primary importance and which cannot be disregarded in order to achieve reliable results. A further aspect to consider is the difficulty of measuring the processes underlying the development of T2DM. In fact, direct measurement of these processes is possible through experimental procedures that are extremely expensive and sometimes very invasive and therefore not feasible in humans. To overcome this, mathematical methods and models have been developed that allow the quantification of these processes from easily measurable data (such as plasma concentrations) [14]–[16] but on the other

hand, may require specific bio-engineering skills to be applied. The application of data mining techniques to databases that contain not only raw data but also features that quantify specific processes, is highly desirable.

Therefore, this study aimed to identify hidden patterns for the study of diabetes pathophysiology through data mining techniques in a database constituted by features that quantify specific metabolic processes in a population of women with a previous history of gestational diabetes and thus, particularly prone to progress toward T2DM. Interest was reserved to insulin clearance process as relevant feature for progression to the T2DM. In particular, a systematic review has been conducted to examine the epidemiological determinants, phenomena and processes categories affecting insulin clearance.

Chapter 1

Physiology of glucose homeostasis

Glucose is a simple sugar (molecular formula: $C_6H_{12}O_6$) which belongs to the monosaccharide carbohydrates together with the fructose and galactose. Glucose is used by human body to produce energy through different mechanisms depending on the possible oxygen requirement: the glycolysis (it doesn't require oxygen and the final energy release is 2 ATP molecules) or glucose phosphorylation (it requires oxygen and it produces 38 ATP molecules). As carbohydrate, glucose is the main and preferable source of energy in our body (4 Kcal/g) considering that the final products derived from glucose oxidation are not toxic (carbon dioxide and water), while the other two energy substrates that are protein (starting from amino acids) and fats (starting from fatty acids) produce body dehydration and blood acidification, respectively. The concentration of blood glucose into the plasma, called glycemia, should be maintained in a narrow range (70-110 mg/dL). The reason behind the importance of maintaining blood glucose level stable in this range (called "glucose homeostasis"), despite the possible perturbations (mainly food ingestion and physical activity), relies on the fact that possible lower value (< 70 mg/dL) can cause the malfunction of brain and red blood cells since they are organs which use glucose as the main energy substrate, while higher value (> 180 mg/dL) causes the urine loss of glucose (glucosuria) and consequent body dehydration (osmotic effect). Glucose homeostasis requires the involvement of some organs such as the pancreas, the liver, the adipose tissue, the skeletal muscle and the gastro-intestinal tract and some glucoregulatory hormones like insulin, glucagon, amylin, epinephrine (or adrenaline), cortisol, GIP (glucose-dependent insulinotropic polypeptide) and GPL-1 (glucagonlike-peptide-1).

Insulin is a 51-amino acid pancreatic hormone derived from proinsulin and formed by two chains, A (21 residues) and B (30 residues), linked by two disulphide bonds (**Figure 1**) while glucagon is a 29-amino acid pancreatic peptide, derived from proglucagon. Insulin and glucagon are produced by different cells of pancreas, beta cells and alfa cells, respectively and they act on glucose regulation having different roles: insulin decrease the blood glucose levels, while glucagon increases it.

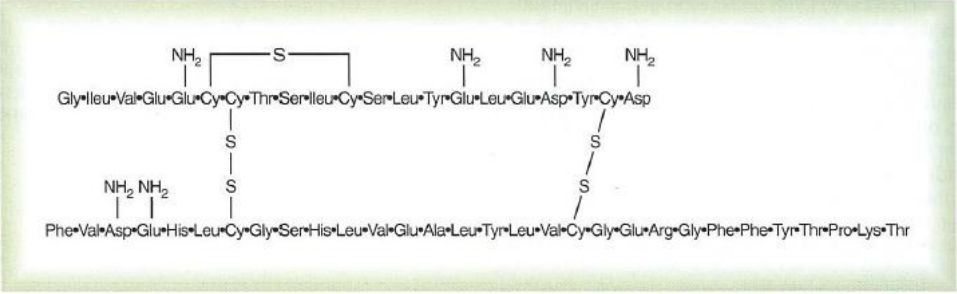


Figure 1. Human insulin molecule.

During the phase of absorption (anabolic) insulin is involved in the carbohydrate and fat metabolism. It allows plasma glucose coming from the intestine to enter the muscle, heart and adipose tissue; in addition, insulin allows glucose storage as glycogen into the muscles and into the liver, suppresses endogenous glucose production (glycogenolysis; gluconeogenesis) and indirectly suppresses glucagon secretion and lipolysis (**Figure 2**).

During the phase of post-absorption (catabolism), the glucagon acts on the liver to break the glycogen molecule allowing glucose to be released into the plasma and after that to enter the brain and erythrocytes.

Moreover, it allows the hydrolysis of triglycerides (TG) into fatty acids (FA) and glycerol (Gli): FA is used for the synthesis of ketone bodies (KB) utilized by the brain as a substrate of energy, while the glycerol together with amino acids, lactate and pyruvate is involved into glucose formation (gluconeogenesis) inside the liver (**Figure 3**)**.**

Amylin is a peptide hormone produced by beta cells of pancreas which acts on the gastric-intestinal tract slowing the gastric emptying and promoting satiety.

Epinephrine (or adrenaline) is a neurotransmitter secreted by the medullary gland which inhibits insulin secretion; thus, it could be released during the physical activity.

Cortisol is a steroid hormone which increases glycemia.

GIP and GLP-1 are hormones released into the blood after glucose ingestion from the k-cells and L-cells of the small intestine, respectively. They are called incretin hormones and their action is called incretin effect or insulin potentiation since they enhance insulin secretion after glucose transition inside the gastro-intestinal tract (glucose-stimulated insulin secretion).

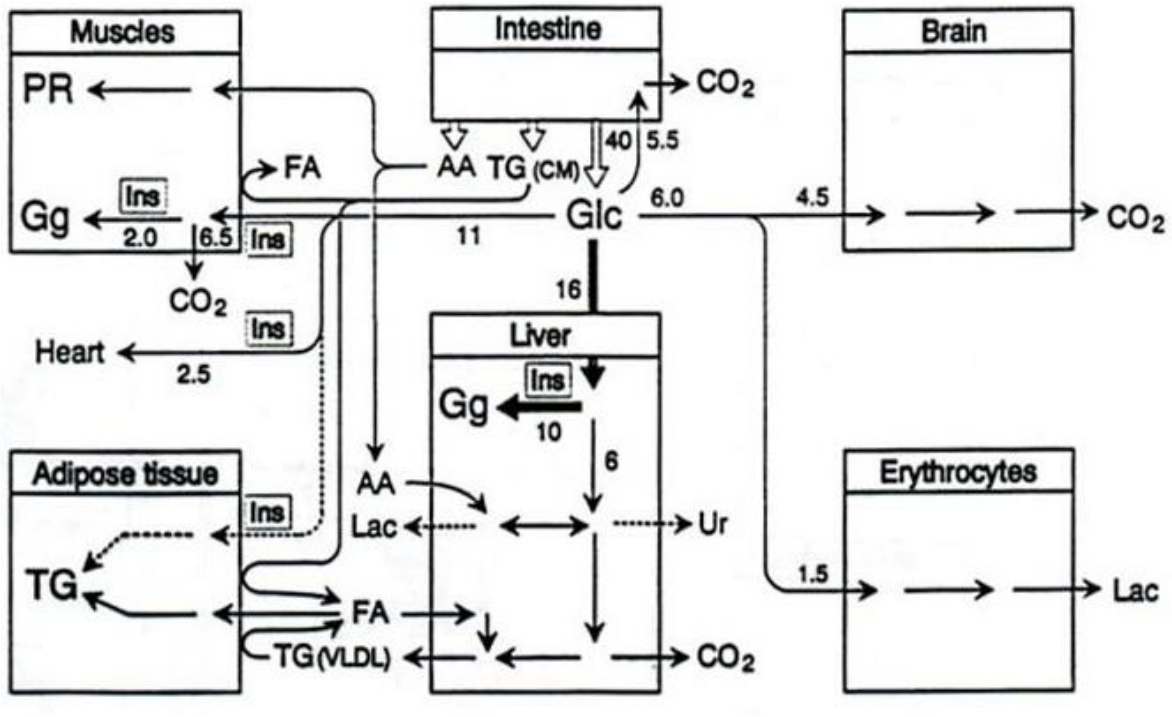


Figure 2. Phase of absorption (Anabolic).

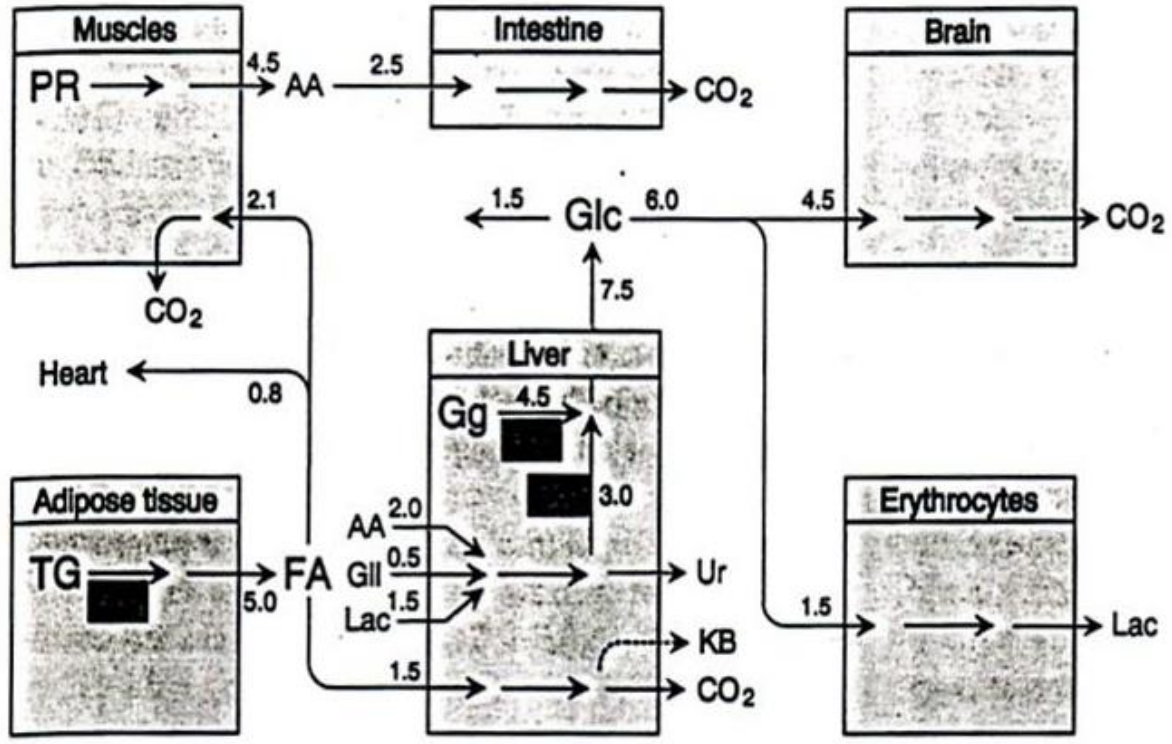


Figure 3. Phase of post-absorption (catabolism).

Despite of the fact that all these hormones take part on glucose regulation, only the two major hormones, insulin and glucagon, are generally taken into consideration into the so called “Glucose insulin regulatory system”, acting through a feedback mechanism (**Figure 4**).

Different stimuli could perturbate glucose homeostasis in a different manner: for example, after glucose ingestion, the blood glucose level rises to the normal range and the pancreas secerns insulin until the blood glucose level returns to the normal range; instead, during the physical activity, the blood glucose level goes down to the normal value and the pancreas secerns glucagon until the normal blood glucose level is restored.

After glucose ingestion, glucose disappears from blood (glucose disappearance or uptake) through two categories of processes: the insulin-dependent processes and the insulin-independent processes. The insulin dependent processes take place into the organs having the receptors for insulin: adipose tissue, skeletal muscle and liver (insulin-sensitive tissues). The insulin-independent processes act on the organs having or not the insulin receptors: adipose tissue, skeletal muscle and liver (insulin-sensitive tissues) and erythrocytes and brain (non- insulin-sensitive tissues).

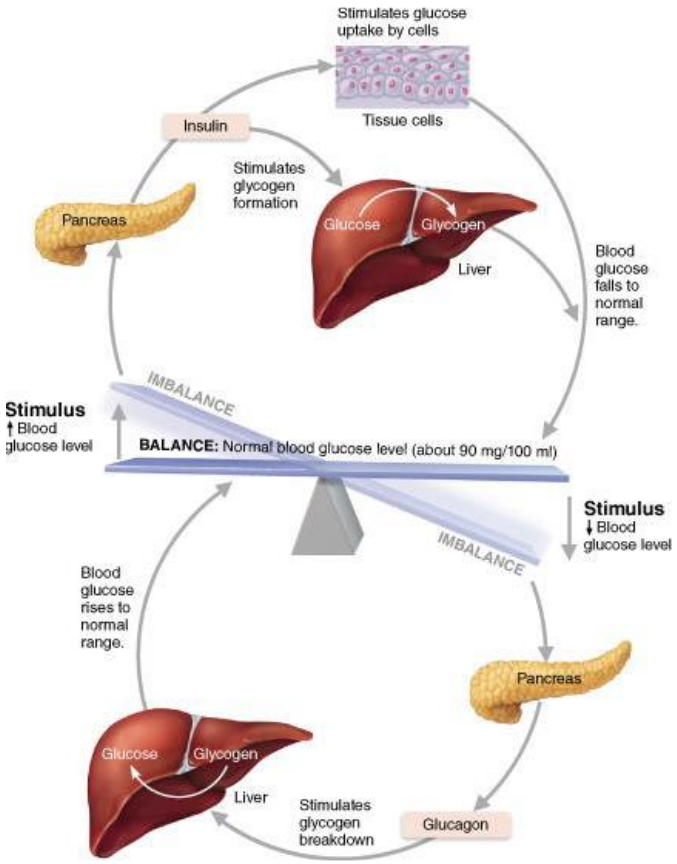


Figure 4. Blood glucose regulation.

Insulin dependent processes account for insulin action (or insulin sensitivity or insulin resistance), insulin secretion and insulin clearance, while the insulin-independent processes account for glucose effectiveness.

Glucose effectiveness quantifies the ability of glucose per se to enhance its own metabolism independently from any change in insulin concentration, suppressing the hepatic glucose production and stimulating glucose uptake by the peripheral tissue.

Insulin sensitivity quantifies the ability of insulin to enhance glucose metabolism, suppressing the hepatic glucose production (gluconeogenesis) and stimulating glucose uptake by the peripheral tissues. Insulin resistance accounts for the reduced sensitivity of tissues to insulin action, very common denominator of T2DM and cardiovascular disease (cardiometabolic disorder).

Insulin is secreted by the pancreas in response to the variation of glucose concentration and to the rate of change of glucose concentration, thus the feedback mechanism by which insulin secretion is controlled by glucose is a proportional + derivative type. Since insulin secretion undergoes hepatic clearance and the pancreas is not an accessible compartment, insulin secretion is quantified by the plasma C-peptide which is secreted by the pancreas in equimolar quantity with respect to insulin, but the hepatic clearance can be considered negligible. Insulin secretion response after a meal shows three peaks: the first and the second called “the cephalic phase” and “gastrointestinal phase” are the response of insulin already synthesized and present into the secretory granules of pancreas before glucose absorption. These phases are related to the response of the Autonomic Nervous System (ANS) and incretin hormones released (first and second peaks, respectively). The last one called “the substrate phase” is the new response of insulin secreted after glucose absorption (**Figure 5**).

Insulin clearance accounts for the degradation of insulin by liver, kidney and other tissues diminishing the plasma insulin about 50% of its secretion.

