Glucose Metabolism Derangements in Pediatric Age

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SECTION 1:

TYPE 1 DIABETES

SECTION 1 CHAPTER 1

EPIDEMIOLOGY AND PATHOGENESIS OF TYPE 1 DIABETES MELLITUS IN CHILDREN

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Key messages

- **Type 1 diabetes mellitus** (T1DM) is characterized by progressive destruction of insulin secreting β cells, leading to absolute insulin deficiency
- **Lifelong** treatment with insulin and intensive glucose management is necessary
- **The incidence** of T1DM is still rising in most parts of the world
- **Genetic susceptibility** in combination with environmental factors leads to an immune-mediated process and β cell destruction
- Diabetes pathogenesis is **heterogeneous**
- Prevention is not (yet) possible

Introduction

Type 1 diabetes mellitus (T1DM) is one of the most frequent chronic diseases in pediatric age. Current estimates of T1DM suggest that 1,110,100 young people live with T1DM (1). Each year around 128,900 new cases are diagnosed and, in most regions, the incidence of diabetes in the pediatric population is still rising. T1DM is characterized by progressive destruction of pancreatic insulin secreting β cells, leading to absolute insulin deficiency. This requires lifelong insulin treatment and intensive disease management. Without insulin, T1DM is lethal and without continuous intensive management of the disease, the risk of acute and chronic comorbidities is high (2). It is assumed that the health

expenses of diabetics are on average twice as much as those of non-diabetics, which adds a financial component to the burden of illness. Much progress has been made over the past 30 years, in particular in terms of treatment and understanding of the disease process. Yet, the cause of T1DM remains an enigma and prevention is not—yet—possible. In this chapter, a summary of T1DM epidemiology and the pathogenesis will be provided.

Epidemiology

Epidemiology is defined as the discipline that studies the distribution of health-related determinants states or events in specific populations and the use of these data to control health problems (3). The incidence rate represents the number of new cases of a disease over a period of time in relation to the population at risk for the disease. (3). The incidence rate represents the number of new cases of a disease within a time period in relation to the population at risk of the disease. Prevalence refers to the proportion of persons in a population with a disease (or other characteristic). When comparing reported incidence data of a disease—such as T1DM—it is important to consider the methodology which has been used to obtain the incidence data. The reliability of the numerator and denominator is a key element. For the numerator, a clear definition of the disease is required and all cases with the disease must be included.

For T1DM the criteria are well defined:

- Fasting glucose plasma value ≥ 7 mmol/l
- Classical symptoms and plasma glucose ≥ 11.1 mmol/l, or HbA1c (in NGSP certified lab) ≥ 6.5% (48 mmol/mol)
- At least one diabetes specific autoantibody
- Exclusion of monogenic diabetes, type 2 diabetes, or other forms of secondary diabetes

A person with T1DM will only survive when treated with insulin. In regions where children die before diagnosis, the numerator will be lower, leading to an underestimation of the incidence rate. When providing or analyzing incidence rates of T1DM, it is important to keep in mind where the data have been collected. A reported incidence rate can reflect local (for example hospital recruitment area), regional or national data. The data collection may or may not be exhaustive for that given population. Longitudinally collected data in a registry, for example in a hospital registry, can provide relevant information, when the data collection has

been consistent and within a well-defined recruitment area. Changes over time ('temporal trend') and geographical differences can be detected with registry data, provided that the registry includes mergeable data, a standardized dataset, rules for data collection, an inclusion principle (including long term outcome/clinical benchmarking for longitudinal studies), observations associated over time, and knowledge of outcome (4).

Although registries as surveillance systems have the advantage of providing fast (and continuous) information on temporal changes in the incidence of a disease, a disadvantage is the cost of maintaining them. This may be prohibitive. An alternative method of continuous surveillance is the capture-mark-recapture method, as suggested by LaPorte (5). This methodology was used (and is still used) to study the incidence rates of T1DM in Europe (EURODIAB substudy 2). It requires the inclusion of two independent data sources for the identification of newly diagnosed children with T1DM, to optimize data quality and comparability. The outcome of the EURODIAB substudy 2 confirmed a North-South gradient in Europe (2009–2013 incidence rates varied between 7.7/105 in Macedonia and 60.9/105 in Finland), with the exception of Sardinia. Over the 25 years of incidence data collection (1989–2013), this European project has recently shown that the estimated annual increase in incidence rate is 3.4% with a regional range of 0.5% (Spain) to 6.6% (Poland) (6). Although the suspected increase in the incidence of type 2 diabetes mellitus (T2DM) in the USA amongst Native Americans prompted the SEARCH study (SEARCH for diabetes in youth) (7,8), this study found an annual increase in the incidence of T1DM in that continent of 1.8% per year between 2002 and 2012 compared to an increase of 4.8% in T2DM amongst 0- to 19-year-olds. Another USA study, reporting on persons with private health care insurance between 2001 and 2015 (Clinformatics Data Mart Database), reported a 1.9% increase in the incidence of T1DM amongst young people (9). Incidence rates varied across the USA (2.4% to 3.8%) and, interestingly, a decrease in the incidence rate in adults was found (-1.3% between 2001 and 2015). This data set only reflects the population with private health insurance, and not the population as a whole, which may influence the outcome.

The DiaMond study reported that one of the lowest T1DM incidence rates is observed in China. Using the capture-mark-recapture method, Weng et al. reported the incidence of T1DM between 2010 and 2013 based on 10% of the population living in 13 areas across China (133×106 persons) (10). The incidence of T1DM among those aged 0–14 years was 1.93/105.

Importantly, while still among the lowest global incidence rates in those aged 0–14 years, the incidence has increased since 1985 (1985–1994 = 0.51/105; 1988–1996 = 0.59/105) and is an important consideration for adjusted health care planning and expenditure.

For a global overview of all forms of diabetes and estimates of absolute numbers of patients, every two years the International Diabetes Federation provides the IDF ATLAS (1). In the most recent update (2019) the high incidence of T1DM in Scandinavian countries was again confirmed with 62.3/105 children in Finland, 43.2/105 Sweden, 33.6/105 in Norway and 27/105 in Denmark, but it also reported high incidence rates of T1DM in the Middle East (Kuwait 41.7/105, Saudi Arabia 31.4/105, Qatar 28.4/105). Clearly, the genetic susceptibility and environmental factors that contribute to the development of T1DM need to be re-evaluated and cannot be only attributed to a North-South gradient. Disease burden expressed in absolute numbers shows that the largest number of children aged 0–14 years with T1DM live in India (95,600), followed by the USA (94,200), Brazil (51,500) and China (28,700). Consistent with the reports above, a recent systematic review confirmed a global increase in T1DM prevalence and incidence (11). The pace of this global increase demands responses at national and international levels to ensure that health care systems are equipped to provide lifesaving treatment and the necessary guidance to meet T1DM treatment targets. The increase in T1DM incidence also suggests that environmental factors that are as yet unidentified have a role in the pathogenesis of this multifactorial condition. Although much has been learned over the last decades, we are still faced with many gaps in our understanding. Continued surveillance of the incidence rates remains essential, as it may help to fill in some of these gaps.

Pathogenesis of type 1 diabetes: heterogeneity

Over the last four decades, more data on type 1 diabetes and its pathogenesis have been collected, confirming the heterogeneity of the disease pathway (12). So far, a genetic susceptibility, in combination with one or multiple environmental factor(s) seems to lead to the immune-mediated process with the subsequent progressive destruction of the pancreatic β cells. The temporal changes in incidence and the geographical variations confirm that environmental factors play an important role.

Figure 1. The pathogenesis of type 1 diabetes. Adapted from the Natural history of type 1 diabetes (NEJM, G Eisenbarth 1986).