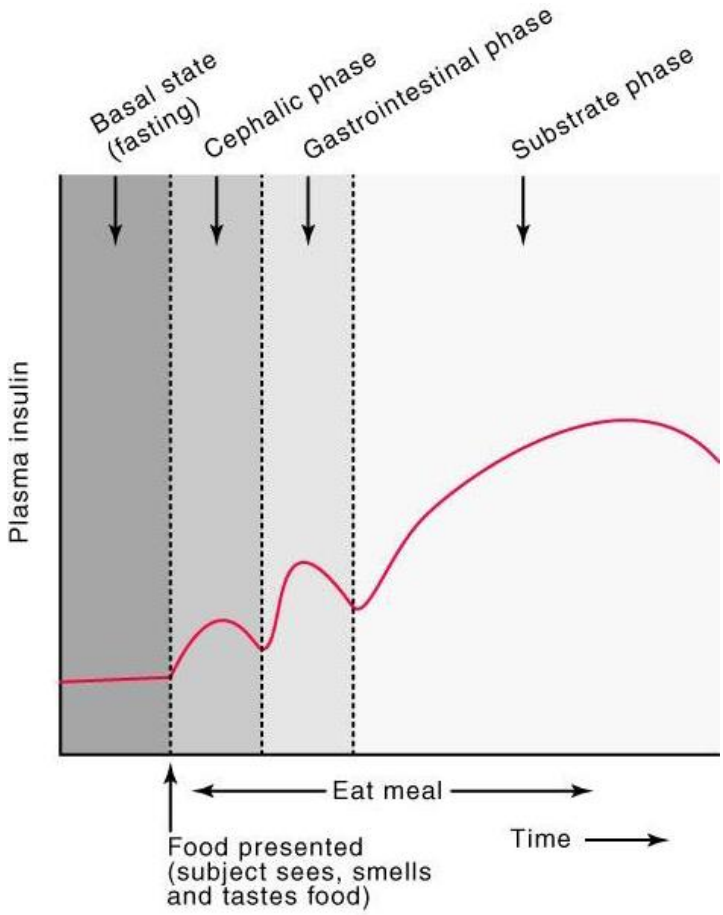


Figure 5. Phases of insulin secretion.

Chapter 2

Insulin clearance and methods for its assessment

The pharmacological definition of clearance is a pharmacokinetic measurement of the volume of plasma from which a substance (drug) is completely removed per unit of time (mL/min). It can be calculated as the ratio between the rate of elimination of a substance and the plasma concentration:

$$Clear = \frac{rate\ of\ elimination}{concentration} \quad (1)$$

$$Cl_T = \int_{t=0}^{t=\infty} \frac{(dx/dt)\ dt}{C.dt} = \frac{Total\ Elimination\ (Dose)}{Total\ Area\ under\ the\ curve} \quad (2)$$

Insulin clearance (L/min) is defined as "the disappearance of insulin from the bloodstream in the entire organism" [17]. Other terms related to insulin clearance are insulin degradation or insulin extraction; Insulin extraction is defined as the fractional amount (%) of secreted insulin that it is removed by the bloodstream while insulin degradation is the defined as the amount of insulin entering into the organs per unit of liter (pmol/L).

After its secretion from the beta cell of the pancreas, insulin reaches the liver through the portal vein where it is cleared (first pass); the remained insulin enters the systemic circulation through the hepatic vein and it reaches the insulin sensitive tissues performing its action. After insulin action, insulin is degraded by the insulin sensitive tissues including skeletal muscle, kidneys and liver (after recirculation). Insulin clearance is composed of two independent processes: hepatic clearance and extrahepatic clearance. Hepatic clearance (80% of insulin clearance) includes the removal of a portion of secreted insulin by the liver during the first pass through the hepatic portal vein (50 %) and later through the hepatic artery (after recirculation). Extrahepatic clearance (20 % of insulin clearance) includes the removal of a portion of secreted insulin by other organs, mainly by kidney (15 %) and muscle (5%) (**Figure 6**).

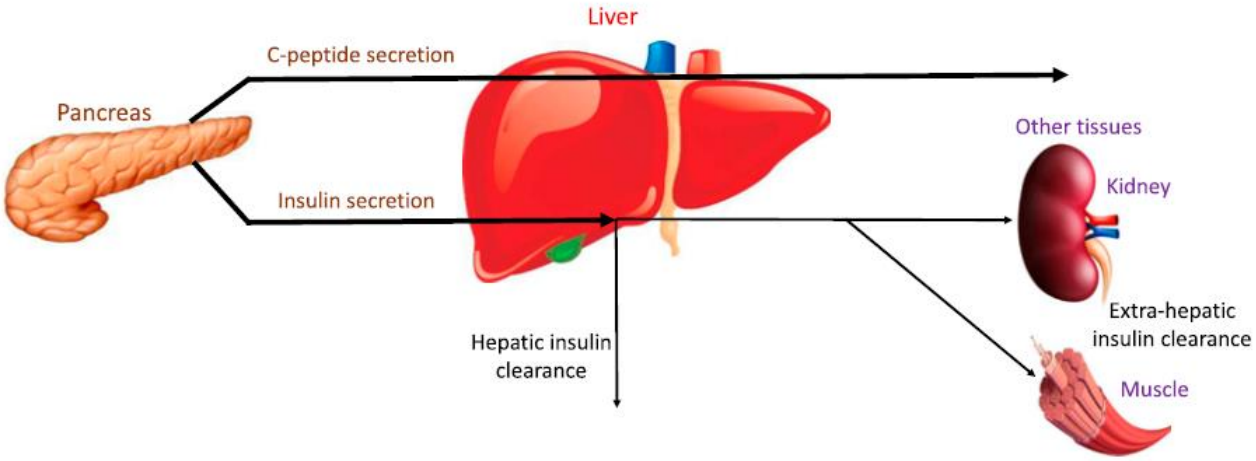


Figure 6. Insulin secretion and clearance rationale [17].

The assessment of insulin clearance in humans can be performed through several methods using data coming from oral (OGTT) or intravenous (IVGTT) glucose tolerance tests.

The OGTT consists of administering an oral glucose dose to the patient (usually 75 grams) and taking blood samples in the next 2-3 hours.

The general experimental procedure is:

- Fasting withdrawals - usually two: 10 and 5 min before taking glucose.
- At time 0, oral glucose (in aqueous solution) is taken at a total dose of 75 grams (duration of intake 30 seconds to 5 minutes).
- Collection of blood samples: 30, 45, 60, 90, 120, 150, 180 min.
- Measurement of glucose, insulin and possibly C-peptide concentrations.

The classical IVGTT test consisted of an injection in the vein of a bolus of glucose (usually 0,3 grams per kilo of weight) with blood samples taken frequently during the three-hour duration of the test.

The insulin modified IVGTT, IM-IVGTT, is a classical IVGTT with an infusion for 5 minutes of insulin (dose 0,03- 0,05 U/kg) started 20 minutes after glucose bolus.

The general experimental procedure is:

- Fasting samples - usually two: 10 and 5 minutes before glucose injection.
- At time 0, start of glucose infusion (50% in H_2O) at the total dose of 0.3 grams per kilogram of body weight (infusion time from 30 seconds to minute).
- For classic IVGTT, blood samples taken at the time: 3, 4, 5, 6, 8, 10, 14, 19, 22, 25, 30, 35, 40, 50, 60, 70, 80, 90, 120, 150, 180 min.

- For modified IVGTT, blood samples taken at the time: 3, 4, 5, 6, 8, 10, 14, 19 min; insulin infusion (0,03-0,05 U/kg) from the time 20 to 25 min and then blood samples at 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 120, 150, 180 min.
- Measurement of glucose, insulin and possibly C-peptide concentrations.

Some modelling-based techniques or some indexes like the molar ratio between the C-peptide and insulin (3) or the AUC ratio between the C-peptide and insulin (4) both in the fasting state and after OGTT and IVGTT are largely used for the measurement of insulin clearance.

$$\mathrm{IC}\ (\mathrm{t}) = \frac{\mathrm{C-peptide}\ (\mathrm{t})}{\mathrm{Insulin}\ (\mathrm{t})} \tag{3}$$

$$\mathrm{IC}_{\mathrm{AUC}} = \frac{\mathrm{C-peptide\ area\ under\ the\ curve}}{\mathrm{Insulin\ area\ under\ the\ curve}} \tag{4}$$

The mentioned indexes (3) and (4) give an inaccurate estimate of insulin clearance due to the different kinetics of C-peptide and insulin. Moreover, they do not allow the estimation of the extrahepatic component of insulin clearance, although the hepatic component is the major one. Among the models proposed in the literature they have some limitations because most of them permit only the estimation of the hepatic insulin clearance, do not include the hepatic insulin recirculation and have individual parameter estimation issues [17]. Further studies are developing for insuring more reliable estimate of insulin clearance, allowing a better prevention of some diseases related to its impairment.

Insulin homeostasis can be achieved with a coordinated activity between insulin secretion and insulin clearance which in turn is linked to insulin action. The mechanisms of insulin clearance in all insulin sensitive tissues are insulin uptake and insulin degradation and the organs involved are mainly the liver and the kidney, but other tissues are also involved like the muscle, the adipose tissue and the gastrointestinal tract. Under normal conditions, insulin is degraded intracellularly or by membrane processes, while in case of wound, insulin can be degraded extracellularly having a wound-healing activity [18]. Insulin uptake could be mediated by insulin receptor or by nonspecific

processes, the latter achieving greater importance at higher insulin concentrations. Once insulin entering the cell, insulin can undergo degradation or can be released back into the circulation. The mean residence time of insulin endogenously secreted is estimated equal to 72 min with 62 min for liver receptor binding, 6 min for peripheral receptor binding and 3 min in blood or interstitial fluid. The liver (mainly hepatocytes) contributes to insulin clearance mainly by receptor mediated processes although other processes like pinocytosis may be involved especially in case of higher insulin concentration. The kidney removes insulin by two mechanisms: by glomerular clearance from the glomerular capillaries through the mechanisms of filtration-reabsorption-degradation (99% of filtered insulin) and filtration-urine execration (1% of filtered insulin) and by post-glomerular, peritubular clearance from the peritubular capillaries. They remove insulin via receptor mediated processes and non-receptor mediated processes (**Figure 7**). Although in normal subjects first pass hepatic clearance plays a major role in insulin clearance, in insulin-treated patient with diabetes, kidney clearance has the main role since subcutaneous injection of insulin escapes first-pass removal by the liver.

The insulin metabolism is composed of two mechanisms: insulin uptake and insulin degradation. As said previously, insulin uptake is mainly mediated by the insulin receptor process, but other possible mechanisms are also found especially in case of high concentration of insulin. Insulin degradations involves multiple pathways inside the cell: endosomes (or even the cell membrane) initiate the degradation through IDE (Insulin-Degrading Enzyme) and other proteases action. The insulin fragments which are not fully degraded or intact insulin which escape from endosomal degradation can reach other subcellular compartments including cytosol, nucleus, Golgi or lysosome where final degradation can be completed. Not all insulin which is internalized into the cell is degraded, but a significant portion is released into the circulation intact or partially degraded by diacytosis or retroendocytosis.

In liver and kidneys organs, insulin uptake and insulin degradation are regulated by the cooperative action of CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1), IDE and other proteases [19].

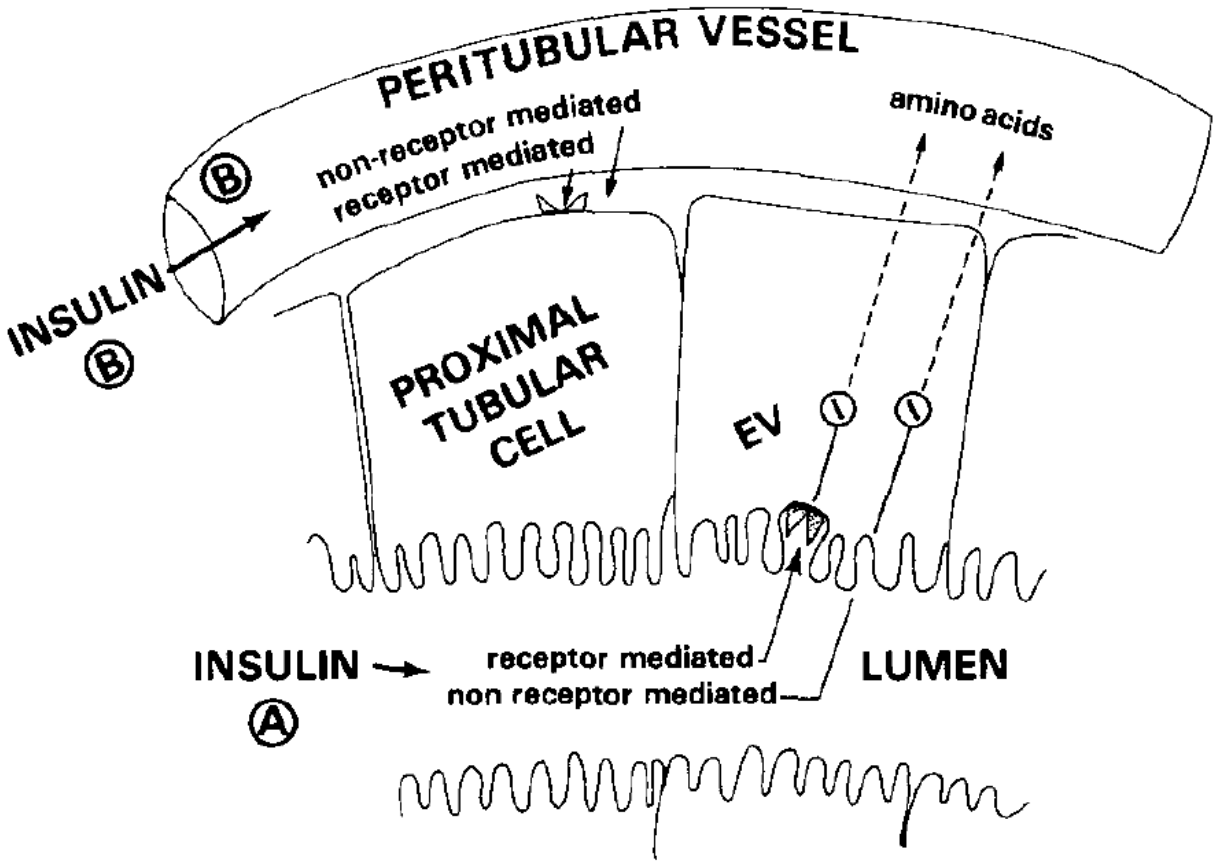


Figure 7. Intra-renal pathways of insulin clearance [20].

CEACAM1 is a transmembrane glycoprotein devoid of enzymatic activity, expressed mainly into the liver and kidneys in two alternatively spliced isoforms: CEACAM1-L and CEACAM1-S.

CEACAM1-L is constituted by a longer chain of amino acids and its role is to target insulin clearance to the degradation pathways (acting as a chaperone) and to mediate the synthesis of fatty acids (suppressing lipogenesis). CEACAM1-S is formed by a smaller chain of amino acids and it is involved into the receptor-mediated insulin uptake. IDE (also known as insulin protease, insulinase, metalloendoprotease) is a Z_n^{2+} metallo-endopeptidase which has two functions: the proteolytic one is referred to its ability to degrade intact insulin into several fragments mainly after internalization of insulin into early endosomes (non-acidic environment); the non-proteolytic one is hypothesised to be related to the regulation of the level of insulin receptors. The coordinating action of the CEACAM1 and IDE in the insulin metabolism is composed of 4 steps (**Figure 8**)**:**

First step is the endocytosis: once insulin binds the insulin receptor, the insulin-insulin receptor (IR) complex is internalized into the cell via endocytosis. This action is increased by the insulin-induced phosphorylated CEACAM 1 likely by stabilizing the insulin-IR complex.

Second step is the early endosome: CEACAM 1 targets the insulin-IR complex to early endosomes where its non-acidic environment elicits the IDE action in degrading insulin into fragments.

Third step is the late endosome: CEACAM-1 delivers the insulin-IR complex to late endosomes where it acts destabilizing the insulin-IR complex and provoking the dissociation of insulin from its receptor; an acidic protease and not IDE acts to fully degrade insulin into the late endosome.

Fourth step is the receptor recycling or degradation: depending on the conditions, IDE regulates the insulin receptor sorting process in terms of lysosomal degradation (in non-physiological condition) or plasma membrane recycling (in physiological condition).

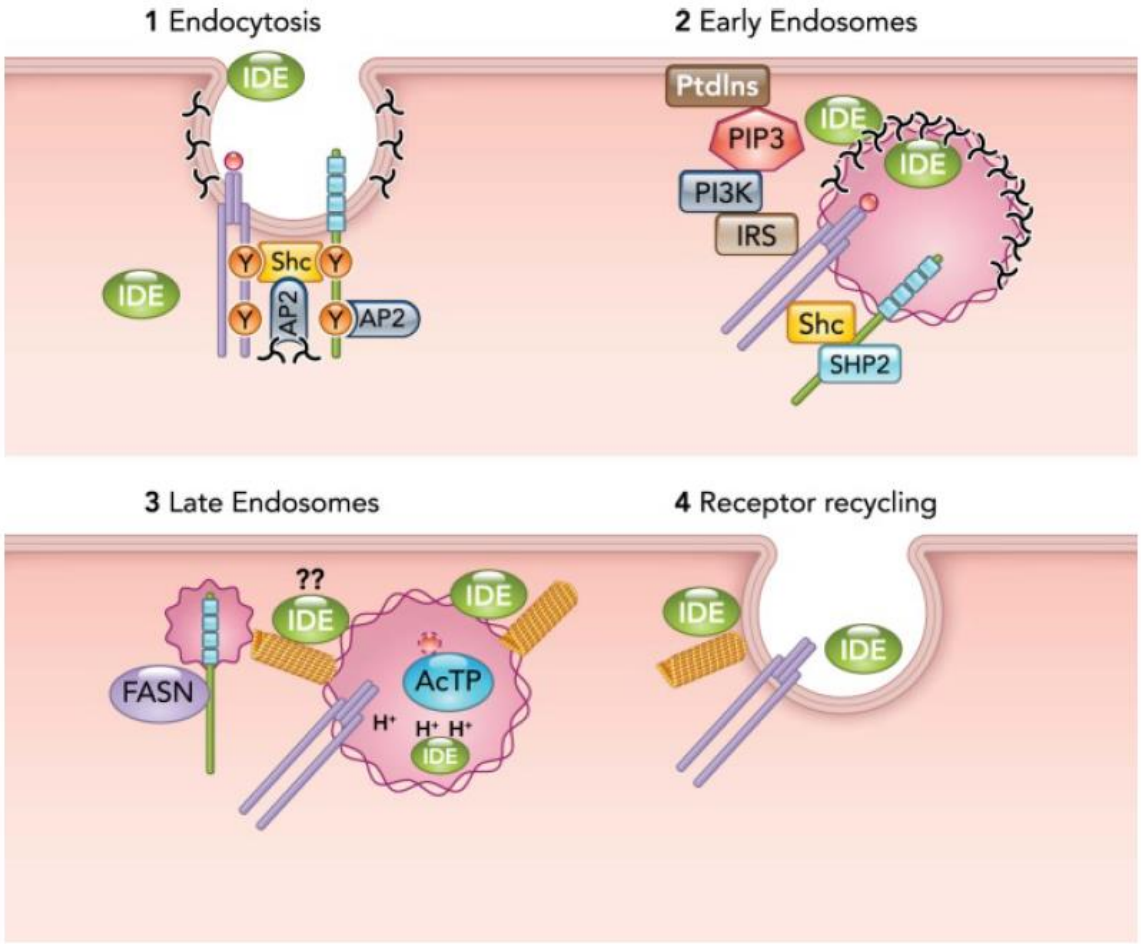


Figure 8. Coordinated regulation of receptor mediated insulin intracellular trafficking by CEACAM1 and IDE *[19]*.

Chapter 3

Determinants of insulin clearance: a systematic review of the literature

A systematic review of the literature has been conducted to find out the determinants of insulin clearance from a PubMed, Scopus and Web of Science search.

3.1 Methods of the systematic review

Eligibility criteria

The studies included for the full-text eligibility were in English language and they dealt with human individuals who fell in the following categories:

- Age: from childhood to elderly people
- Status: a) healthy individuals; b) normal, overweight and obese individuals; c) non-diabetic (NGT), impaired glucose tolerance (IGT), T1DM, T2DM and previous gestational diabetes (pGDM) individuals; chronic renal failure individuals; NAFLS (non-alcoholic fatty liver syndrome) individuals; metabolic syndrome individuals
- Race: African American, European American, Black, White, Caucasian, Indians, Chinese, European, non-European.
- Epidemiological determinants and Phenomena and Processes categories (**Figure 9**)

Exclusion criteria

The studies dealing with modelling technique to measure insulin clearance were excluded from the full text eligibility.

Search strategy

The studies were searched into PubMed, Scopus and Web of Science databases with the date of publication from January 1985 to November 2020; the string chosen among all the possible ones was reported into the **Table 1** in the 14th row.

Screening

The studies were screened based on the title and abstract and considering the eligibility criteria; the process was shown in **Figure 9**.

Data extraction

The information extracted from the studies regarded the author and date of publication, the method applied for insulin clearance measurement, the data analysis (determinant), the outcome (the effect of the determinant in insulin clearance), the participant characteristics and the source database.

Table 1. Strings: number (n°), search string, database, results (n°) and rationale.

Search n°	Search string	Database	Results n°	Rationale
1	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND (("determinants") OR ("determination") OR ("determine") OR ("predictors"))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	97	• General determinants and predictors • No animals
2	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	773	• Without determinants • No animals
3	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((**determinant, epidemiologic [MeSH Terms]**) OR ("determination") OR ("determine") OR ("predictors") OR ("covariate"))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	126	• **Epidemiologic determinant** • General determinants, predictors and covariates • No animals
4	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic [MeSH Terms]) OR (**Health Social Determinant [MeSH Terms]**) OR ("determination") OR ("determine") OR (predictors) OR (covariate)) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	134	• **Health social determinant** • Epidemiologic determinant • General determinants, predictors and covariates • No animals
5	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic [MeSH Terms]) OR (Health Social Determinant [MeSH Terms]) OR (**lifestyle [MeSH Terms]**) OR ("determination") OR ("determine") OR (predictors) OR (covariate)) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	137	• **Lifestyle determinant** • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates • No animals
6	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR **("Behavior" [MeSH**]) OR **("Biological Factors" [MeSH**]) OR **("Nutritional Physiological Phenomena" [MeSH**]) OR **("Diseases Category" [MeSH**]) OR ("determinant") OR ("determination") OR ("determine") OR (predictors) OR (covariate))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	636	• **Behaviour determinant** • **Biological Factors** • **Nutritional Physiological Phenomena** • **Diseases Category** • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates • No animals

Search n°	Search string	Database	N° results	Rationale
7	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR ("Behavior" [MeSH]) OR ("Biological Factors" [MeSH]) OR ("**Glucose Metabolism Disorders"[Mesh]**) OR ("**Nutrition Disorders"[Mesh**]) OR ("determinants") OR ("determinant") OR ("determination") OR ("determine") OR (predictors) OR (covariate))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	582	• **Glucose Metabolism Disorders** • **Nutrition Disorders** • Behaviour determinant • Biological Factors • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates • No animals
8	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR ("Behavior" [MeSH]) OR ("Biological Factors" [MeSH]) OR ("Nutritional Physiological Phenomena" [MeSH]) OR ("**Diabetes Mellitus" [MeSH**]) OR (**"obesity"**) OR (**"determinants"**) OR ("determinant") OR ("determination") OR ("determine") OR (predictors) OR (covariate))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	521	• **Diabetes Mellitus** • **Obesity** • **Determinants** • Behaviour determinant • Biological Factors • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates • No animals
9	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin hepatic extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR (Behavior [MeSH]) OR ("Biological Factors" [MeSH]) OR ("Nutritional Physiological Phenomena" [MeSH]) OR ("Diabetes Mellitus" [MeSH]) OR (**obesity**) OR (determinant*) OR (**determination**) OR (determine) OR (predictor*) OR (covariate*))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	705	• **Diabetes Mellitus** • **Obesity** • Behaviour determinant • Biological Factors • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates (asterisks) • No animals
10	(clearance[ti] OR extract*[ti] OR degradat*[ti]) AND insulin[ti] AND (determinant* OR predictor* OR covariate* OR association* OR determinant, epidemiologic [MeSH Terms])	PubMed	135	• **General determinant, predictor, covariate, association** • **Epidemiologic determinant**
11	(clearance[ti] OR ((extract*[ti] OR degradat*[ti]) AND (hepatic[ti] OR liver[ti]))) AND insulin[ti] AND (determinant* OR predictor* OR covariate* OR association* OR determinant, epidemiologic [MeSH Terms])	PubMed	85	• **General determinant, predictor, covariate, association** • **Epidemiologic determinant** • **Liver**

Search n°	Search string	Database	N° results	Rationale
12	(((((("insulin clearance") OR ("insulin extraction") OR ("insulin hepatic extraction") OR ("insulin degradation")) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR (Behavior [MeSH]) OR ("Biological Factors" [MeSH]) OR ("Nutritional Physiological Phenomena" [MeSH]) OR ("Diabetes Mellitus" [MeSH]) OR (**"Obesity"[Majr**]) OR (determinant*) OR (predictor*))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	455	• **Obesity [Majr]** • Diabetes Mellitus • Behaviour determinant • Biological Factors • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates (asterisks) • No animals
13	(((((**clearance[ti] OR extract*[ti] OR degradat*[ti]**) AND insulin[ti]) AND ((determinant, epidemiologic[MeSH Terms]) OR (Health Social Determinant[MeSH Terms]) OR (Behaviour [MeSH]) OR ("Biological Factors" [MeSH]) OR ("Nutritional Physiological Phenomena" [MeSH]) OR ("Diabetes Mellitus" [MeSH]) OR ("Obesity") OR (determinant*) OR (predictor*))) AND ((humans) OR (individuals) OR (subjects))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	326	• **[ti]** • Obesity • Diabetes Mellitus • Behaviour determinant • Biological Factors • Health social determinant • Epidemiologic determinant • General determinants, predictors and covariates (asterisks) • No animals
14	((((clearance[ti] OR extract*[ti] OR degradat*[ti]) AND insulin[ti]) AND ((determinant, epidemiologic [MeSh]) OR (**"Phenomena and Processes Category"[Majr]**) OR (determinant*) OR (predictor*) OR (association*))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	298	• **Phenomena and Processes categories [Majr]** • Epidemiologic determinant • General determinant, predictor, association No animals
15	((((clearance[ti] OR extract*[ti] OR degradat*[ti]) AND insulin[ti]) AND ((determinant, epidemiologic [MeSh]) OR ("Physiological Phenomena") OR (determinant*) OR (predictor*) OR (association*))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	85	• **Physiological Phenomena** • Epidemiologic determinant • General determinant, predictor, association • No animals
16	((((clearance[ti] OR extract*[ti] OR degradat*[ti]) AND insulin[ti]) AND ((determinant, epidemiologic [MeSh]) OR ("Phenomena and Processes Category"[Majr]) OR (**health**) OR (determinant*) OR (predictor*) OR (association*))) NOT (("animals") OR ("mice") OR ("dog") OR ("rat")))	PubMed	337	• **Health** • Phenomena and Processes categories [Majr] • Epidemiologic determinant • General determinant, predictor, association • No animals

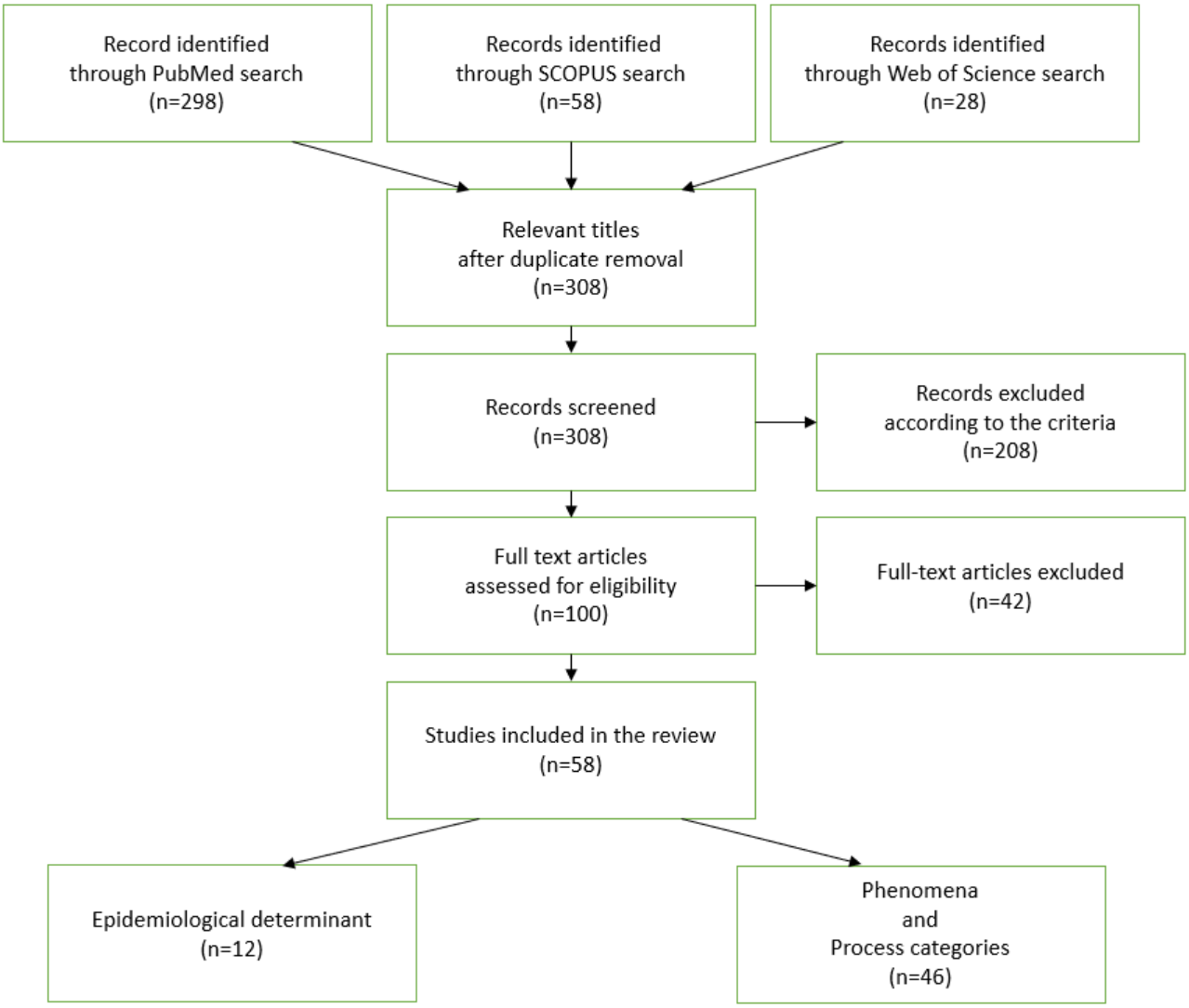


Figure 9. Systematic review procedure flowchart.

3.2 Results of the systematic review

The determinants resulting from the research string chosen (14th string of **Table 2**) were reported below with a definition coming from the PubMed database. Moreover, the effect (outcome) of each determinant on insulin clearance can be visualized in **Table 2**.