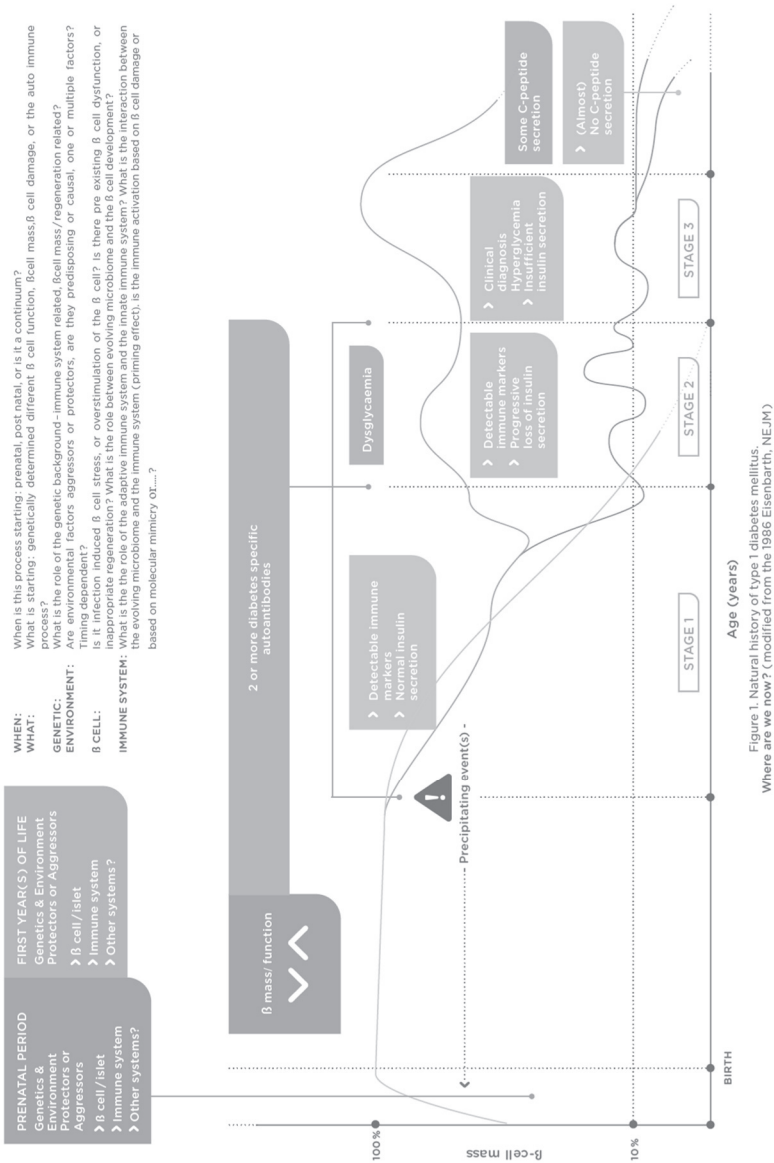

Figure 1. Natural history of type 1 diabetes mellitus.
Where are we now? (modified from the 1986 Eisenbarth, NEJM)

Although the endpoint is β cell destruction and insulin deficiency, many options, as listed in figure 1, are possible, confirming the complexity and heterogeneity of the disease. In the next paragraphs the key players, β cells, genetic predisposition, immune system and environmental factors are discussed.

β cells

Pancreatic cells, including β cells, develop from the endoderm through a complex interplay of signaling pathways (13). Throughout this development, a fine balance exists between proliferation and differentiation into the different islet cells. At birth, all populations of endocrine cells are formed and, shortly after birth, functional mature endocrine cells are grouped into islet structures. In the postnatal phase, β cells and their surrounding islet cells mature and become the key players in glucose homeostasis. The endocrine part of the pancreas is only a small part of the organ (<5%) compared to the exocrine pancreas, which excretes enzymes in the gut to support the digestion of food carbohydrates, lipids, and proteins. In humans, the expansion of β cells continues throughout the first years of life, reaching a stable mass at around the age of 5 years. Understanding the complex regulation of the expansion and maturation of β cells, ultimately determining β cell mass and function, may be essential to understand their fate in health or disease. Rodent data suggest that the maturation and development of glucose responsiveness of the β cells are determined by selective changes in micro-RNA profiles, occurring around the time of weaning (14). The micro-RNA molecules (miRNAs) are short non- coding RNAs, which support the regulation of gene expression post-transcriptionally. As such, they play a key role in a wide range of biological processes such as cell growth, proliferation, differentiation, development, and apoptosis and not only β cell function but also the regulation of the diverse immune cells. Their expression is influenced by environmental factors such as starvation, infections, etc., and this may point to a potential role in the pathogenesis of diabetes.

Shortly after the discovery of the islets and their role in diabetes, research into the transplantation of islets was initiated. One of the important lessons learned from the isolation and transplantation of islets and/or β cells was the detrimental impact of many environmental factors on the function of islets and β cells. Islets of Langerhans are part of a complex system, interacting continuously with neighboring cells and beyond. This interaction determines the function and viability of the cells within islets

(15). Loss of this interaction leads to changes in islet morphology and integrity, ultimately resulting in β cell apoptosis. The destruction of insulin secreting β cells is central in T1DM. Understanding their characteristics alone or in combination with the adjacent islet cells is essential when analyzing potential pathways leading to T1DM.

Immune-mediated process

Over 50 years ago, W. Gepts demonstrated the presence of "insulitis" in 15/22 pancreata of deceased patients with recent onset T1DM under 40 years of age, suggesting the presence of an immune reaction in association with insulin secreting islet cells. After this observation, diabetes specific autoantibodies were identified and considered to be involved in β cell death. Bottazzo was one of the first scientists to discuss whether β cell death was "homicide" or "suicide." In support of "homicide," he suggested that an environmental factor might stimulate the release of autoantigens by the β cell. These "self-antigens" would then be scavenged by macrophages and presented through their human leukocyte antigen (HLA) class II molecules (HLA-DR) to T helper cells. B lymphocytes would be activated by these T helper cells to secrete specific antibodies and activate cytotoxic T cells, ultimately leading to β cell death. In contrast, "suicide" would involve genetic or other predisposing characteristics of the β cell itself leading to its destruction. In the endocrine and the exocrine pancreatic tissue of newly onset patients' islet specific CD4+ and CD8+ T cells have been isolated with a predominance of CD8+ T cells. Major histocompatibility complexes (MHC) on antigen presenting cells bind the antigen peptides and present them to T cells by stimulating the activation of CD4+ and CD8+ T cell populations, necessary for disease induction. This implicates cytotoxic T cells (CTLs) in β cell destruction. Activated B lymphocytes, secreting disease specific antibodies, are detected as well. The process initiating this/these activation(s) remains uncertain. Potentially different pathways, such as infection, toxins, β cell stress due to high demand or even molecular mimicry should be considered. The immune system is trying to maintain a fundamental balance between host and environment. During evolution, the immune system has learned to cope with all kind of pathogens present in its vicinity. Through the innate immune system, a fast reaction can be mounted to clear the system of the potential pathogen. Different immune cells such as monocytes/macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells and humoral components are part of this fast-acting innate immune system. The immune cells express different pattern recognition receptors (PRRs) that can detect

danger via recognizing specific pathogen-associated molecular patterns (PAMPs). The humoral components include the circulating complement system proteins/components, and cytokines and chemokines secreted by innate immune cells along with various antimicrobial peptides. Toll-like receptors (TLRs) are part of these PRRs, and TLR mediated recognition of pathogens by innate immune cells plays a very important role in the induction of the pro-inflammatory immune response required to clear the system of the presumed pathogen or altered self-antigens. However, under special circumstances, this a-specific response can be uncontrolled or exaggerated leading to the development of severe inflammation and cell death (16). Not only can this first a-specific response of the innate immune system contribute to the process leading to β cell death, but the adaptive immune system may also play a role. Through the adaptive immune system, specific T and B cells can be selected out of a large repertoire and they can be activated after exposure to detected antigens, whether the antigens are altered self or microorganism related. Many environmental factors such as microorganisms and viral infections have been discussed in relation to T1DM, but during periods of stress or increased demand for insulin new hybrid peptides may be secreted by the stressed β cell (17,18). The secretion of these hybrid molecules may lead to this activation of the autoreactive T cells and initiate loss of self-tolerance and autoimmune β cell destruction. This contributes to the heterogeneity of the disease.

Nowadays, the emergence of cellular and humoral autoimmunity of islet cells is the distinguishing feature of the disease process. The number of diabetes specific autoantibodies has increased to at least five, including the ICA, (directed against cytoplasmic proteins in the beta cell), antibodies to glutamic acid decarboxylase (GAD- 65), insulin autoantibodies (IAA), IA-2A, protein tyrosine phosphatase as well as the antibody against Zinc Transporter 8 (ZnT8). In people with at least two autoantibodies, symptomatic disease develops within five years in 44% of those people; within ten years in 70%. Over a lifetime, 100% conversion to overt disease is expected. Based on these observations a new definition for T1DM has been proposed distinguishing three stages of the disease, not limited to the clinical onset (as mentioned in figure 1) (19):

- Stage 1: the presence of β cell autoimmunity as evidenced by the presence of two or more islet autoantibodies with normoglycemia and in absence of any symptoms,
- Stage 2: the presence of β cell autoimmunity with dysglycemia and no symptoms,
- Stage 3: onset of symptomatic disease.

Although this may help to identify persons at risk at an earlier stage, one should keep in mind that not all persons will develop the disease within the next five to ten years.

Both the innate immune system and the adaptive immune system are involved in this complex interplay leading to β cell death. The early detection of autoantibodies and careful follow-up of people with potential early stages of T1DM may help to better understand the ongoing process and subsequently facilitate the development of new drugs for the appropriate population to prevent further β cell destruction and overt disease.