The determinants of insulin clearance were divided into: 1) epidemiological (including sex, age and ethnicity) and 2) phenomena and processes categories which can be summarized in **Figure 10**

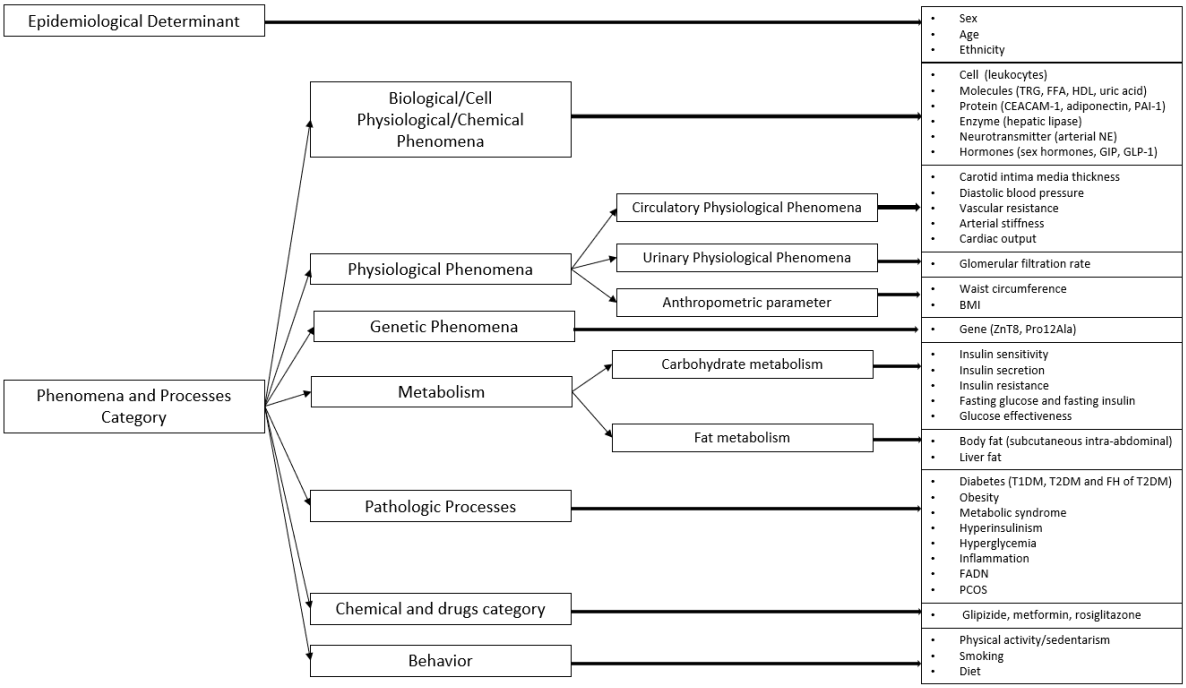


Figure 10. Insulin clearance determinants.

Sex

The totality of characteristics of reproductive structure, functions, phenotype, and genotype, differentiating the male from the female organism.

Age Factors

Age as a constituent element or influence contributing to the production of a result. It may be applicable to the cause or the effect of a circumstance. It is used with human or animal concepts but should be differentiated from aging, a physiological process, and time factors which refers only to the passage of time.

Ethnology

Used with diseases for ethnic, cultural, or anthropological aspects, and with geographic headings to indicate the place of origin of a group of people.

Fatty Acids, Nonesterified

Fatty acids found in the plasma that are complexed with serum albumin for transport. These fatty acids are not in glycerol ester form.

Cholesterol, HDL

Cholesterol which is contained in or bound to high-density lipoproteins (HDL), including cholesterol esters and free cholesterol.

Uric Acid

An oxidation product, via xanthine oxidase, of oxypurines such as xanthine and hypoxanthine. It is the final oxidation product of purine catabolism in humans and primates, whereas in most other mammals urate oxidase further oxidizes it to allantoin.

Apolipoproteins

Protein components on the surface of lipoproteins. They form a layer surrounding the hydrophobic lipid core. There are several classes of apolipoproteins with each playing a different role in lipid transport and lipid metabolism. These proteins are synthesized mainly in the liver and the intestines.

Plasminogen Activator Inhibitor 1

A member of the serpin family of proteins. It inhibits both the tissue-type and urokinase-type plasminogen activators.

Adiponectin

A 30-kDa complement C1Q-related protein, the most abundant gene product secreted by fat cells of the white adipose tissue. Adiponectin modulates several physiological processes, such as metabolism of glucose and fatty acids, and immune responses. Decreased plasma adiponectin levels are associated with insulin resistance, type 2 diabetes mellitus, obesity and atherosclerosis.

Lipase

An enzyme of the hydrolase class that catalyzes the reaction of triacylglycerol and water to yield diacylglycerol and a fatty acid anion. It is produced by glands on the tongue and by the pancreas and initiates the digestion of dietary fats.

Alanine Transaminase

An enzyme that catalyzes the conversion of L-alanine and 2-oxoglutarate to pyruvate and L-glutamate.

Norepinephrine

Precursor of epinephrine that is secreted by the adrenal medulla and is a widespread central and autonomic neurotransmitter. Norepinephrine is the principal transmitter of most postganglionic sympathetic fibers, and of the diffuse projection system in the brain that arises from the locus ceruleus. It is also found in plants and is used pharmacologically as a sympathomimetic.

Growth Hormone

A polypeptide that is secreted by the adenohypophysis (pituitary gland, anterior). Growth hormone, also known as somatotropin, stimulates mitosis, cell differentiation and cell growth. Species-specific growth hormones have been synthesized.

Glucagon-Like Peptide 1

A peptide of 36 or 37 amino acids that is derived from proglucagon and mainly produced by the intestinal l cells. GLP-1(1-37 or 1-36) is further N-terminally truncated resulting in GLP-1(7-37) or GLP-1-(7-36) which can be amidated. These GLP-1 peptides are known to enhance glucose-dependent insulin release, suppress glucagon release and gastric emptying, lower blood glucose, and reduce food intake.

Gastric Inhibitory Polypeptide

A gastrointestinal peptide hormone of about 43-amino acids. It is found to be a potent stimulator of insulin secretion and a relatively poor inhibitor of gastric acid secretion.

Glucagon

A 29-amino acid pancreatic peptide derived from proglucagon which is also the precursor of intestinal glucagon-like peptides. Glucagon is secreted by pancreatic alpha cells and plays an important role in regulation of blood glucose concentration, ketone metabolism and several other biochemical and physiological processes.

Androgens

Compounds that interact with androgen receptors in target tissues to bring about the effects similar to those of testosterone. Depending on the target tissues, androgenic effects can be on sex differentiation, male reproductive organs, spermatogenesis, secondary male sex characteristics libido, development of muscle mass, strength and power.

Gonadal Steroid Hormones

Steroid hormones produced by the gonads. They stimulate reproductive organs, germ cell maturation and the secondary sex characteristics in the males and the females. The major sex steroid hormones include estradiol, progesterone and testosterone.

Leukocytes

White blood cells. These include granular leukocytes (basophils, eosinophils and neutrophils) as well as non-granular leukocytes (lymphocytes and monocytes).

Carotid Intima-Media Thickness

A measurement of the thickness of the carotid artery walls. It is measured by B-mode ultrasonography and is used as a surrogate marker for atherosclerosis.

Blood Pressure

Pressure of the blood on the arteries and other blood vessels.

Vascular Stiffness

Loss of vascular elasticity due to factors such as aging and arteriosclerosis. Increased arterial stiffness is one of the risk factors for many cardiovascular diseases.

Vascular Resistance

The force that opposes the flow of blood through a vascular bed. It is equal to the difference in blood pressure across the vascular bed divided by the cardiac output.

Cardiac Output

The volume of blood passing through the heart per unit of time. It is usually expressed as liters (volume) per minute so as not to be confused with stroke volume (volume per beat).

Waist Circumference

The measurement around the body at the level of the abdomen and just above the hip bone. The measurement is usually taken immediately after exhalation.

Waist-Hip Ratio

The waist circumference measurement divided by the hip circumference measurement. For both men and women, a waist-to-hip ratio (WHR) of 1.0 or higher is considered "at risk" for undesirable health consequences, such as heart disease and ailments associated with overweight. A healthy WHR is 0.90 or less for men and 0.80 or less for women.

Glomerular Filtration Rate

The volume of water filtered out of plasma through glomerular capillary walls into Bowman's capsules per unit of time. It is considered to be equivalent to inulin clearance.

Weight Loss

Decrease in existing body weight.

Body Fat Distribution

Deposits of adipose tissue throughout the body. The pattern of fat deposits in the body regions is an indicator of health status. Excess abdominal fat increases health risks more than excess fat around the hips or thighs, therefore, waist-hip ratio is often used to determine health risks.

Adiposity

The amount of fat or lipid deposit at a site or an organ in the body, an indicator of body fat status.

Fatty Liver

Lipid infiltration of the hepatic parenchymal cells resulting in a yellow-colored liver. The abnormal lipid accumulation is usually in the form of triglycerides, either as a single large droplet or multiple small droplets. Fatty liver is caused by an imbalance in the metabolism of fatty acids.

Diabetes Mellitus

A heterogeneous group of disorders characterized by hyperglycemia and glucose intolerance.

Obesity

A status with body weight that is grossly above the acceptable or desirable weight, usually due to accumulation of excess fats in the body. The standards may vary with age, sex, genetic or cultural

background. In the body mass index, a BMI greater than 30.0 kg/m^2 is considered obese and a BMI greater than 40.0 kg/m^2 is considered morbidly obese (morbid obesity).

Metabolic Syndrome

A cluster of symptoms that are risk factors for cardiovascular diseases and type 2 diabetes mellitus. The major components of metabolic syndrome include abdominal obesity, atherogenic dyslipidemia, hypertension, hyperglycemia, insulin resistance, a proinflammatory state and a prothrombotic (thrombosis) state.

Hyperinsulinism

A syndrome with excessively high insulin levels in the blood. It may cause hypoglycemia. Etiology of hyperinsulinism varies, including hypersecretion of a beta cell tumor (insulinoma), autoantibodies against insulin (insulin antibodies), defective insulin receptor (insulin resistance) or overuse of exogenous insulin or hypoglycemic agents.

Inflammation

A pathological process characterized by injury or destruction of tissues caused by a variety of cytologic and chemical reactions. It is usually manifested by typical signs of pain, heat, redness, swelling and loss of function.

Metabolic Diseases

Generic term for diseases caused by an abnormal metabolic process. It can be congenital due to inherited enzyme abnormality (metabolism, inborn errors) or acquired due to disease of an endocrine organ or failure of a metabolically important organ such as the liver.

Polycystic Ovary Syndrome

A complex disorder characterized by infertility, hirsutism, obesity and various menstrual disturbances such as oligomenorrhea, amenorrhea, anovulation. Polycystic ovary syndrome is usually associated with bilateral enlarged ovaries studded with atretic follicles, not with cysts. The term, polycystic ovary, is misleading.

Non-alcoholic Fatty Liver Disease

Fatty liver finding without excessive alcohol consumption.

Genes

A category of nucleic acid sequences that function as units of heredity and which code for the basic instructions for the development, reproduction and maintenance of organisms.

Tofogliflozin

Inhibits sodium-glucose cotransporter 2.

Glipizide

An oral hypoglycaemic agent which is rapidly absorbed and completely metabolized.

Metformin

A biguanide hypoglycaemic agent used in the treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification. Metformin improves glycaemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose.

Rosiglitazone

A thiazolidinedione that functions as a selective agonist for ppar gamma. It improves insulin sensitivity in adipose tissue, skeletal muscle and the liver of patients with type 2 diabetes mellitus.

Exercise

Physical activity which is usually regular and done with the intention of improving or maintaining physical fitness or health. Contrast with physical exertion which is concerned largely with the physiologic and metabolic response to energy expenditure.

Sedentary Behaviour

Behaviours during waking hours that have low energy expenditure and are often performed in a sitting or reclining posture.

Smoking

Wilful or deliberate act of inhaling and exhaling smoke from burning substances or agents held by hand.

Diet

Regular course of eating and drinking adopted by a person or animal.

Insulin Resistance

Diminished effectiveness of INSULIN in lowering blood sugar levels: requiring the use of 200 units or more of insulin per day to prevent hyperglycemia or ketosis.

Insulin Secretion

Production and release of insulin from pancreatic beta cells that primarily occurs in response to elevated blood glucose levels.

Table 2. Studies on the determinants of insulin clearance: method, details, outcomes and participant characteristics.

Author/year	Method/Test	Data analysis	Outcome	Participant characteristics	Database
Acerini et al. (2000)	Index (EGHIC)	Hormone: Growth Hormone (GH)	GH↓→IC↑	T1DM	PubMed
		Age	Age↓→IC↑		
Basu et al. (2006)	(HGC)	Age	Age↓→HIC↑	Healthy	PubMed
		Sex	No sex effect		
Bergaman et al. (2019)	Model (FSIGTT)	Ethnicity: African Americans (AA) vs European Americans (EA)	AA→HIC↑ EA→HIC↓	Healthy	PubMed
Camilo et al. (2018)	Index (HGC)	Adipose tissue: Body fat (BF)	BF↑→HIC↓	Overweight, Obese	PubMed
		Diabetes: Family History of T2DM (FH of T2DM)	FH of T2DM no effect		
Escobar et al. (1999)	Indirect estimation	Weight Loss (WL)	WL↑→HIC↓	Obese	PubMed
Finucane et al. (2014)	Index (OGTT)	Liver fat (IHL)	IHL↑→HIC↓	Healthy Older white	PubMed
Fiorentino et al. (2018)	(EGHIC)	Uric acid (UA)	UA↑→IC↓	Non-diabetic	PubMed
Galderisi et al. (2019)	Model (EGHIC)	Obesity	No-effect	Young obese	PubMed
Gin et al. (1994)	(EGHIC)	Diet: Low protein and Low phosphorus (LPLP)	LPLP→MCR↑ LPLP→PIC↑	Chronic renal failure, non-diabetic	PubMed
		Heritability (H)	H→MCR		PubMed

Goodarzi et al. (2014)	(FSIGTT)	Metabolic parameter: Insulin sensitivity (S_I)	S_I↑→MCR↑	Mexican Americans	
Goodarzi et al. (2020)	(EGHIC)	BMI+lipids (BMI+L)	(BMI+L) →IC↑	Non-diabetic, Mexican American	PubMed
Guo et al. (2012)	(EGHIC)	Heritability (H)	H →IC	Hispanic	PubMed
Hakim et al. (2019)	(HGC)	Liver fat (IHL)	IHL↑→HIC↓	Black West-African T2DM	PubMed
Henry et al. (1988)	Index	Weight Loss (WL)	WL→HIC↑	Obese T2DM	PubMed
Huber et al. (2020)	(EGHIC)	glomerular filtration rate (GFR)	GRF↓→IC↓	nondiabetic persons with chronic kidney disease (SUGAR)	PubMed
Jensen et al. (2012)	(EGHIC)	Sex: female (F), male (M)	M→ sp IC↑ F→PIC↑ F→IC↑	Healthy, black and white	PubMed
Jiang et al. (1996)	Index	Obesity (O)	O→HIC↓	Adolescent, Black and white	PubMed
		Hyperinsulinemia (I)	I→HIC↓		
		Ethnicity: Black (B) and White (W)	B→HIC↓		
Kaga et al. (2017)	(EGHIC)	Adipose tissue: Body fat (BF)	BF↑→MCR↓	Healthy, non-obese, Japanese men	PubMed
Keyhani-Nejad (2020)	Index (Oral isomaltulose or sucrose injestion)	Hormone: GIP	GIP↑→HIC↓	NGT, IGT, T2DM	PubMed
		Hormone: GLP-1	GLP-1↑→HIC↑		
Kopylov et al. (2020)		Proteins: CEACAM1	CEACAM1↓→IC↓	GDM and T2DM	PubMed
Kotronen et al. (2007)	(EGHIC)	Liver fat (IHL)	IHL↑→IC↓	Non-diabetic, non-obese adults	PubMed
Kotronen et al. (2008)	(EGHIC)	Liver fat (IHL)	IHL↑→IC↓	T2DM	PubMed
		Diabetes (D)	(D) →IC↓		
Kuhl et al. (1981)	Index (fasting)	Pregnancy	No effect	Late pregnancy	PubMed
Labadzhyan et al. (2016)		Enzyme: hepatic lipase (HL)	HL↑→IC↓	Mexican American coronary artery disease (MACAD)	PubMed
		Enzyme: alanine aminotransferase (AAt)	AAt↑→IC↓		
		Adipose tissue: Body fat (BF)	BF↑→IC↓		
		Diabetes (D)	(D) →IC↓		