Genetic predisposition

T1DM is a polygenic disease. Susceptibility and resistance to develop T1DM are first of all associated with genetic polymorphisms in the major histocompatibility complex within the HLA classes. The potential role of HLA variants in the susceptibility and resistance to infectious diseases is well described as they bind and present processed peptides derived from phagocytosis to the CD4+ T cells, triggering the specific adaptive immune response. The HLA haplotypes linked to the highest risk are the heterozygous HLA DR4-DQ8 and HLA DR3-DQ2. Differences between ethnicities in high risk and protective variants are observed. The protective variants may act through the inability to present the relevant antigens to the T helper cells. It is important to note the observation that the relationship between HLA class II genotypes and T1DM is mainly based on the development of islet cell autoantibodies or seroconversion. The time between detection of the autoantibodies and progression to clinical disease is not affected by these polymorphisms. Recent observations suggest a shift in genetic markers in newly diagnosed patients to less frequent HLA subtypes. More than fifty non-HLA genes are associated with type 1 diabetes as well. Some of these are involved in the immune reaction (INS, PTPN22 IL2RA, CTLA4, IFIH1, UBASH3A) whereas others contribute to the regulation of life/death or function of the β cells (ERBB3, PTPN2, CTSH, BACH2) (12). This suggests that β cell death may be the effect of very different processes, confirming the heterogeneity.

The environment

The incidence of T1DM continues to increase. This supports the implication of fast changing factors such as environmental factors as a

cause. Different observations support the importance of the environment. An interesting example is the huge increase in incidence in Poland over a short time span after the transition to Western Europe. Many changes in lifestyle occurred simultaneously. This has led to new hypotheses, for example the hygiene hypothesis which associates improved hygiene with an increase in diabetes incidence (in animal models a germ-free environment may increase the risk of developing diabetes) (20, 21). It may be linked to changes in the ongoing interaction (from birth onwards) between our microbiome and immune system. It may also reflect changes in nutrition, lifestyle, infections, etc. Another example is the change in incidence observed in migrating populations. When people migrate from a country of low incidence to a country with a high incidence, there tends to be an increase in T1DM incidence. Diabetes incidence shows a seasonal variation and a North-South gradient. Seasonal variation has been associated with viral infections—an increase in the number of new cases in association with viral infections has supported a viral cause of diabetes. Mumps, rubella and enteroviruses and more (recently even SARS-CoV2 virus infection) have been linked to the onset of the disease. However, only in a limited number of cases has the virus been detected in the pancreatic tissue of the newly diagnosed patient. Furthermore, the presence of a virus is not enough proof that it has caused the disease. It could be a bystander or accelerator of an ongoing process. Modes of delivery and infant feeding have been associated with the risk of diabetes. Children who have been vaginally delivered and/or breastfed show a reduced risk of developing diabetes. The exclusion of cow milk proteins appeared to have a positive impact in some populations, although this was not confirmed in the global TRIGR study (22). This may suggest a specific genetic background. One may also consider a role for the colonization and succession of the infant's microbiome and the interaction between the microbiome and the immune system. In zebrafish a conserved bacterial protein induces pancreatic β cell expansion, suggesting a role of the microbiome on the developing β cell mass (23). Weaning in rodents has demonstrated an effect on the β cell function (glucose responsiveness). Both observations suggest a role for interaction at the gut level early in life (14). Whether these factors play a role in the human remains to be investigated. Many more interesting and relevant observations have been reported, linking T1DM to climate, vitamin D, viral or other microorganism infections, lifestyle and dietary habits (20, 24). The timing and duration of exposure to these factors may determine their effect. Genetic predisposition or previous priming by earlier exposures can influence the outcome. These risk factors and more information on

when/how/in whom need to be collected to improve our understanding of the environmental factors and the disease process. T1DM is a heterogeneous disease with a single outcome: β cell destruction. Carefully designed studies are needed, with "out of the box thinking" to move forward and obtain a more complete picture of this complex disease.

Conclusion

The incidence of T1DM is increasing globally. This needs to be addressed at national and international levels to ensure that appropriate care can be provided. The pace of the increase suggests that environmental factors play a key role in this immune-mediated disease. In a person with a genetic predisposition, exposure to one or more environmental factor(s) can lead to immune-mediated β cell destruction, necessitating lifelong insulin treatment. Despite huge progress in our understanding of T1DM, many gaps in our knowledge persist and further research to understand the pathogenesis of this chronic disease is needed.

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SECTION 1 CHAPTER 2

DIABETIC KETOACIDOSIS: PROPER MANAGEMENT TO AVOID MISTAKES

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Diabetic ketoacidosis (DKA) is the most serious complication that can occur at the time of clinical onset of type 1 diabetes in children. The frequency of DKA is highly variable between countries, ranging from 80% to 12.8% (1), and despite the new progresses in diabetes management, no reduction through time has been reported. DKA is associated with increased morbidity and mortality (2), unsatisfactory long-term metabolic control (3), anomalies in brain imaging, low cognitive scores even after only one moderate or severe DKA episode (4), and high health care costs for DKA (5). This evidence should help as a call to promote early detection of diabetes in order to prevent DKA in children and adolescents worldwide (6). DKA is also a major problem in children and adolescents with established type 1 diabetes, with significant morbidity, mortality (7), and associated costs to patients, families, and health care systems. This chapter summarizes clinical and laboratory investigations and proper management to avoid errors that occur both at diagnosis of diabetes and in children and adolescents with established diabetes.

DKA definition and diagnostic criteria

Diabetic ketoacidosis (DKA) is combined with the absolute or relative insulin deficiency and increased levels of counter-regulatory hormones. This condition induces hypercatabolism (increased glycogenolysis and gluconeogenesis), reduced peripheral use of glucose, increased lipolysis and ketogenesis with hyperglycemia and consequent osmotic diuresis and dehydration, loss of electrolytes, hyperosmolarity, ketonemia and metabolic acidosis. The DKA clinical presentation is caratterized by serious dehydration (due to the imbalance between polyuria and polydipsia), tachycardia, tachypnea, Kussmaul breathing, acetone breath, nausea, vomiting, abdominal pain, blurred vision, confusion, drowsiness, progressive reduction of the level of consciousness and coma (2).

The biochemical criteria that define DKA are (2):

- Hyperglycemia (blood glucose > 11 mmol/L [>200 mg / dL])
- Venous pH <7.3 or serum bicarbonate <15 mmol/L
- Ketonemia (blood ß-hydroxybuyrate ≥3 mmol/L) or moderate or large ketonuria (8)

Summary of recommended procedures to manage DKA

Clinical and Biochemical assessments

- Confirmation of diagnosis of DKA (medical history, clinical examination, hyperglycemia, glycosuria, ketonuria)
- Evaluation of patient's weight, level of dehydration and level of consciousness (Glasgow coma scale)
- Biochemical monitoring (azotemia, EGA, glycemia, serum creatinine, electrolytes, osmolarity, CBC, glycosylated hemoglobin and, if available, blood beta hydroxybutyrate)
- ECG monitoring

Recommended procedures for treatment

Table 1 summarizes the main points for DKA management. Children with DKA should be managed by a specialized pediatric team properly trained for its management, composed of at least one senior pediatric diabetologist and supported by specialized nursery staff and other healthcare professionals involved in diabetes education, such as dietitians and

psychologists (2). The restoration of the circulating volume and the replacement of metabolic balance are the main goals of therapy.

Table 1. Summary of recommendations for DKA management

Hints for DKA management
• Manage the patient with severe DKA only if you have an expertise in treatment • Start with rehydration • Be careful not to overload liquids and do not use dilutions lower than hemiphysiological • Do not give insulin bolus • Do not be in a hurry to rebalance • Beware of blood sugar falling too fast • Monitor for signs of ketoacidosis complications and treat them right away • Vomiting in a patient with diabetes should first suggest a DKA onset before gastroenteritis

Rehydration

The initial use of rehydration reduces the dehydration-induced insulin resistance, decreases glycemia, avoiding too rapid falls both in glycemia and in plasma osmolarity, increases glucose renal excretion and dilutes counter-regulatory hormones (2). The addition of 5% glucose solution is suggested if blood glucose values fall to 250–300 mg/dL, 10% glucose solution is indicated if blood glucose falls rapidly (>90 mg/dL/h).

Insulin treatment

An intravenous infusion of regular insulin is recommended one hour after rehydration at a dosage from 0.1 to 0.025 U/kg/h, with wide variability in individual response (2). The blood glucose fall should not exceed 70–100 mg/dL/h. The solution with regular insulin (1 UI/cc or 0.5 UI/cc) must be replaced every 24 hours. As oral feeding is restarted and DKA is solved, subcutaneous insulin therapy should be started.

Potassium

The reintegration of potassium is necessary regardless of its blood concentration and should be initiated with insulin therapy, because children

with DKA have a total body potassium impairment on the order of 3-6 mmol/kg (2). If hyperkalemia is present, potassium supplementation should be delayed until diuresis resumes. Depending on potassium levels and the degree of ketoacidosis, a dose of 20 or 40 mEq/L of potassium replacement is indicated. The maximum dose should not be more than 0.5 mEq/kg/h (2).

Sodium bicarbonate

Bicarbonate supplementation should be reserved for cases of resistant and persistent acidosis (pH <7.0 and bicarbonates <5 mEq/L), because acidosis corrects spontaneously with rehydration alone and/or initiation of insulin therapy (2).