		Protein: apoliproteine (AL)	AL↑→**I**C↑		
Lamprinou et al. (2020)	Model (OGTT)	Liver fat (IHL)	No effect	Non-diabetic individuals	Web of Science
		Inflammation (infl)	Infl→HIC↑		
		Molecule: HDL cholesterol	HDL↑→HIC↑		
Lee et al. (2012)	FSIVGTT	Protein: inflammatory protein (PAI1)	PAI1↑→MCRI↓	Non-diabetic, white, hispanic and African american (IRAS)	PubMed
Liew et al. (2000)		Ethnicity: Indians (I) Chinese (Ch) and Caucasian (Ca)	I_IC ↓ vs Ch_IC I_IC ↓ vs Ca_IC	Healthy, non-diabetic, lean, Asian living	PubMed
Marini et al. (2013)	(EGHIC)	Vascular variable: carotid intima media thickness (CIMT)	CIMT↑→IC↓	Adult, non-diabetic caucasian	PubMed
Marini et al. (2014)	(EGHIC)	Metabolic abnormalities (MA)	MA→IC↓ MA→HIC↓	Obese	PubMed
Matsubayashi et al. (2020)	Index (OMTT)	Treatment: SGLT2-I (TOFO)	SGLT2-I →HIC↑ TG↓→HIC↑ BHB↑→HIC↑	T2DM	PubMed
Matsubayashi et al. (2018)	Index (fasting)	Obesity (O) + Fatty liver (IHL)	O+IHL↑→HIC↓	T2DM	PubMed
Meier et al. (2007)	Index (OGTT)	Hormones: GIP and GLP-1	No effect	healthy	PubMed
		Metabolic parameter: insulin secretion (Isec)	Isec↑→MrHIC↓		
Merovci et al. (2020)		physiologic hyperglycaemia for 72 hours (ph72)	Ph72→MCR↓	Healthy NGT	PubMed
Morettini et al. (2019)	IM-IVGTT	Metabolic parameter: Glucose effectiveness (S_G)	S_G↑→IC↓	pGDM	PubMed
		Disposition index (Disp Index)	Disp Index↑→IC↓		
Neyazi et al. (2018)		Bacteria: Lactobacillus casei	No effect on ID		PubMed
Okura et al. (2020)	(EGHIC)	Blood HbA1c	HbA1c↑→HIC↑	Hba1c T2DM	PubMed
		Hormone: GLP1 and GIP	No effect		
		Hormone: Glucagon (G)	G ↑→HIC↓		
		Protein: Adiponectin (A)	A↑→HIC↑		
		Gene: ZnT8 polymorphism	No effect		
		Metabolic parameter: insulin secretion (Isec)	Isec↓→HIC↑		

		Metabolic parameter: insulin resistance (INS.R)	INS.R↓→HIC↑		
Osei et al. (1994)	(t-m. FSIVGTT)	Ethnicity: black (B) and White (W)	HIC_B↓**wrt** HIC_W	Healthy black and white American	PubMed
Osei et al. (1997)	Index (OGTT)	Race and ethnicity: West African Ancestry (WAA) and White American (WA)	HIC_{WAA}↓ **wrt** HIC_{WA} IC_{WAA}↓ **wrt** IC_{WA}	West African Ancestry and White American	PubMed
Osei et al. (2003)	(FSIVGTT)	GITS Treatment: Glipizide (Gl)	Gl →HIC↓	Non-diabetic (NGT) first relative of African American patients with T2DM	PubMed
Osei et al. (2007)	(OGTT)	Treatment: Sulfonylure (S)	S →HIC↓	African American IGT or T2DM	PubMed
		Treatment: Metformin (M)	M →HIC↑		
		Treatment: Rosiglitazone (TZD)	TZD →HIC↑ (not in basal)		
Piccinini et al. (2018)	(model) FSIGTT	Ethnicity: African American (AA) and European American (EA)	HIC_{AA}↓**wrt** HIC_{EA}	Adult African American and European American women	PubMed
		Ethnicity /Genetic-Epigenetic factor	FEL_{AA}↓ **wrt** FEL_{EA}	Childhood African American and European American	
Pimenta et al. (2003)	Index (HGC)	Diabetes: FH of T2DM	HIC no effect	First relative of T2DM patients	PubMed
Pivovarova et al. (2013)	Index (OGTT)	Metabolic syndrome (MS)	MS →HIC↓	No diabetic individuals, metabolic Syndrome	PubMed
		Anthropometric parameter: waist circumference (wc)	wc↑→HIC↓		
		Vascular variable: diastolic blood pressure (dBP)	dBP↑→HIC↓		
		Metabolic parameter: Fasting glucose: (fG)	fG↑→HIC↓		
		Molecule: tryglicerides (TRG)	TRG↑→HIC↓		
		Metabolic parameter: insulin secretion (Isec)	Isec↑→HIC↓		

Polonsky et al. (1988)	(HGC)	Obesity (O)	O →HIC↓	Obese	PubMed
		Metabolic parameter: fasting insulin (fI)	fI↑→HIC↓		
Rossel et al. (1983)	Index (basal)	Hyperinsulinemia(I)	I↑→HIC↓	Obese	PubMed
Semnani Azad et al. (2019)	Index (OGTT)	Ethnicity: non-European (nE) vs European(E)	HIC_{nE} ↓wrt HIC_{E}	Adult at high risk for T2DM (Cohort)	PubMed
		Anthropometric parameter: waist circumference (wc)	wc↑→HIC↓		
		Cell count: White cell (WC)	WC↑→HIC↓		
		Enzyme: alanine aminotransferase (AAt)	AAt↑→HIC↓		
		sex	No effect		
		age	No effect		
		Physical activity	No effect		
		smoking	No effect		
		Diabetes: FH of T2DM	No effect		
		Obesity (O)	O→IC↓		
		FADN	FADN→IC↓		
		Inflammation (Infl)	Infl↑→IC↓		
Straznicky et al. (2015)	(EGHIC)	Neurotransmitter: arterial norepinephrine (aNE)	[aNE] →IC↓	Obese individuals with metabolic syndrome	PubMed
		sex	No effect		
		Vascular variable: arterial stiffness (AI)	AI ↑→IC↓		
		Metabolic parameter: insulin sensitivity (S_I)	S_I ↑→IC↑		
		Vascular variable: cardiac output (CO)	CO↑→IC↑		
Straznicky et al. (2015)	(EGHIC) Index (fasting)	Diet (D)	D→IC↑	Weight Loss individuals	PubMed
		Diet (D)	D→HIC↑		
		Neurotransmitter: arterial norepinephrine (aNE)	[aNE] →IC↓		
		Vascular variable: Vascular Resistance (VR)	VR↓→IC↑		

		Diabetes: Glucose tolerance (GT)	GT↑→IC↑		
Tosi et al. (2020)	(EGHIC)	Polycystic Ovary Syndrome (PCOS)	PCOS →IC↓	Women with PCOS (NGT)	PubMed
		Adipose tissue: Body fat (BF)	BF↑→IC↓		
		Metabolic parameter: insulin secretion (Isec)	Isec↑→IC↓		
		Hormone: androgen (An)	An↑→IC↓		
Tricò et al. (2020)	Model (OGTT)	Liver fat (IHL)	IHL↑→EIC↓	Youth obese (NAFLD cohort)	PubMed
		NAFLD	NAFLD→EIC↓		
Tschritter et al. (2003)	Model (EGHIC) Index (OGTT) (HGC)	Treatment: Pro12Ala	Pro12Ala →IC↑ FFA↓→IC↑	IGT, NGT	PubMed
Tuominen et al. (1997)	(EGHIC)	Physical activity (Pa)	Pa→IC↑	Healthy and T1DM	PubMed
Tura et al. (2020)	Index (IVGTT)	2°Isec phase (2Ip)	2Ip↑→IC↓	pGDM to T2DM	PubMed
Utzschneider et al. (2019)		NAFLD	No effect on ExIC end HIC	No diabetic, NAFLD	PubMed
		Liver fat (IHL)	IHL no effect on ExIC end HIC		
		Metabolic parameter: Insulin sensitivity (S_I)	S_I ↑→HIC↑		
		Protein: Adiponectin (A)	A↑→IC↑		
		Adipose tissue: Body Fat (BF)	BF↑→**Ex**IC↑		
		Adipose tissue: Subcutaneous fat (SF)	SF↑→**Ex**IC↑		
		Anthropometric parameter: Body mass index (BMI)	NO effect on ExHIC		
		Adipose tissue: Intra-abdominal Fat	NO effect on ExHIC		
		Liver fat	NO effect on ExHIC		
		rate of glucose disposal (R_d)	NO effect on ExHIC		
		Protein: Adiponectin	NO effect on ExHIC		
		NEFA	NO effect on ExHIC		
Van Gaal et al. (1991)	Index (OGTT)	Anthropometric parameter: waist	WHC↑→HIC↓		PubMed

		to hip circumference (WHC)			
		Hormone: sex hormone binding globulin (SHBG)	SHBG↑→HIC↓		
		Hormone: Androgen (An)	An↑→HIC↓		
Viskochi et al. (1985)		Physical activity (Pa) + Metformin (M)	Pa+M →IC↑	Adult prediabetes	PubMed
Viskochi et al. (2019)	Index (OGTT)	sedentarism (S)	S↑→HIC↓	Healthy adults	PubMed
Xiao et al. (2016)		Diet: fat ingestion (MU, PU, S)	MU↑→IC↓ PU↑→IC↓ S↑→IC↓	Overweight and obese, non-diabetic	PubMed

Legend: ExIC: extrahepatic insulin clearance, HIC: hepatic insulin clearance, IC: extrahepatic and hepatic insulin clearance; EGHIC: euglycemic hyper insulinemic clamp; HGC: Hyperglycaemic clamp; IVGTT: intravenous glucose tolerance test; FSIVGTT: frequently sample intravenous glucose tolerance test; t-m. FSIVGTT: tolbutamide-modified FSIVGTT; OGTT: oral glucose tolerance test; OMTT: oral meal tolerance test; IM-IVGTT: insulin modified intravenous glucose tolerance test.

Chapter 5

Methods

5.1 Subjects

A population of 78 women with previous GDM (pGDM) was analysed early postpartum (4–6 months after delivery) and at the end of a follow-up period (from 1 to 5 years). During the follow-up period, some pGDM developed T2DM, thus they were classified in two groups: those progressing to diabetes (progressors, PROG, n=19) and those not progressing to diabetes (non-progressors, NONPROG, n=59).

5.2 Metabolic test

All women underwent an insulin modified intravenous glucose tolerance test (IM-IVGTT) both early postpartum and at the end of the follow-up period. Glucose was injected at time 0-0.5 min (300 mg/kg) and insulin (0.03 IU/kg) was infused intravenously at time 20 for 5 min. Venous blood samples were collected at fasting and frequently for 180 min after glucose injection (at 3, 4, 5, 6, 8, 10, 14, 19, 22, 27, 30, 35, 40, 50, 70, 100, 140, 180 min) for the measurement of glucose ($mmol \cdot L^{-1}$), insulin ($pmol \cdot L^{-1}$) and C-peptide ($pmol \cdot L^{-1}$) plasma concentrations.

5.3 Feature extraction

The original dataset was filtered out from glucose, C-peptide and insulin measurements; redundant data were discarded from the analysis. Thus, the final dataset was composed of 13 features.

The features were age (age), body mass index (BMI), first phase mean of insulin clearance (CL-mean_0-10), second phase mean of insulin clearance (CL-mean_10-180), insulin sensitivity (S_I), basal insulin effect of glucose effectiveness (BIE), glucose effect at zero insulin of glucose effectiveness (GEZI) and finally, disposition index (Disp Index). Moreover, rate of glucose disappearance before and after insulin injection (KG (1) and KG (2) respectively), first phase insulin secretion (F1c), basal secretion rate (BSR) and distribution volume of glucose (DIST VOL) were included in the analysis. The name, the acronym, the unit of measure and the method of assessment of the features were reported in **Table 3**.

CL-mean_0-10 and CL-mean_10-100 were obtained by the ratio between the area under the curve (AUC) of insulin secretion rate (ISR (t)) and plasma insulin (I(t)) in the 0-10 min interval and in 10-

180 min of the IM-IVGTT, respectively. They are expressed as ($L \cdot min^{-1}$) whereas ISR(t) as ($pmol \cdot min^{-1}$).

$$\mathrm{CL-mean\_0-10} = \frac{\int_0^{10} ISR(t)dt}{\int_0^{10} I(t)dt} \quad (5)$$

$$\mathrm{CL-mean\_10-100} = \frac{\int_{10}^{180} ISR(t)dt}{\int_{10}^{180} I(t)dt} \quad (6)$$

ISR is equal to the C-peptide secretion rate (CPSR(t)), being C-peptide co-secreted with insulin in equimolar quantity but not extracted by the liver significantly.

CPSR(t) was computed according to Van Cauter et al. [21] by deconvolution from plasma C-peptide concentration using individualized C-peptide kinetic parameters computed for each woman considering her anthropometric characteristics.

Metabolic parameters such as S_I , S_G were assessed by the minimal model analysis of IM-IVGTT data [23] described in the following section, while metabolic parameters accounting for beta-cell function such as the acute insulin response (AIR) and the acute C-peptide response (ACPR) were computed as the mean of suprabasal insulin and C-peptide curve, respectively in the time interval 3–8 min during the IM-IVGTT test.

The metabolic parameter accounting for both S_I and AIR called disposition index (Disp Index) was computed according to Kahn et al. [24] as follows:

$$\mathrm{Disp\ Index} = \mathrm{SI} \cdot \mathrm{AIR} \quad (7)$$

5.3.1 Minimal Model of glucose kinetics (IVGTT)

Glucose kinetics is described using a glucose compartment and an insulin remote compartment (**Figure 11**). Connections between compartments represent matter flows (continuous line) and control signals (dotted line).