Complications of DKA

Cerebral edema is the most frequent and disabling complication of DKA, which in 0.6–5% of cases is associated with an unfavorable neurological outcome, while mortality ranges from 21 to 24% (10). The hyperglycemic hyperosmolar state is another serious complication of DKA (10); it is characterized by a very high concentration of glucose in the blood and hyperosmolarity without significant ketosis. Hypokalemia, hypocalcemia, hypomagnesemia, severe hypophosphatemia especially in the hyperglycemia hyperosmolar state, hypochloremic alkalosis, thrombosis, rhabdomyolysis and hypoglycemia are other life-threatening complications of DKA.

Cerebral edema, practical hints on the diagnosis and treatment

Early diagnosis, careful clinical monitoring and appropriate treatment are essential to prevent adverse outcomes. Signs and symptoms include the onset or worsening of headache following the start of the treatment, focal neurological deficits, reduction in O2 saturation; in addition, the Cushing triad may occur (bradycardia, rising blood pressure and Cheyne-Stokes breathing) (2). Bedside evaluation allows clinicians to define a diagnosis according to diagnostic criteria, major criteria and minor criteria (Table 1). The diagnosis of ketoacidosis can have a sensitivity of 92% and a false positivity rate of 4% in the case of presence two major criteria or one major and two minor criteria (13). The administration of cerebral edema is summarized in Table 2.

Table 2. Criteria for clinical diagnosis of cerebral oedema

Diagnostic criteria	Major criteria	Minor criteria
• Abnormal motor or verbal response to pain • Decorticate or decerebrate posture • Cranial nerve palsy (especially III, IV, and VI) • Abnormal neurogenic respiratory pattern (e.g., grunting, tachypnea, Cheyne-Stokes respiration, apneusis)	• Altered mentation, confusion, fluctuating level of consciousness • Sustained heart rate deceleration (decrease more than 20 beats per minute) not attributable to improved intravascular volume or sleep state • Age-inappropriate incontinence	• Vomiting • Headache • Lethargy or not easily arousable • Diastolic BP >90 mm Hg • Age <5 years

DKA at the clinical diagnosis of diabetes

The frequency of DKA at the diagnosis of type 1 diabetes shows a huge variation among regions (7) with differences ranging from 80% in the United Arab Emirates and Romania to 12.8% in Sweden, Canada and the Slovak Republic. A recent population-based study (7) reporting results of temporal trends across thirteen countries of three continents highlighted a high prevalence of children presenting with DKA at diagnosis and a slight increasing trend in the prevalence of DKA at diagnosis of type 1 diabetes during 2006–2016. The main risk factors associated with DKA at type 1 diabetes onset (1,7,9) are age under five years, lower socioeconomic status, ethnic minority status, and living in a country with a lower prevalence of type 1 diabetes.

DKA in children with established diabetes

Most cases of DKA occur in patients already diagnosed with diabetes (7). Around half of hospitalizations could be avoided by better adherence to self-

medication and improved outpatient treatment (7). Insulin omission and incorrect management of the insulin pump are the major causes of DKA in children with diabetes (2). The incidence of DKA in patients with diabetes shows a large variation ranging from 1.4 to 15 episodes per 100 patients (7). The main risk factors include peripubertal and adolescent age, high HbA1c, previous DKA episodes, female gender, high insulin dose, issues in family and school, eating disorders, psychiatric disorders, alcohol abuse, limited access to medical services, and gastroenteritis with many episodes of vomiting. Since almost all episodes of DKA are due to voluntary or non-voluntary omissions of insulin injections, the main causes of omission should be considered for the prevention of DKA:

- Incorrect insulin administration;
- Difficulties in taking their insulin injection in pump users when hyperglycemia and hyperketonemia occur;
- Pump technical issues due to bubbles in the cannula, tunnelling, pump blockage;
- Psychological causes of insulin omission (for example eating disorders in adolescents, the expression of unpleasant home situations, refusal or depression caused by the chronic disease);
- Difficulties in diabetes management while treating infections or other diseases.

Diabetes education is the cornerstone of DKA prevention. Intensifying structured diabetes education appears to be a way to prevent the recurrence of episodes, as it is effective in improving metabolic control (18). Educational training for patients and their families should be age-appropriate, guaranteed by quality and always available for children's needs (18).

How to avoid false steps during DKA management

DKA management requires specific consecutive steps to promote a rapid diagnosis and proper approach to treatment to avoid complications. The responsibility of treatment has to be shared with a senior pediatric diabetologist in order to prevent false steps. The most common mistakes in DKA management can be summarized as followed:

1. The underestimation of the symptoms of diabetes and DKA onset, which may lead to a delayed diagnosis.
2. The lack of communication of suspected DKA to a senior pediatric diabetologist. Children and adolescents with DKA diagnosis should

be sent to a pediatric intensive care unit or an expert center for diabetes and DKA treatment (2).

3. The treatment of the child with DKA without defining the DKA severity level (by diagnostic exams and laboratory data, pH level), which is fundamental in order to assess the risk of developing cerebral oedema in a short time (2).
4. Inaccurate or delayed treatment and a lack of monitoring. These are the basis of the unfavorable events, which lead to increased mortality, morbidity and management costs (find further indications in Table 3).
5. The omission of screening for DKA risk factors in children and adolescents with diabetes. This screening is important to prevent further DKA episodes (7,16): critical situations should be recognized and identified during ambulatory periodical visits in order to correct them as soon as possible and to support the child with diabetes and their family.
6. Discharging the patient, once recovered from DKA, without knowing the cause of insulin omission in children with established diabetes (psychological issues, problems with pumps, incorrect insulin administration).
7. Disregard of a focused education or re-education about diabetes management, after DKA recovery hospitalization (18). Intensification of structured diabetes education seems to be a way to prevent the recurrence of episodes, as it is effective in improving metabolic control (18).

Ultimately, understanding possible errors increases the level of accuracy in managing DKA and decreases the risk of complications.

Table 3. Recommended procedures and treatment of cerebral oedema

Management of Cerebral oedema
• Fluid restriction • Mannitol: 0.5–1 g / Kg i.v. infusion (20 min), it can be repeated if necessary, after 30 minutes • Hypertonic saline solution (3%) 2.5–5 mL/kg in 30 minutes (alternatively, if there was no response to mannitol) • Keep the patient in a semi-sitting position • Evaluate intubation and ventilation

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SECTION 1 CHAPTER 3

GLUCOSE MONITORING: SBGM, CGM, FGM

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Abbreviations:

SMBG	Self-Monitoring of Blood Glucose
CGMS	Continuous Glucose Monitoring System
FGM	Flash Glucose Monitoring
isCGM	Intermittently Scanned CGM
rtCGM	Real-time Continuous Glucose Monitoring
CV	Coefficient of glucose Variation
TAR	Time above range
TIR	Time in range
TBR	Time below range

The foundations of modern diabetes management can be traced back to the early attempts to quantify blood glucose during the mid-1800s, which involved measuring glucose levels in urine. In particular, the most important technological advancement of urinary glucose tests occurred in 1908, when Benedict developed a copper-based reaction system, which was used for over fifty years (1). Subsequently, in 1965, Ames developed the first test for the measurement of blood glucose by test strips, a semi-quantitative system called Dextrostix; five years later, the first glucose meter was introduced that could be used with the Dextrostix strips. With the introduction of the first digital display glucometer in 1980, named the Dextrometer, a progressive technological update was subsequently developed that led to devices that can be used directly by patients. Subsequently, various blood glucose meters were developed requiring smaller amounts of blood with lower costs and greater accuracy; self-monitoring of blood glucose (SMBG) has become the standard method for measuring blood sugar at home and has

assumed fundamental importance in the management of diabetes, together with the measurement of glycated hemoglobin (as a clinical control variable). In this way, since 1980, SMBG technologies showed significant improvements, until they became practically painless and recommended for all diabetic patients who are intensively insulin treated (2). In 1999, the U.S. Food and Drug Administration approved the first "professional" CGMS (Continuous Glucose Monitoring System): a blinded glucose monitoring system aimed at collecting glucose data over three days; the data were downloaded and retrospectively analyzed. Since the landmark Diabetes Control and Complications Trial (DCCT) (3) was published, the need to implement and optimize insulin therapy in order to delay the development of the micro and macrovascular complications of diabetes became evident. Glucose monitoring represented a fundamental step towards achieving this goal, even in children. Accurate measurement of blood glucose is essential in order to achieve the goal of good metabolic control—this was established by the Clinical Practice Consensus Guidelines 2018 of the International Society of Pediatric and Adolescent Diabetes (ISPAD) as a value of HbA1c <7% (53 mmol/mol) (or higher, up to 7.5%, 58 mmol/mol, in particular conditions such as hypoglycemia unawareness, inability to detect symptoms of hypos, lack of access to rapid analogue insulin) (4).

Glucose monitoring can be performed in two ways:

A) SMBG
B) Continuous Glucose Monitoring
   1. Flash Glucose Monitoring (FGM) or intermittently scanned CGM (isCGM)
   2. real-time Continuous Glucose Monitoring (rtCGM)

A) Self-Monitoring of Blood Glucose (SMBG):

Through the development of blood glucose tests, diabetics were able to immediately obtain news about their blood glucose levels and control their insulin therapy accordingly. This procedure must always be performed before main meals and several times a day. The recommendations for self-monitoring by the Italian Society of Pediatric Endocrinology and Diabetology, ISPED (5) highlighted the timing of SMBG as a crucial factor: they suggested capillary blood glucose checks should be executed before and two hours after meals and generally whenever hypo- or hyperglycemia is suspected and finally to evaluate post-correction blood glucose. Increased frequency of glucose self- monitoring by fingertip glucose is associated with lower HbA1c in patients with type 1 diabetes due to the improved insulin

dosing at meals and better capacity to rapidly rectify out-of-range glucose values (5). The frequency of BG determinations must be individualized for each child's needs; nevertheless, national (5) and international guidelines (4) recommend at least 4–6 to 10 determinations daily (more than 10 seems to offer no additional information). Substantial differences are sometimes observed between the patient's average blood glucose values and HbA1c levels. These discrepancies can be related to comorbidity that alters the average life of red blood cells, as in the case of hemoglobinopathies, or heritable alterations in the dynamics of the application processes. The more accurate an instrument is from an analytical point of view, the closer the value generated by it approaches the value generated by the reference instrument indicated by the manufacturer (accuracy) and the closer the values of subsequent measurements are between them (precision). Several external factors influence the functioning of a glucometer (6). Innovative systems use electrochemical or colorimetric signal detection principles, which allow correcting within certain limits the influence on glucose data of any external interfering factors, such as the hematocrit, thanks to sophisticated algorithms. Accuracy of blood glucose evaluation is crucial for appropriate decisions. The International Standards Organization (ISO) specified the standards for accuracy in ISO 15197:2013. These standards demand that ≥95% of glucose values measured by a blood glucose monitoring (BGM) system fall within 15 mg/dL as compared to a reference procedure for glucose concentrations <100 mg/dL, and within 15% for glucose concentrations ≥100 mg/dL. The ISO requires that three strip lots must be tested twice and that all lots conform to the accuracy criteria (7). Clinical accuracy, represented graphically by the Clarke-Parkes error grid, represents the probability of making a correct therapeutic decision based on the values obtained with the self-monitoring system. The grid comprises five risk zones that identify the overlap between a self-monitoring system and a reference standard. To meet ISO 2013 standards, ≥99% of all the measurements from a BGM system need to fall in zones A and B of the grid for patients with type 1 diabetes (7). The clinical accuracy of SMBG has been evaluated in silico. The impact of accuracy on glucose control and its variability can be predicted by mathematical models based on real information. The accuracy of the BGM system inversely correlates with HbA1c and influences the number of severe hypoglycemic events. Diabetic patients and their physicians should know the analytical and clinical accuracy of the blood glucose meter they are going to use. Systems operating according to ISO 15197:2013 criteria offer advantages in the management of the disease. Although SMBG has been demonstrated to improve glycemic control and quality of life in subjects with diabetes (6) it

has some limitations: first, it requires fingersticks to obtain blood samples, and it may be painful, especially for young children. This can make the procedure less acceptable for patients. Moreover, it only provides a single "point-in-time" measurement, with no information about glucose trends or rate of change of glucose levels. Thus, using SMBG data alone may result in inappropriate therapy decisions (8). Finally, SMBG is highly dependent on individual patient decisions. Consequently, SMBG is often not effective in detecting nocturnal and asymptomatic hypoglycemia (8). Real-time Continuous Glucose Monitoring (rtCGM) and Flash Glucose Monitoring (FGM), also called intermittently scanned CGM (isCGM) allow many of the limitations of SMBG to be bypassed (8).

B) Continuous Glucose Monitoring Systems (CGMSs)

CGM let patients to measure glucose levels and their variations without the need for finger pricking. It provides information about current glucose levels, glucose trends and rates of change, as well as warnings when glucose concentrations are not within the predetermined and modifiable target ranges and/or change too quickly, upwards or downwards (8). CGMSs are composed of three parts: a glucose sensor just beneath the skin that measures glucose levels in the subcutaneous interstitial space; a transmitter connected to the sensor able to transmit data (via wireless) to a receiver in order to visualize glucose values, trend arrows and glucose graphs; and a receiver that can be a hand-held device (even a smartphone) or an insulin pump. The majority of glucose sensors are transcutaneously self-inserted and have a lifetime of 6 to 14 days (8). Because CGM sensors measure glucose values in the interstitial fluid, changes in sensor readings typically lag 10–15 min behind changes in blood glucose (5,8,9). Therefore, the use of CGM allows the direct observation of real-time and daily glycemic excursions, with the possibility of intervention with immediate therapy decisions and/or lifestyle changes. CGM is also able to assess glucose variability and recognize patterns of hypo- and hyperglycemia (9). Appropriately factory calibrated, some CGM and FGM devices are now approved for real-time non- adjunctive (complete replacement of fingerstick monitoring) use in some settings, although depending on the accuracy and labelling of the technology used, some CGM values must still be confirmed by fingerstick BG monitoring (10). The revision of CGM data is a very helpful tool to teach patients about the effects of food, insulin timing, and exercise on glucose levels (10) and several studies have shown significant clinical benefits of CGM use in in diabetics regardless of the insulin delivery method (8–10). Not astonishingly, the frequency of sensor use predicts

HbA1c lowering; in fact, CGM should be considered in conjunction with HbA1c for glycemic status evaluation and therapy adjustment in all patients with type 1 diabetes (4,5,10). HbA1c is the result of average glucose levels over the last 2–3 months, but it shows no information about intra- and inter-daily glucose excursions and the acute complications such as hypo- and hyperglycemia (4,5,8), as the use of CGM does. The 2017 ATTD consensus conference provided important information about various aspects and parameters of glycemic status detected by CGM (8,9).

The expert panel identified 10 CGM metrics for clinical care:

1. Number of days CGM worn
2. Percentage of time CGM is active (recommend 70% of data from 14 days)
3. Mean glucose
4. Glucose Management Indicator (GMI)
5. Glucose variability, expressed as a coefficient of glucose variation (CV)
6. Time above range (TAR) Level 2: % of readings and time >250 mg/dL (>13.9 mmol/L)
7. Time above range (TAR) Level 1: % of readings and time between 181–250 mg/dL (10.1–13.9 mmol/L)
8. Time in range (TIR): % of readings and time between 70–180 mg/dL (3.9–10.0 mmol/L)
9. Time below range (TBR) Level 1: % of readings and time between 54–69 mg/dL (3.0–3.8 mmol/L)
10. Time below range (TBR) Level 2: % of readings and time <54 mg/dL (<3.0 mmol/L)

In order to streamline and standardize data interpretation, the consensus panel identified “time in range” as the metric that provides more actionable information as compared to A1C alone (9).

Some authors identified a relationship between TIR and A1C—some datasets from randomized trials were analyzed (11,12) and TIR values (70–180 mg/dL [3.9–10.0 mmol/L]) of 70% and 50% strongly corresponded with A1C values of approximately 6.7–7% (50–53 mmol/mol) and 7.9–8.3% (63–67 mmol/mol), respectively. An increase in TIR of 10% (2.4 h per day) corresponded to a decrease in A1C of approximately 0.5–0.8% (5.5–8.7 mmol/mol). However, it may be appropriate to customize this interval for some patients or situations (8,9). The information provided by TIR alone cannot be considered satisfactory if it does not also include data

on glucose variability, as it contributes to the risk for complications independently of HbA1c (8.9). It has been suggested that the coefficient of variation (the SD of the BG values divided by the mean) may be the most descriptive of overall excursions and that "stable" glucose values can be defined as having coefficients of variation <36% with greater values being "unstable" (8).

Flash Glucose Monitoring (FGM) or intermittently scanned CGM (isCGM)

In 2014, a new type of device was introduced: the FreeStyle Libre Flash Glucose Monitoring System (Abbot Diabetes Care, Alameda, CA). Calibration free, the available FGM is a disc (the glucose sensor) worn on the upper part of the arm for 14 days which is designed to substitute the recommended daily 4–10 painful finger-stick blood glucose tests for the self-management of diabetes (13,14). A reader device is swiped close to the sensor and the sensor transmits both an instantaneous glucose level and a graph showing glucose data for the preceding eight hours to the reader, producing real- time on-demand glucose data ("Flash" Glucose Monitoring). FGM uses a similar methodology to show continuous glucose measurements, but retrospectively at the time of checking through the scanning. A new model of FGM (Freestyle Libre 2) has been recently released with the possibility of alarms that send a signal when the set limits for hypo- and hyperglycemia have been exceeded, without showing the glucose value. In patients with type 1 and type 2 diabetes on insulin therapy, FGM use was linked to a significant reductions of HbA1c (in direct correlation with HbA1c levels at baseline (14), time below 70 mg/dL (14) and glucose variability (15)). Studies showed a higher treatment satisfaction with FGM in adults, children and adolescents (15). The accuracy of an FGM on the thigh is comparable to that of an FGM on the arm but is significantly reduced if on the abdomen (14).