The mathematical equations are:

$$\begin{cases} \dfrac{\mathrm{dG(t)}}{\mathrm{dt}} = \mathrm{NHGB}\,(t) - \mathrm{KG}\,(t) \\ \dfrac{dI'(t)}{dt} = -k_3 I'(t) + k_2(I(t) - I_b) \\ G(0^+) = G_b + \dfrac{D}{DIST\ VOL} \\ I'(O^+) = 0 \end{cases} \tag{8}$$

$$NHGB(t) = NHGB_0 - (k_5 + k_6 I'(t))G(t) \tag{9}$$

$$NHGB_0 = (\,k_5 + K_1)G_b \tag{10}$$

$$KG\,(t) = (k_1 + k_4 I'(t))G(t) \tag{11}$$

G(t) is glucose concentration, G_b is glucose basal value; D is the injected glucose dose; DIST VOL is the distribution volume of the glucose, NHGB(t) is the net hepatic glucose balance; $NHGB_0$ is the net hepatic glucose balance at zero glucose, KG (t) is the disappearance rate of glucose in plasma; I'(t) is the remote insulin representing the decay of insulin action on glucose disappearance, I(t) is the plasma insulin concentration and I_b is the insulin basal value [25] (**Figure 12**).

$$\begin{cases} \dfrac{\mathrm{dG(t)}}{\mathrm{dt}} = -[(\mathrm{k_5} + \mathrm{k_1}) + (\mathrm{k_6} + \mathrm{k_4})\,I'(t)]G(t) + (\mathrm{k_1} + \mathrm{k_5})Gb \\ \dfrac{dI'(t)}{dt} = -k_3 I'(t) + k_2(I(t) - I_b) \\ G(0^+) = G_b + \dfrac{D}{DIST\ VOL} \\ I'(O^+) = 0 \end{cases} \tag{12}$$

From the new model parametrization

$$\begin{cases} p_1 = (\mathrm{k}_1 + \mathrm{k}_5) \\ p_3 = -k_3 I'(t) + k_2 (I(t) - I_b) \\ p_2 = k_3 \\ p_4 = DIST\ VOL \end{cases} \quad (13)$$

Considering

$$X(t) = (\mathrm{k}_6 + \mathrm{k}_4)\, I'(t)$$

Formulation of minimal model of glucose kinetic

$$\begin{cases} \dfrac{\mathrm{dG(t)}}{\mathrm{dt}} = -[p_1 + X(t)]\, G(t) + p_1 G_b \\ \dfrac{dX(t)}{dt} = -p_2 X(t) + p_3 (I(t) - I_b) \\ G(0^+) = G_b + \dfrac{D}{DIST\ VOL} \\ X(O^+) = 0 \end{cases} \quad (14)$$

Estimation of glucose effectiveness (S_G) and insulin sensitivity (S_I)

$$S_G = - \left. \frac{\partial \left[\frac{dG(t)}{dt}\right]}{\partial\, \mathrm{G(t)}} \right| b \quad (\mathrm{min}^{-1}) \quad (15)$$

$$S_G = [p_1 + X(t)]_b = p_1 = (\mathrm{k}_1 + \mathrm{k}_5)$$

$$S_I = -\left.\frac{\partial S_G}{\partial \mathrm{I(t)}}\right| b = -\left.\frac{\partial^2 \left[\frac{dG(t)}{dt}\right]}{\partial \mathrm{G(t)}\,\partial \mathrm{I(t)}}\right| b \qquad (\mathrm{min^{-1}/\ (\mu U \cdot mL^{-1})}) \quad (16)$$

$$S_I = -\left.\frac{\partial^2 \{[p_1 + X(t)]\, G(t) + p_1 G_b\}}{\partial \mathrm{G(t)}\,\partial \mathrm{I(t)}}\right| \mathrm{b}$$

$$= -\left.\frac{\partial [p_1 + X(t)]}{\partial \mathrm{I(t)}}\right| \mathrm{b}$$

$$= -\left.\frac{\partial X(t)}{\partial \mathrm{I(t)}}\right| \mathrm{b}$$

$$= -\left.\frac{\partial (\mathrm{k_6 + k_4})\, I'(t)}{\partial \mathrm{I(t)}}\right| \mathrm{b}$$

$$= -\left.\frac{\partial \left[(\mathrm{k_6 + k_4}) \cdot \frac{\mathrm{k_2}}{\mathrm{k_3}} (I(t) - I_b)\right]}{\partial \mathrm{I(t)}}\right| \mathrm{b}$$

$$= (\mathrm{k_6} + \mathrm{k_4}) \cdot \frac{\mathrm{k_2}}{\mathrm{k_3}}$$

$$= \frac{p_3}{p_2}$$

$$S_G = BIE + GEZI \qquad (\mathrm{min^{-1}}) \quad (17)$$

$$BIE = S_I \cdot I_b$$

BIE accounts for the basal insulin effect, while GEZI for glucose effect at zero insulin.

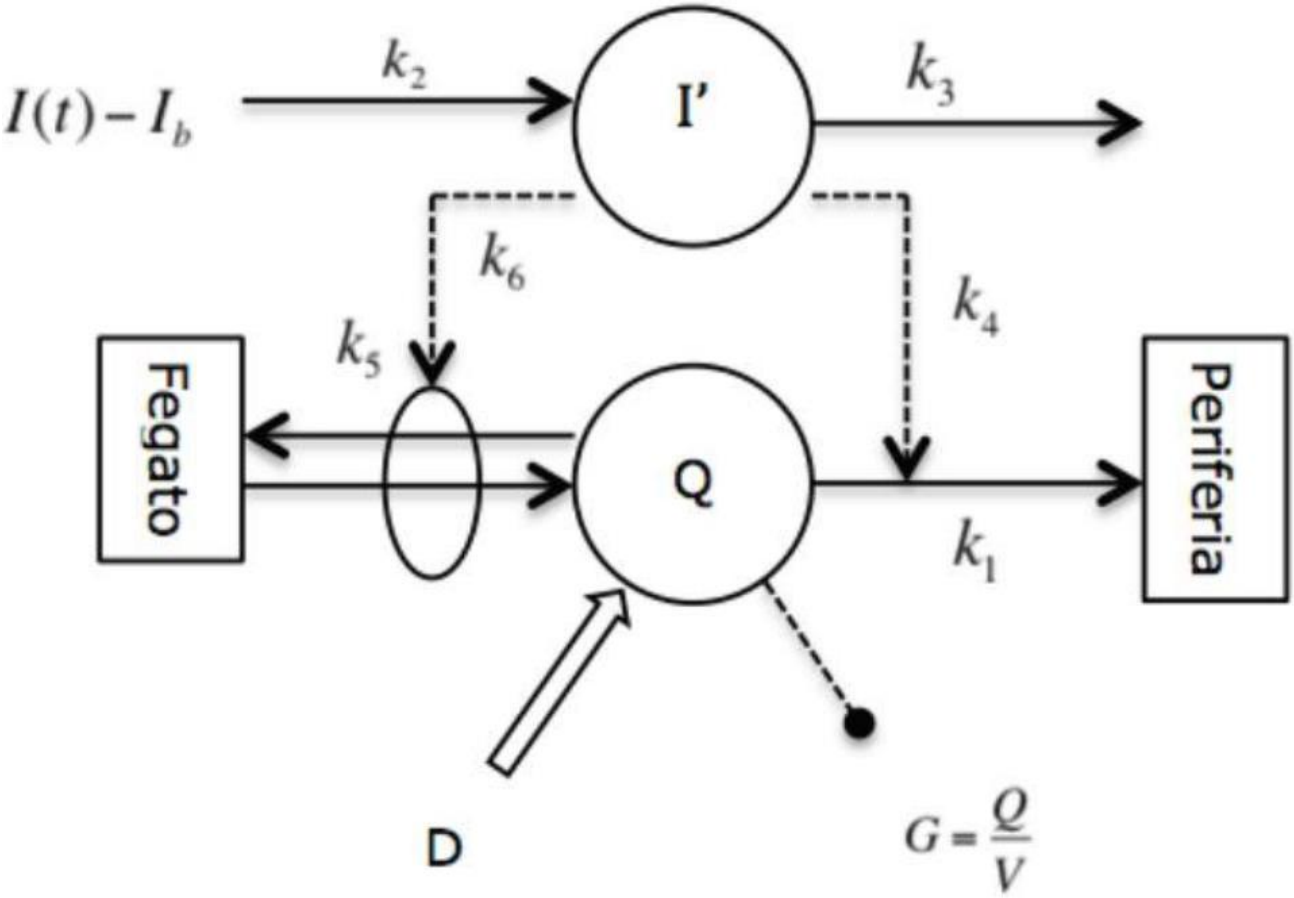


Figure 11. Minimal model of glucose kinetics.

5.6 Classification algorithm

The algorithms used for the data analysis were the decision tree and the logistic regression.

The decision tree algorithm predicted the classes PROG or NONPROG splitting the data into nodes by class purity. The tree parameters set inside the algorithm were the minimum number of instances in leaves equal to 2, the maximum number of subsets splits equal to 5 and the maximal tree depth to 100. Moreover, the “induce binary tree” and “stop when majority reaches 95%” were selected.

The logistic regression algorithm predicted the classes PROG or NONPROG with the LASSO regularization. The parameter set was the cost strength to C=1.

5.7 Feature selection and feature scoring

The features selection was based on the scoring and ranking of the information gain for the decision tree and of model based logistic regression for the logistic regression algorithm.

They can rank features through assignment of weights; feature weights for logistic regression correspond to the absolute value of coefficients of their linear model while for decision tree correspond to a measure of impurity. The top 10 features with the highest scores were included in the analysis.

5.8 Orange data mining and flowchart

The software used for the data analysis was the Orange (phyton based SW). Initially, two excel files were created: both contained the anthropometric data and derived data and the class PROG or NONPROG related to them, but one file included insulin clearance and the other one does not include insulin clearance as feature. Each dataset file was used two times for the analysis: once for the logistic regression classification algorithm and the other one for the decision tree algorithm. The first step in Orange was the loading of the excel file on the Orange file widget. The dataset was initially inspected in Data Table widget, after that, the class (PROG or NONPROG) was selected as the target variable in the Select Columns widget; then the data was split into two parts in Data Sampler widget: 70 % of the data (Data sample) was used for the training part, 30 % (Remaining Data) for the testing part. The Data Sampler was linked to the Rank widget; the Rank widget had the information gain as a default option while it did not have the logistic regression-based scoring, thus it must be given as input. Inside the Rank, the top 10 features with the highest score were selected. After that, the new dataset (Reduced Data), composed of the observations related to the 10 features could be visualized in the Data Table widget. The following step was the connection between the Reduced Data and the classification algorithm. Then, the classification algorithm widget and the Reduced Data were linked to the Test and Score widget for the evaluation of the model on the training data. In the Test and Score widget, the cross validation with 5 number of folds (stratified) was selected. Finally, the classification algorithm and the Remaining Data were linked to the Prediction widget for the validation of the classification model on the test data. The flowchart for each dataset and classification algorithm was reported in **Figure 12-13-14-15.**

Table 4. Features: name, acronym and unit of measure.

Name	Acronym	Unit of measure
body mass index	BMI	kg/m^2
age	age	years
mean of first phase insulin clearance	CL-mean_0-10	$L \cdot min^{-1}$
mean of second phase insulin clearance	CL-mean_10-180	$L \cdot min^{-1}$
insulin sensitivity	S_I	$min^{-1}/(\mu U \cdot mL^{-1}$
basal insulin effect of glucose effectiveness	BIE	min^{-1}
glucose effectiveness at zero insulin	GEZI	min^{-1}
disposition index	Disp Index	
disappearance rate of glucose before insulin injection	KG (1)	%/min
disappearance rate of glucose after insulin injection	KG (2)	%/min
first phase insulin secretion	F1c	mg/dL
basal secretion rate	BSR	pM/min
distribution volume of glucose	DIST VOL	L

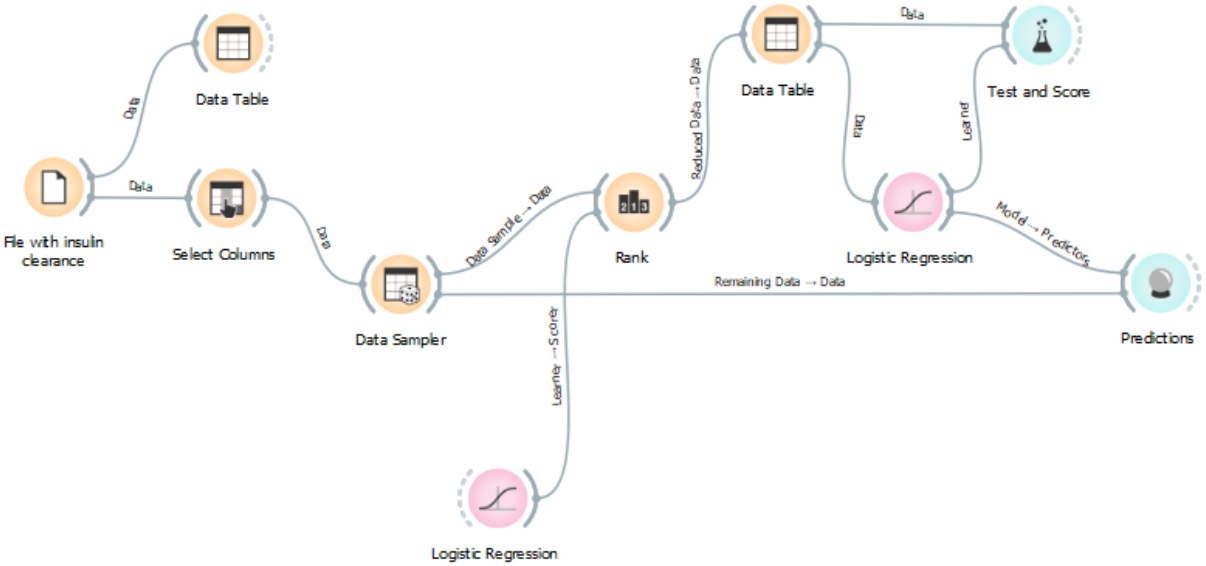


Figure 12. Flowchart of the data analysis performed by the logistic regression with insulin clearance.

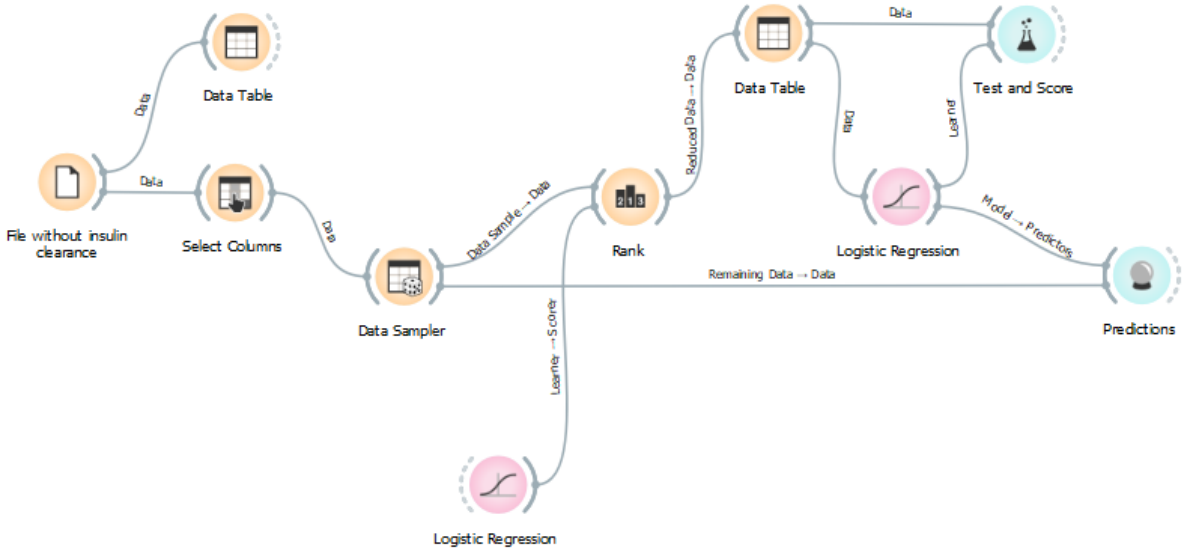


Figure 13. Flowchart of the data analysis performed by the logistic regression without insulin clearance.

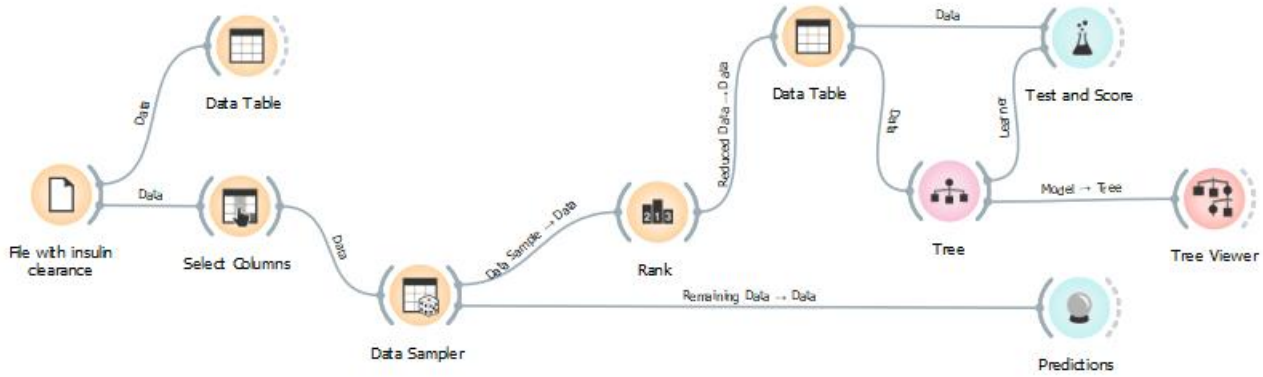


Figure 14. Flowchart of the data analysis performed by the decision tree with insulin clearance.

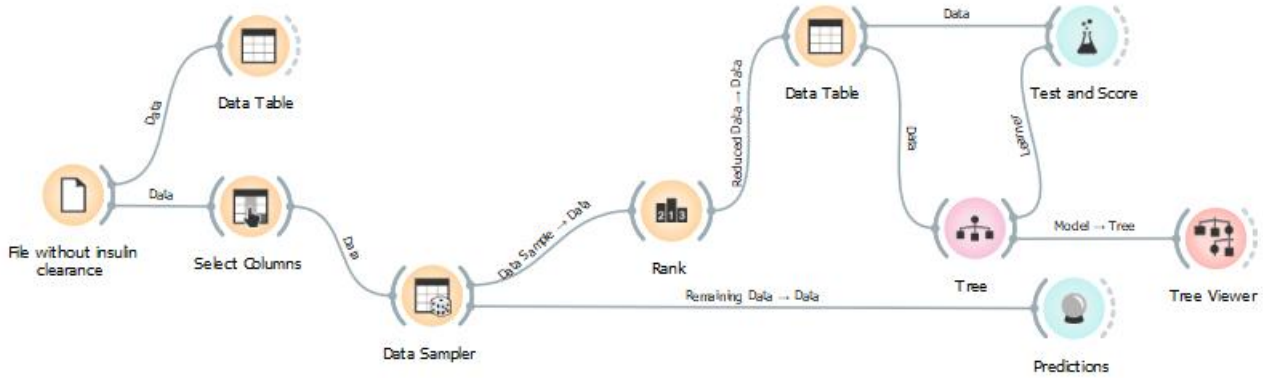


Figure 15. Flowchart of the data analysis performed by the decision tree without insulin clearance.

Chapter 6

Results

The central value, the dispersion, the minimum and the maximum value of the data were reported in **Figure 16**.

The ranking and scoring of the top 10 features for each dataset analysed were reported in **Figure 17-18-19-20-21-22**. The evaluation and score results for each dataset analysed with the specific classification algorithm were reported in **Figure 23-24.** The visualization of the decision trees was reported in **Figure 25-26**.

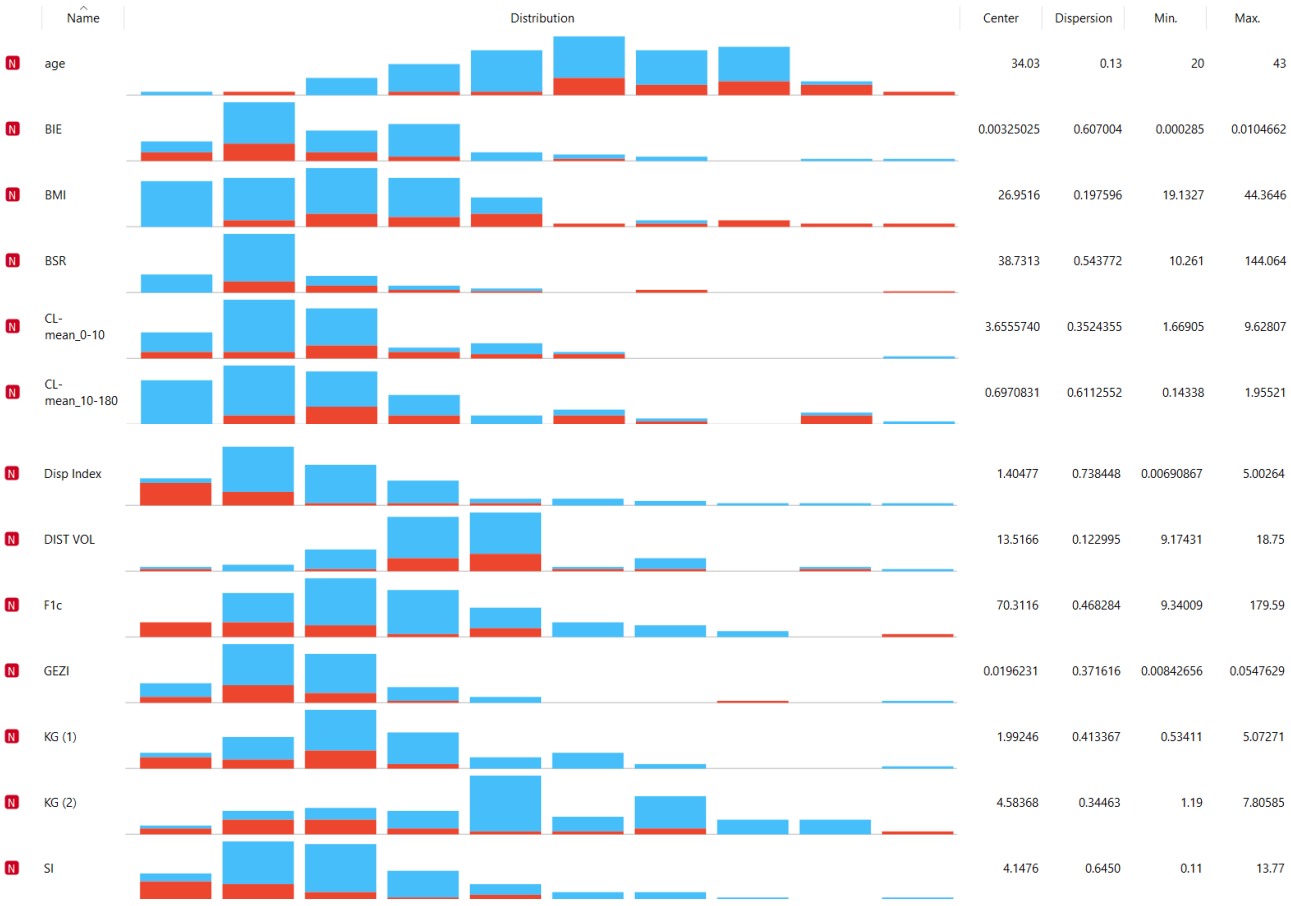
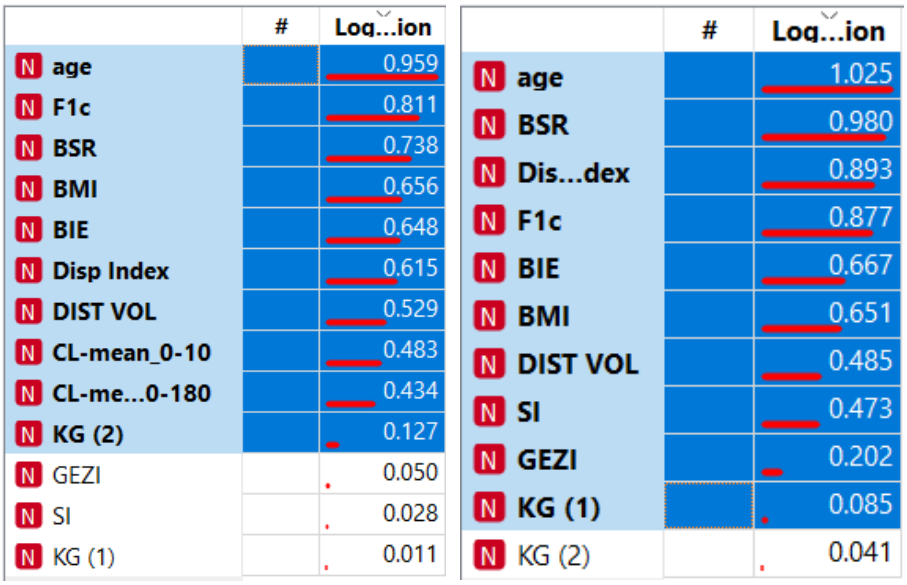


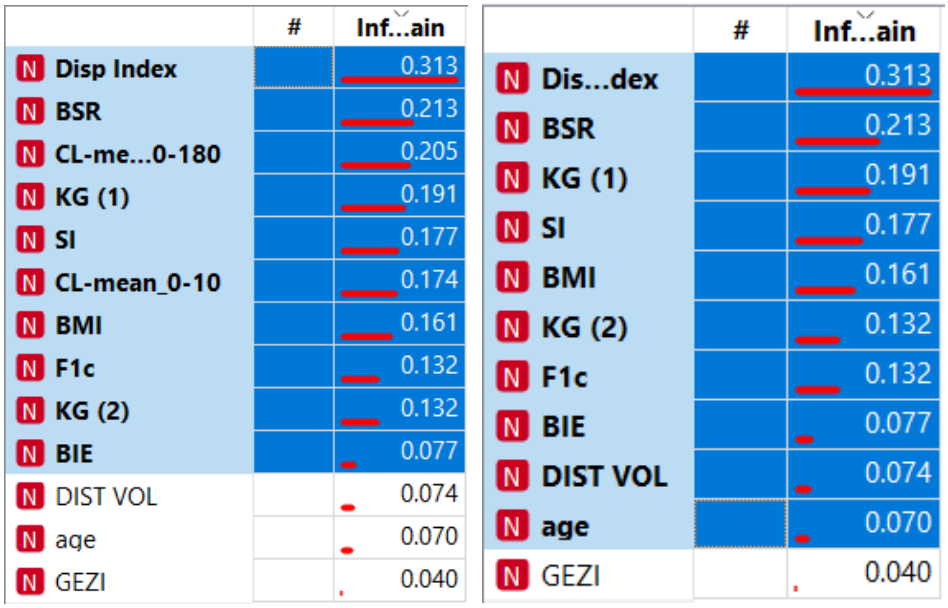
Figure 16. Data statistics. Name, distribution, center, dispersion, minimum and maximum value of the data were reported from left to right. Blu colour referred to NONPROG; red colour referred to PROG. "N" referred to numeric data.



	#	Log...ion
age		0.959
F1c		0.811
BSR		0.738
BMI		0.656
BIE		0.648
Disp Index		0.615
DIST VOL		0.529
CL-mean_0-10		0.483
CL-me...0-180		0.434
KG (2)		0.127
GEZI		0.050
SI		0.028
KG (1)		0.011

	#	Log...ion
age		1.025
BSR		0.980
Dis...dex		0.893
F1c		0.877
BIE		0.667
BMI		0.651
DIST VOL		0.485
SI		0.473
GEZI		0.202
KG (1)		0.085
KG (2)		0.041

Figure 17. Feature ranking and scoring for the logistic regression.



	#	Inf...ain
Disp Index		0.313
BSR		0.213
CL-me...0-180		0.205
KG (1)		0.191
SI		0.177
CL-mean_0-10		0.174
BMI		0.161
F1c		0.132
KG (2)		0.132
BIE		0.077
DIST VOL		0.074
age		0.070
GEZI		0.040

	#	Inf...ain
Dis...dex		0.313
BSR		0.213
KG (1)		0.191
SI		0.177
BMI		0.161
KG (2)		0.132
F1c		0.132
BIE		0.077
DIST VOL		0.074
age		0.070
GEZI		0.040

Figure 18. Feature ranking and scoring for the decision tree.

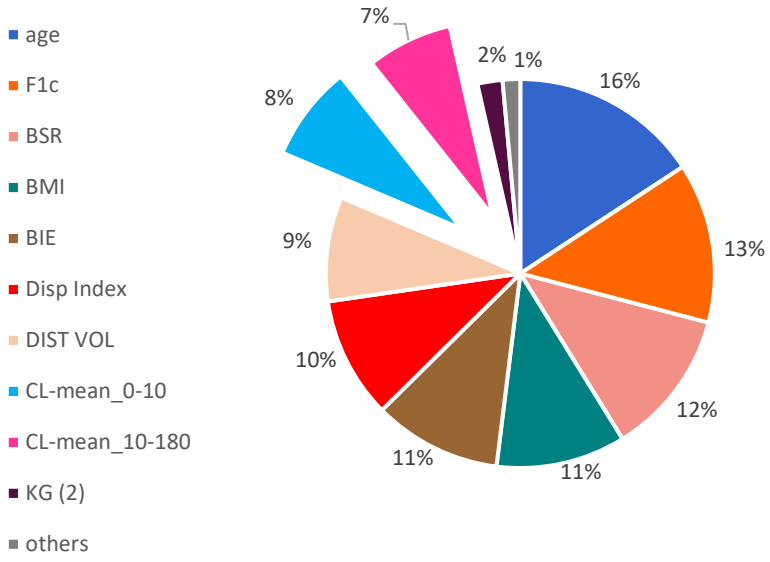


Figure 19. Top 10 features listed in descending order of percentage importance according to the logistic regression on the dataset with clearance.

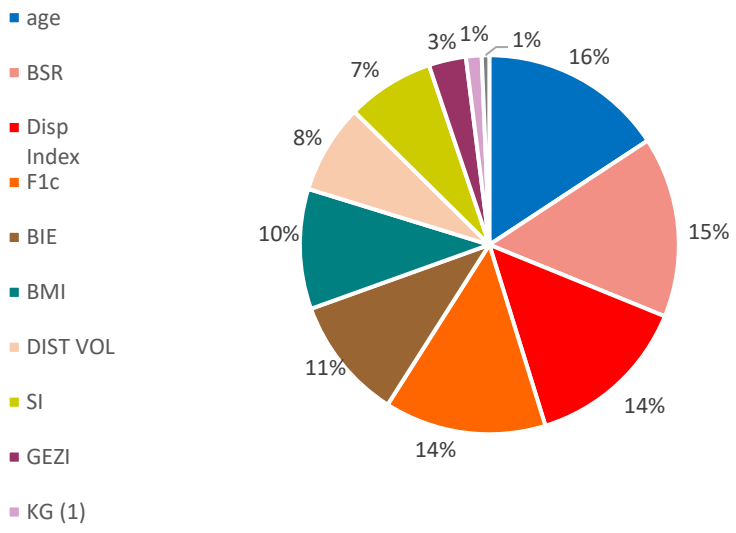


Figure 20. Top 10 features listed in descending order of percentage importance according to the logistic regression on the dataset without clearance.

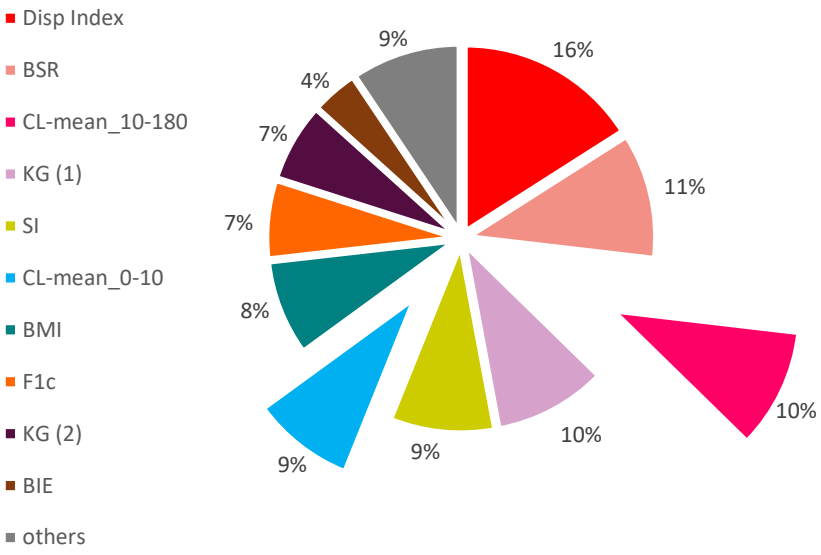


Figure 21. Top 10 features listed in descending order of percentage importance according to the decision tree on the dataset with clearance.