Real-time Continuous Glucose Monitoring (rtCGM)

rtCGM uses minimally invasive devices that measure subcutaneous interstitial fluid glucose at one to five minute intervals. rtCGM provides near real-time glucose data. All rtCGM systems permit BG targets to be set so that an alarm will alert the wearer in case of a glucose value projected to fall below or rise above the target in 10 to 30 minutes, based on the rate of change of the interstitial glucose (13). Real-time CGM systems were originally approved for adjunctive use, meaning the sensor glucose results

needed to be verified by capillary SMBG before taking action. More recent devices show mean relative differences (MARD) of less than 10 % and, consequently, an accuracy similar to that of capillary BG (10).Some studies have shown that the use of rtCGM improves glycemic control and quality of life in both children and adults with type 1 diabetes treated with either continuous subcutaneous insulin infusion or multiple daily insulin injection therapy, improving HbA1c, shortening the time spent in hypoglycemia and hyperglycemia, and reducing moderate-to-severe hypoglycemia (4,7,8) CGM use has been associated with lower HbA1c compared to fingerstick BG measurements alone; greater improvements in HbA1c correlate with increasing hours per week of CGM use. Real-time CGM use with immediate corrections to keep glucose levels in range has been shown to more effectively improve glycemic control (7,8) The use of rtCGM, compared to FGM, highlighted a higher efficacy in reducing the time in hypoglycemia and, especially in cases of hypoglycemia unawareness, the CGM is recommended (9,10). This is related to the possibility of setting threshold or predictive alarms. Most of the published data on the use of rtCGM in multiple daily injection treatments (MDI) confirmed the reduction of the time spent in hypoglycemia and hyperglycemia, improving TIR, and ensuring the achievement of a better metabolic control expressed in terms of HbA1c (4,7–9). Furthermore, other studies did not report any difference in terms of reducing the time in hypoglycemia and metabolic control when the sensor is independently associated with the insulin pump or MDI, suggesting that the effectiveness of the treatment is more linked to the use of the sensor than to the type of insulin administration regimen used. There are no studies examining the efficacy of CGM in patients receiving MDI as a primary endpoint in pediatric age. Quite recently a few clinical trials on adult populations affected by T1D reported that the use of CGM results in a reduction in HbA1c ranging from 0.4 to 1.1%, a reduction of the time spent in hypoglycemia, and an increase in TIR (16). The benefit of using this device is directly related to the persistence and frequency of use (9,16). Current limitations of real-time CGM include economic and behavioral barriers and imperfect accuracy; moreover, discomfort with the wearability of some sensors may discourage routine use. Currently, these devices, while approved for pediatric use, are expensive and may not be available in many countries. Insurance coverage may also be limited. Pump and CGM technology is now so advanced that it is possible to regulate automatically insulin delivery based on CGM data using computerized algorithms. These are important steps toward an eventual true artificial pancreas system. Such devices reduce the risk of severe and moderate hypoglycemia, particularly overnight, and hold promise to reduce the burden of care and improve

glucose control (10). A new type of long-term implantable sensor for real-time use (Eversense, Senseonics Inc, Germantown, Maryland) in adults with type 1 diabetes is available as an alternative for transcutaneous CGM, with approval in Europe for up to six months. Implantable sensors are perhaps more manageable than standard transcutaneous CGMs since they are long lasting. However, the need for an invasive subcutaneous implant makes it less feasible in a pediatric population (10).

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SECTION 1 CHAPTER 4

MULTI-INJECTION INSULIN THERAPY, BIOSIMILARS AND NEW INSULINS IN TYPE 1 DIABETES

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Abbreviations:
pharmacokinetic (PK) profile, pharmacodynamic (PD) profile, fast-acting insulin aspart (Fiasp®), aspart insulin (IAsp), insulin degludec (IDeg), blood glucose (BG,) postprandial blood glucose (PPG)

Patients with type 1 diabetes (T1D) receive multi-injection insulin therapy. Long-acting basal insulins are used to manage fasting glucose, whereas rapid-acting insulins are typically used to control postprandial plasma glucose excursions.

Long-acting basal insulins

First-generation long-acting basal insulin analogs

The basal insulin analogs to date available are glargine U100, detemir, glargine U300, and degludec. Insulin glargine U100 (IGlar U100) is a long-acting insulin analog, extensively used as a basal insulin, with a good safety profile. After injection, cIGlar U100 precipitates in situ and this ensuring its effect for up to 24 hours in adults; although about 20 hours after injection a declining effect may be noted. Occasionally, a burning sensation may be experienced after glargine injection due to its acidic pH. This insulin is approved for children over two years of age. Detemir insulin is an insulin analog in which a fatty acid (myristic acid) is bound to the amino acid lysine at the B29 position. Because of this substitution, detemir is rapidly absorbed and binds to albumin in the blood and slowly dissociates from this complex. The time to action is between six and twenty-three hours when doses between 0.1 and 0.8 U/kg are given. In pediatric patients, detemir is sometimes administered twice daily. These two analogs reduced daily variability in absorption, resulted in a lower risk of hypoglycemia, and improved glycemic control compared with NPH insulin (1).

Second-generation long-acting basal insulin analogs

Insulin degludec (IDeg) is a long-acting basal analogue composed of multiesters accumulated in subcutaneous tissue. These multiesters are soluble and as a result monomers gradually separate, offering slow and continuous uptake. The mechanism of protraction that allows the prolonged release of insulin degludec is peculiar: in the pharmaceutical formulation, in the presence of phenol and zinc, degludec forms a soluble and steady dihexamer that reorganizes into multi-hexamer chains when phenol diffuses after injection, allowing a long stay in the injection site. With the slow diffusion of zinc, these multihexamer chains gradually unbind releasing monomers from the terminal ends (as the zinc ions of the terminal ends are exposed due to their T3 conformation). The action time of IDeg, approximately 42 hours, is significantly longer than IGlar U100.Degludec allows a more flexible timing of administration of basal insulin from day to day and can be mixed with short-acting insulins. This insulin is approved in children over one year of age. Compared with Detemir, IDeg achieved the same long-term glycemic control as disclosed by HbA1c with a significant reduction in fasting plasma glucose with a

30% lower basal insulin dose. Rates of hypoglycemic events were not statistically different between the two groups, whereas hyperglycemic events with ketosis were significantly lower in the IDeg group. Compared with insulin glargine U100, in the SWITCH 1 study, T1D affected adult patients with also risk factors for hypoglycemia, treatment with IDrg showed a lower incidence of total symptomatic hypoglycemic events (3). IGlar U300 is a new ultra-long-acting basal insulin. Thanks to its three times more concentrated formulation, when subcutaneously injected the microprecipitates of IGlar U300 have a longer residence time compared with IGlar U100 and its duration of action is >24 hours. The full glucose-lowering effect may not be apparent for at least the first three to five days of use. IGlar U300 provides glucose control comparable to IGlar U100, and it can be administered at any time of day. Hypoglycemia event rates do not differ between treatment groups, except for nocturnal hypoglycemia which is lower with IGlar U300 compared to IGlar U100. CGM studies in T1DM patients demonstrated lower glucose variability over 24h with IGlar U300 compared to IGlar U100. Basal insulin dose is 20% higher with IGlar U300 than with IGlar U100, probably because of local degradation of IGlar U300 at the injection site. IGlar U300 is approved above the age of eighteen years (2).

In conclusion, second-generation basal insulins (IGlar U300 and IDeg), compared with previous insulin analogs, have improved pharmacokinetic/pharmacodynamic profiles and have led to clinical benefits, primarily by reducing the nocturnal-hypoglycemia risk. Reducing the risk of hypoglycemia is an important treatment goal because it is the main obstacle to achieving good glycemic control.

Head-to-head comparison

Compared with the previous basal insulin analog IGlar U100, IGlar U300 that IDeg show steady and more protracted PK/PD ratio although there were conflicting results when the PK/PD profiles of the two second-generation basal insulins were compared at once even if it can reflect differences in study methods and analyses. Heise et al. (2017), in a Novo Nordisk initiated study carried out using a clamp, demonstrated lower day-to-day and within-day variability and a more stable glucose- lowering efficacy for IDeg versus IGlar U300 (0.4 U/kg, evening). Conversely, in a study by Bailey TS (2018) and co-workers, which was supported by Sanofi and conducted at the same research center as the former, smaller within-day variability was demonstrated with IGlar U300 compared to IDeg in adults with T1D. Insulin glargine 300 U/mL (0.4 U/kg, morning)

revealed a more stable pharmacodynamic profile and more even 24-h distribution than insulin degludec. It remains to be fully ascertained whether degludec and glargine U300 are equivalent concerning the glycemic control and the risk of hypoglycemia. In real-world clinical practice, the change of basal insulin therapy to either glargine U300 or degludec in adults with type 2 diabetes resulted in similar improvements in glycemic control and reductions in hypoglycemic events. Two randomized, controlled, head-to-head comparisons between the two insulin analogs in T2D are now available. The BRIGHT study in insulin-naive patients with uncontrolled type 2 diabetes revealed that the two basal insulins have similar effects on HbA1c improvement (primary outcome). Hypoglycemia incidence and rates were comparable with both insulins during the full study period but they were lower in favor of IGlar U300 during the titration period. In the CONCLUDE trial, 1609 patients affected by type 2 diabetes, were randomized to degludec 200 U/ml or glargine U300. During the maintenance period, HbA1c improved in the two groups with no significant difference in the rate of overall hypoglycemia (the primary endpoint of the study), while rates of nocturnal symptomatic and severe hypoglycemia (secondary endpoints) were lower with degludec than with glargine U300. The study design had to be modified because of the poor reliability of the glucometers initially used, particularly in the low blood glucose ranges, so the potential implications of these changes in the subsequent conduct of the trial cannot be excluded.

A multi-center, randomized, crossover study (Kobe Best Basal Insulin Study 2) is ongoing. This study will be the first trial to compare the effects of IDeg and IGlar U300 on day-to-day FPG variability in patients with type 1 diabetes and c-peptide negative (4).

Rapid-acting insulins

Three rapid-acting insulin analogs are currently available: insulin lispro (Humalog®, Lispro Sanofi®), insulin aspart (NovoRapid®) and insulin glulisine (Apidra®). Insulin aspart is approved for children above the age of one year, glulisine above six years old, while the age for lispro is not specified. The three rapid insulin analogs differ in the way that their molecular structure has been modified from human insulin and in the chemical composition of their formulations, but their pharmacokinetic (PK) and pharmacodynamic (PD) profiles are similar. These three insulins have similar efficacy and safety in the pediatric population. In comparison to regular insulin, they have a more rapid onset, shorter duration of action

and similar effect on blood glucose control in children and adolescents (HbA1c – 0.1%) while a decrease in hypoglycemic episodes per patient per month in adolescents has been reported, as well as more flexibility in daily lives. These analogs give a quicker effect than regular insulin when treating hyperglycemia. The three rapid insulin analogs should be administered before meals to reduce postprandial hyperglycemia and nocturnal hypoglycemia, and in exceptional cases can be given after food when needed, particularly in infants and toddlers (5).

Ultra-rapid-acting insulins

Fiasp® (fast-acting insulin aspart) is an insulin aspart in a new formulation with the purpose to be more similar to the physiologic release of prandial insulin than currently available fast-acting insulin products. Fiasp® is an insulin aspart in which two excipients (L-arginine and niacinamide) have been added. L-arginine is a stabilizing agent, while niacinamide accelerates the initial absorption phase after subcutaneous administration. Insulin concentration in the first thirty minutes is doubled or, when administered via CSII, tripled compared with insulin aspart (IAsp). Fiasp® has a faster onset and offset than IAsp and this gives better control of post-meal spikes in glucose and causes less hypoglycemia hours later. Fiasp® has been approved for patients above eighteen years, but the pharmacokinetic and pharmacodynamic results in adults have been preserved in children and adolescents. In the ONSET 1 trial, in adults with type 1 diabetes, lower postprandial blood glucose (PPG) and HbA1c was reached using Fiasp® compared with IAsp. The trend toward improved PPG control was attributable to both greater early suppression of endogenous glucose production and stimulation of glucose disappearance. Time spent in hypoglycemia was similar, except for a slight increase in postprandial hypoglycemia with Fiasp®. In the Onset 7 trial, in children and young persons (one to seventeen years), Fiasp® versus IAsp gave reduced HbA1c by 0.17%, with a comparable rate of severe or BG-confirmed hypoglycemia; PPG increment at 1 h was reduced. Fiasp® has to be taken up to two minutes before the start of a meal, with the option to be administered up to twenty minutes after starting the meal, and it is as effective as IAsp administered at mealtimes concerning HbA1c reduction and postprandial glucose values. In children, the administration of post-meal Fiasp® was less effective (but still non-inferior) than IAsp (ONSET 7) (6). Regarding the potential competitors for Fiasp®, BioChaperone Lispro® (BCLIS) is an ultra-rapid formulation of insulin lispro which contains the novel excipient BioChaperone BC222 (an oligosaccharide

modified with natural molecules) and citrate to accelerate the absorption of insulin lispro after subcutaneous administration. BCLIS has a faster absorption than insulin lispro formulation and significantly improved postprandial blood glucose compared with Lispro when administered at mealtime in people with type 1 diabetes. BioChaperone Lispro® was originally developed by Adocia together with Lilly, but this collaboration was terminated in January 2017 and the future remains unclear (7). Eli Lilly's new ultra-rapid insulin, LY900014, contains the excipients citrate and treprostinil. Citrate improves vascular permeability at the administration site, while treprostinil improves absorption of lispro through local vasodilation without measurable systemic exposure. In people with T1DM, ultra-rapid lispro was detected in the blood 8.8 min earlier than lispro with a twofold higher insulin exposure over the first 30 min. Time to onset of ultra-rapid lispro exposure occurred earlier than with Fiasp®. Ultra-rapid lispro also showed a greater early glucose-lowering effect, when compared with lispro. In meal tests, ultra-rapid lispro improved PPG control when it is administered immediately before a mixed meal (4).

Biosimilars

Compared to the originator, biosimilar insulin has to respect a consistency in the primary structure (amino acid sequence), in the secondary and tertiary structure (three-dimensional convolution configuration) and comparable quaternary structure (stable association of two or more identical or different molecule units) in order to allow the comparable formation of hexamers after insulin injection. The original product and biosimilar therefore will never be absolutely identical molecules; however, bioequivalence has been established through proper clinical trials and pharmacovigilance on reporting of any side effects observed (1). Long-acting biosimilar Abasaglar® is a biosimilar of insulin glargine and it is the first insulin biosimilar approved in the European Union. The ELEMENT 1 study showed Abasaglar® to have similar efficacy and a comparable safety profile to the insulin glargine Lantus® (8). Abasaglar® has induced significant reductions in the sales of the originator insulin. In a meta-analysis no differences between long-acting biosimilar and originator insulin were found concerning the reduction in glycated hemoglobin, or reduction in fasting plasma glucose hypoglycemia, mortality, injection site reactions, insulin antibodies and allergic reactions (9). Short-acting biosimilar Insulin lispro Sanofi® is a biosimilar of insulin lispro. The SORELLA 1 study showed that biosimilar insulin has

similar efficacy and long-term safety (including immunogenicity) to Lispro Humalog® (9). A recent systematic review concluded that biosimilars have similar clinical efficacy and safety compared to their reference products and they may be considered as alternative options for non-basal and basal insulin therapy. However, these are not approved to be used as "interchangeable" by the dispenser without a prescriber's assent, because studies with a switch design are absent (8).

Take home messages

- Second-generation basal insulins (IGlar U 300 and IDeg), compared with earlier insulin analogs, have improved pharmacokinetic/pharmacodynamic profiles and lead to clinical benefits, primarily reduced nocturnal-hypoglycemia risk. There have been conflicting results when the PK/PD profiles of the two second-generation basal insulins have been compared directly.

- The three rapid-acting insulin analogs currently available have similar efficacy and safety in the pediatric population. In comparison to regular insulin they have a more rapid onset, shorter duration of action and give a reduction of hypoglycemic episodes.

- The only ultra-rapid-acting insulin now available is Fiasp® (fast-acting insulin aspart). It has a faster onset and offset than aspart insulin (IAsp) and this gives better control of post-meal spikes in glucose and causes less hypoglycemia hours later.

- Long-acting and short-acting biosimilars are never absolutely identical to the original product, however bioequivalence has been established through proper clinical trials and pharmacovigilance on reporting of any side effects observed.

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SECTION 1 CHAPTER 5

CONTINUOUS SUBCUTANEOUS INSULIN INFUSION AND SENSOR AUGMENTED PUMP IN TYPE 1 DIABETES

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Continuous Subcutaneous Insulin Infusion (CSII) provides a more physiological insulin delivery than other regimens and closely imitates the action of the pancreas. CSII employs a programmable pump in order to continuously infuse rapid-acting insulin via a subcutaneous infusion set which should be changed every 2–3 days. The basal insulin infusion rate can be preprogrammed and varied at least hourly to match activity and changing requirements (e.g., illness, travelling, hormonal changes, growth spurts). CSII allows the delivery of tiny doses of insulin versus an insulin pen/syringe and is particularly useful for low dose insulin-sensitive children and in childhood.