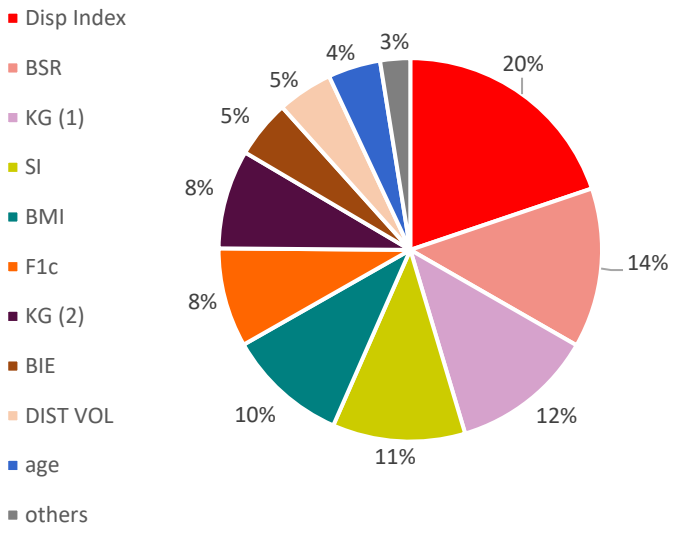


Figure 22. Top 10 features listed in descending order of percentage importance according to the decision tree on the dataset without clearance.

Evaluation Results

Model	AUC	CA	F1	Precision	Recall
Logistic Regression	0.792	0.745	0.732	0.727	0.745

Evaluation Results

Model	AUC	CA	F1	Precision	Recall
Logistic Regression	0.795	0.800	0.793	0.791	0.800

Figure 23. Evaluation results for the logistic regression in the dataset with insulin clearance in the upper level and without insulin clearance in the lower level.

Evaluation Results

Model	AUC	CA	F1	Precision	Recall
Tree	0.746	0.800	0.798	0.796	0.800

Evaluation Results

Model	AUC	CA	F1	Precision	Recall
Tree	0.730	0.818	0.814	0.812	0.818

Figure 24. Evaluation results for the decision tree in the dataset with insulin clearance in the upper level and without insulin clearance in the lower level.

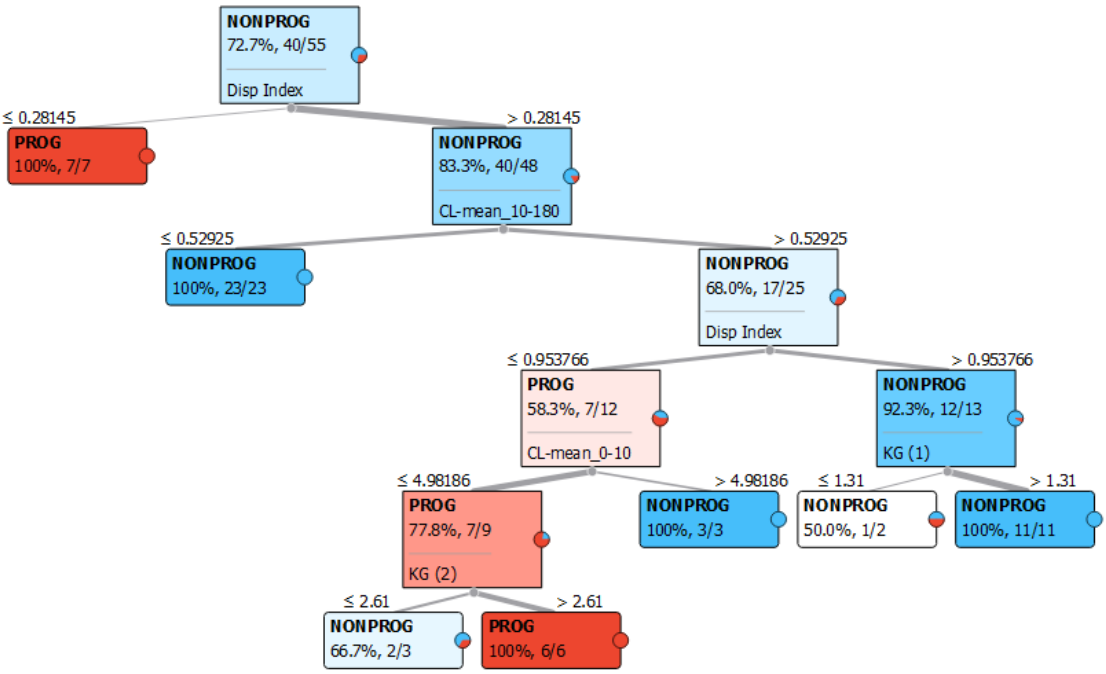


Figure 25. Decision Tree with insulin clearance. Gradation of red referred to PROG probability; Gradation of blue referred to NONPROG probability.

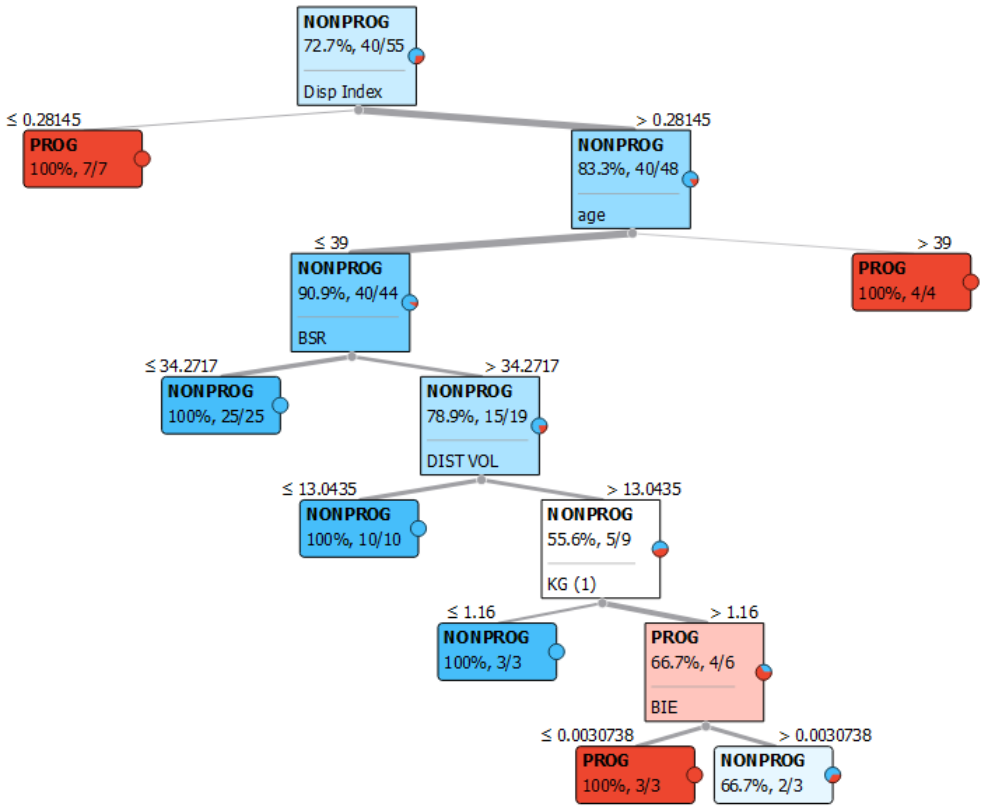


Figure 26. Decision tree without insulin clearance. Gradation of red referred to PROG probability; Gradation of blue referred to NONPROG probability.

Chapter 7

Discussion and conclusion

This study aimed to identify hidden patterns in a database constituted by features quantifying specific metabolic processes and subject characteristics related to a population of women with a history of gestational diabetes progressing to T2DM. To this scope, data mining techniques were applied and specifically, two feature scoring and classifiers were shown: the logistic regression and the decision tree. Interest was reserved to insulin clearance process as relevant feature for progression to T2DM, after reviewing the epidemiological determinants and phenomena and processes categories affecting insulin clearance.

Among the epidemiological determinants, the sex seemed to have no effect on insulin clearance in some studies despite Jensen at al. reported an increase of total and peripheral (but not splanchnic) insulin clearance in female; the increase of age led to a decrease of insulin clearance but not in Semnani-Azad et al. where there was no age effect [26]–[29]; the ethnicity affected insulin clearance comparing African American (AA) vs European American (EA), black vs white, Indians vs chinese and Caucasian, white-african-american vs white American and non-European vs European. In all the cited comparisons, insulin clearance was higher in the second mentioned ethnicity ([27], [30]–[35]). The ethnicity as determinant of insulin clearance seemed to be also hereditable; in fact, the heritability of insulin clearance, correlated with a possible genetic/epigentic factor, was provided by Piccinini et al. taking into consideration AA and EA from childhood to adult [30].

Among the chemical phenomena, the increase of TRG, FFA and HDL cholesterol, PAI_1, hepatic lipase, alanine aminotransferase, arterial norepinephrine, growth hormones, glucagon, androgens, sex hormones and white cell decreased insulin clearance, while the apoliprotein and adiponectin increased it ([36],[27], [29], [37]–[44]). GIP and GLP-1 seemed to not affect insulin clearance, despite one study reported an opposite effect of the two on insulin clearance: GIP decreased while GLP-1 increased it [42], [45], [46]. The possible influence of GIP and GLP-1 on insulin clearance was important since they were the hormones which were secreted in response to glucose ingestion and not in case of intravenous infusion, thus they could affect the measurement of insulin clearance performed during an OGTT or IVGTT. Thus, a possible incretin hormones action could explain the alterations of insulin clearance resulting from IVGTT and not from OGTT in pGDM people [47]. Moreover, a diet involving an increment of GIP secretion could explain better the impairment in

insulin clearance. This could be the case of AA people diet and the explanation of the decrement in insulin clearance assessed in AA with respect to European American [45].

Among the circulatory physiological phenomena, the increment of carotid intima media thickness, diastolic blood pressure, arterial stiffness and vascular resistance decreased insulin clearance while the increase of cardiac output increased it ([36], [40], [48]). Thus, the cardiovascular system was a main factor on insulin clearance.

Among the anthropometric parameters, the increase of waist circumference and waist to hip ratio led to a reduction of insulin clearance while the BMI seemed to not affect insulin clearance [49]. This finding could be correlated with the body fat determinants in which the increase of body fat seemed to decrease insulin clearance ([39], [43], [50], [51]). The increase of liver fat which could lead to hepatic steatosis, seemed to decrease insulin clearance despite Utzschneider et al. reported a no effect on it ([49], [52]–[55]). The possible explanation of the influence of body fat and liver fat on insulin clearance could be attributed to the CEACAM-1 disfunction in suppressing lipogenesis. In fact, a possible weigh loss could lead to a reduction of adiposity and consequently to an increase of hepatic insulin clearance [56].

Among the urinary physiological phenomena, Huber at al. reported the possibility to predict insulin clearance from the glomerular filtration rate, being the kidneys, the organ mainly involved into the process of peripheral insulin extraction. The increase of glomerular filtration rate seemed to increase insulin clearance since 99% of insulin which undergo filtration was reabsorbed to undergo clearance into the kidneys [20].

As discussed above, Piccinini et al. reported a possible genetic/epigenetic factor as explanation for the decrement of African American insulin clearance with respect to European American, visible not only in case of adults but also in childhood [30]. The possible genetic phenomena as determinant of insulin clearance was also investigated in other studies where some genes were analysed: Znt8 gene did not affect insulin clearance, while Pro12Ala gene could be a potential factor [37], [42].

Some pathological processes were involved into the impairment of insulin clearance like the hyperglycaemia, hyperinsulinemia, diabetes (T1DM, T2DM but not the case of a family history of T2DM), obesity, metabolic syndrome, metabolic abnormalities, systemic inflammation, NAFLD and PCOS [27], [29], [32], [36], [39], [43], [50], [54], [57]–[62]. Some metabolic parameters seemed to be involved in the impairment of insulin clearance like the decrement in S_I (or the increase of insulin

resistance), the rise of insulin secretion (Isec), S_G, Disp Index, fasting glucose (fG) and fasting insulin (fI) ([16], [36], [40], [42], [43], [46], [49], [59], [63]). Thus, a consideration comes out: to understand the mechanism underlining insulin clearance, all the processes of insulin should be considered.

Some drugs were studied for possible treatments of pathological states involving an impairment of insulin clearance such as metformin and thiazolidinediones which increased insulin clearance while glipizide and sulfonylurea decreased insulin clearance[64]–[66].

Behaviour such as a regular physical activity and low protein-low phosphorus diet could improve insulin clearance both in case of pathological state or physiological one, while sedentarism and fat ingestion (being them indistinctly monounsaturated, polyunsaturated and saturated) deteriorated insulin clearance [29], [67], [68].

After reviewing the determinants of insulin clearance as a complex not completely known mechanism, this study analysed the influence of insulin clearance on the prediction of T2DM.

About that, hidden patterns were identified in 2 databases having or not insulin clearance as feature. A population of women with a history of gestational diabetes (pGDM) was chosen for the analysis because particularly prone to progress toward T2DM. Thus, this study included pGDM progressing or not to T2DM (PROG or NONPROG).

The logistic regression feature scoring showed insulin clearance at the 8^{th} and 9^{th} position of the ranking with the scores equal to 0.483 for CL-media_0-10 and 0.434 for CL-media_10-180, while the decision tree feature scoring showed insulin clearance at the 3^{rd} and 6^{th} position with the scores equal to 0.205 for CL-media_10-180 and 0.174 for CL-media_0-10. The type and order of feature selected for the logistic regression prediction of the class PROG or NONPROG were different in the 2 datasets considered. In fact, both datasets showed Disp Index, age, F1c, BIE, BSR, BMI and DIST VOL from the 1^{st} to the 7^{th} position of the logistic regression ranking with a different order except age, BIE and DIST VOL; different features instead occupied the 8^{th} , the 9^{th} and the 10^{th} position: the dataset that considered insulin clearance as feature showed CL-media_0-10, CL-media_10-180 and KG (2) while the dataset that didn't considered insulin clearance as feature presented S_I, GEZI and KG (1). The evaluation of the logistic regression algorithm from the top 10 features selected for the 2 datasets in terms of area under the curve (AUC), classification accuracy (CA), F1, precision and recall showed worse results for the dataset that considered insulin clearance (AUC_{ins}= 0.792 vs

AUC_{notins}= 0.795, CA_{ins}= 0.745 vs CA_{notins}= 0.800, $F1_{ins}$= 0.732 vs $F1_{notins}$= 0.793, $Precision_{ins}$= 0.727 vs $Precision_{notins}$= 0.791, $Recall_{ins}$= 0.745 vs $Recall_{notins}$= 0.800).

In the decision tree, the features shared between the 2 datasets were Disp index, BSR, KG (1), SI, BMI, F1c, KG (2) and BIE with Disp Index and BSR occupied the 1st and the 2nd position for both datasets, while the features not shared were CL-media_10-180 and CL-media_0-10 for the dataset with insulin clearance considered and DIST VOL and age for the dataset with insulin clearance not considered. In addition, the splitting of the tree based on the feature importance showed CL-media_10-180 and CL-media_0-10 on the 2nd and the 4th splits of the decision tree with insulin clearance, while they were replaced by age and DIST VOL in the decision tree without insulin clearance.

The evaluation of the decision tree model in terms of AUC showed a better result for the dataset that considered insulin clearance (AUC_{ins}= 0.746 vs AUC_{notins}= 0.730) while in terms of CA, F1, Precision and Recall the mentioned dataset showed lower value. (CA_{ins}= 0.800 vs CA_{notins}= 0.818, $F1_{ins}$= 0.798 vs $F1_{notins}$= 0.814, $Precision_{ins}$= 0.796 vs $Precision_{notins}$= 0.812, $Recall_{ins}$= 0.800 vs $Recall_{notins}$= 0.818).

For the logistic regression analysis, CL-media_0-10 and CL-media_10-180 seemed to be more informative for the prediction of the PROG or NONPROG with respect to KG (2) (score = 0.127); for the decision tree analysis CL-media_10-180 resulted more informative with respect to KG (1) (score = 0.191) and S_I (score = 0.177), while CL-media_0-10 resulted less informative with respect to KG (1) and S_I.

CONCLUSION

In conclusion, the systematic review conducted to examine the epidemiological determinants as well as the determinants deriving from the phenomena and processes category that affected insulin clearance was fundamental to understand this complex, not completely known process which exerts an important role in diabetes. In particular, being insulin clearance involved in the top ten features in the logistic regression as well as in the decision tree, it resulted a relevant feature for the prediction of type 2 diabetes in a population of women with a history of gestational diabetes. The information obtained from the resultant pattern could be of interest for the diabetes pathophysiology.

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