Bolus can be given with a carbohydrate intake in the form of immediate bolus, long bolus, or a combination of both, to help treat a particular food (e.g., pizza or meals high in fat and/or protein). Most pumps have bolus calculators which take into account the insulin still active from previous bolus to advise the user on the necessary bolus dosage. The greater flexibility in insulin provided by the pumps can enhance quality of life. The

decision to initiate insulin pump therapy should be made following discussion by the pump multidisciplinary team which includes a pump-trained diabetologist, diabetes specialist nurse, dietitian and psychologist. Literature data show that ISCI has been associated with a slight improvement of metabolic control considering only HbA1c reduction. Thus, the real value of CSII is hidden due to the fact that HbA1c should not be the only parameter used to evaluate metabolic control and because in many studies new pumps are considered together with old generation pumps, decreasing the positive effects provided from the most recent technologies. CSII has been compared with multiple daily injections (MDI) with NPH as long-acting insulin, and this showed a decreased number of hypoglycemic events and better HbA1c levels (1). These results were confirmed by randomized controlled trials (RCT) including patients treated with basal analogues (2). One possible bias in favor of CSII comes from the inclusion in the MDI control population of subjects not treated with a basal-bolus regimen. Moreover, CSII can reduce glycemic variability and improve quality of life (3). The VIPKIDS study (14 Italian centers) showed how, even if the metabolic control was not significantly different among subjects in CSII versus MDI, the quality of life was better in patients on CSII (4). Proper metabolism control was associated with the use of advanced features, such as CHO counting and continuous glucose monitoring (CGM), and with pregnancy and longer diabetes duration. Recent data support an association between the use of CSII and decreased mortality regardless of the metabolic control itself (5). NICE guidelines, meta-analysis of RCT, systematic reviews and a Cochrane from 2010 (6) show an improvement of metabolic control (HbA1c – 0.3%) in subjects treated with CSII versus MDI, both adults and children, without significant differences in severe and moderate hypoglycemia. HbA1c improvement is more evident in patients with poor metabolic control in MDI. For example, in patients with HbA1c over 10% at the beginning of CSII, metabolic control can improve greatly with a HbA1c decrease of 3–4% (1). Insulin pumps reduce chronic complications of type 1 diabetes (T1D) in young people, even when compared to those who exhibit similar levels of HbA1c in IMD therapy. A population-based cohort study in the Diabetes Prospective Follow-up Initiative in Germany, Austria, and Luxembourg of 30,579 patients with T1D younger than 20 years compared insulin pump therapy with MDI, showing improved clinical outcomes, lower rates of severe hypoglycemia and diabetic ketoacidosis (DKA), and lower HbA1c (-0.18%) associated with CSII in children, adolescents, and young adults with T1D (8).

In children under the age of six, ISCI is associated with improved long-term metabolic control and a reduced risk of severe hypoglycemia compared to

MDI, especially when children start CSII immediately after T1D onset. After the first two years of T1D, HbA1c tends to worsen in MDI patients compared to CSII (9). Even in toddlers, HbA1c improvement is more evident, with higher HbA1c at the beginning of CSII. Although some randomized studies in pre-school children did not show better glycemic control, parents of pre-school children treated with ISCI reported more flexibility, a better quality of life and less anxiety. SCII is suitable for youth with diabetes of all ages.

Regarding hypoglycemia, most trials were not designed to demonstrate significant differences in hypoglycemia due to the low baseline rates of hypoglycemia reported; however, those patients with the greatest hypoglycemia burden at baseline got the most significant improvement from CSII use.

Adolescents have low compliance with recommendations and good practice about CSII use, resulting in an effectiveness reduction. Data downloaded from 100 insulin pumps of patients with a median age of 13.6 years confirmed that patients' compliance was correlated with age and diabetes duration. Every year of age was associated with a 0.31 decrease in self-monitoring of blood glucose (SMBG) per day and 0.22 bolus events/day. On average, if breakfast insulin was missed ≥4 times per fortnight, HbA1c increased by 1% (11). Insulin pump treatment can be hazardous when education and adherence to treatment are inadequate because the pump contains only fast acting insulin and if the delivery is interrupted for any reason, it will result in hyperglycemia: if this is not detected quickly, ketosis will develop into DKA. The pumps can only be switched off for short periods of time. Failure of the setting may occur and, if not detected, can potentially lead to the development of ketosis/DKA within hours. All pump users should be aware of the potential failure of the system and know how to prevent it, diagnose and manage insulin infusion system failure and all other problems that can contribute to an unexplained hyperglycemia. Patients should be encouraged to explore the reasons for hyperglycemia and should be able to measure β ketones by means of a portable measuring device. In addition, all CSII users should be advised to perform the set changes at the beginning of the day, not in the evening and should be able to administer insulin with a pen or syringe in the event of a suspected pump failure (hyperglycemia and elevated ketones levels). They should also have access to long-acting insulin and be aware of the dose to be taken in the event of a pump failure, especially if they are travelling far from home. If they do not have long-acting insulin with them, they should test their glucose levels and take a rapid-acting insulin injection every three hours.

Lipodystrophy at the insulin delivery site is another problem that can affect insulin absorption leading to aggravation of glycemic control. These issues highlight the importance of the continuum of diabetes education, especially when new technologies are adopted.

The extreme importance of personalizing diabetes treatment is widely recognized. The decision to initiate insulin pump treatment should only be taken after agreement between the multi-disciplinary insulin pump team and patients or their caregivers and they should make an informed decision on the pump model best suited to their needs. According to guidelines, CSII should be considered in some clinical conditions (7) (see Table 1).

Table 1: Clinical Conditions in which CSII is indicated (7)

- Recurrent severe hypoglycemia
- Wide fluctuations in blood glucose levels regardless of HbA1c
- Suboptimal diabetes control
- Microvascular complications and/or risk factors for macrovascular complications
- Good metabolic control but insulin regimen that compromises lifestyle
- Young children and especially infants and neonates
- Children and adolescents with pronounced dawn phenomena
- Children with needle phobia
- Pregnant adolescents, ideally preconception
- Ketosis prone individuals
- Competitive athletes

Modern pumps have integrated bolus calculators, allowing patients to schedule their insulin-carbohydrate ratios and insulin sensitivity factors, preventing them from making complex calculations at meals, reducing glucose variability and increasing the number of post-meal glucose readings within the target (12). In consultation with patients who use CSII, it is useful to make sure that they (or their parents) use the bolus calculator and that the settings are appropriate to the child and up to date. The most common reason why pump users refuse a bolus calculator is mistrust about the parameters of the bolus calculator, so they have to be adjusted with a member of the diabetes team. Insulin pump data should be routinely downloaded as an essential part of the consultation and patients should also be encouraged to take a periodic look at their data at home. It is important that advanced pump functions are taught over time, after patients become confident with the

basic skills, such as changing the infusion set every three days, bolusing before all meals and snacks and how to manage hyperglycemia. Lower proportions of basal insulin and over seven daily bolus are associated with better glycemic control and motivation is crucial for the long-term success of CSII therapy. The decrease in glucose variability observed in patients who switched to CSII may be explained by the possibility to continuously adjust the rate of basic insulin administration by the pump. Moreover, temporary basal rates allow users to adjust the basal insulin delivery upwards (e.g., during intercurrent illness) or downwards (e.g., during exercise), as well as complete suspension, for a fixed period of time. The change in basal insulin delivery should be done 1-2 hours prior to the desired change in blood glucose.. Preprogrammed basal models can be adopted when the days of different insulin requirements are predictable, for example during the weekend or during menstrual periods in women. Usually, rapid-acting insulin analogs are used in pumps but regular insulin can be considered in case rapid-acting analogs are not available.

Sensor augmented pump (SAP) therapy is defined as a combination of CSII and real-time continuous glucose monitoring (rt-CGM) and represents the first step on the road to an engineered pancreas. SAP therapy shows advantages in the reduction of HbA1c without an increase of hypoglycemia episodes in children and adolescents compared both to MDI with SMBG and to the sole use of the pump. However, the positive effects of SAP are closely related to the sensor port with at least 60% use being associated with significant improvement and children and teenagers may find it difficult to carry the sensor at all times. (7). The technological evolution from SAP to SAP with algorithms has improved the metabolic control and beyond the reduction of HbA1c, the reduction of time in hypoglycemia and the increase in time in range (TIR) have been added.

The comparison between SAP therapy and MDI with SMBG shows a reduction of HbA1c in children and adolescents who frequently use the sensor in favor of SAP (>60–70% of the time), without an increase of hypoglycemia episodes (study STAR 3). A reduction of both hyperglycemia and glycemic variability in SAP, regardless of the HbA1c value, was found, with better results in those with moderate glycometabolic control (HbA1c < 8%) (13). Moreover, patients using SAP show greater satisfaction with the treatment and improvement of quality of life (7,13).

The comparison between SAP and CSII with SMBG shows a reduction of HbA1c in children and adults who use the sensor for >60% of the time in

favour of SAP (with even better results if use is >70%), with a reduction of both hypoglycemia and hyperglycemia and glycemic variability in SAP (7).

SAP with algorithms refers to the integration of CGM data and insulin pump delivery into one device in order to modify insulin delivery based on sensor data. It includes low glucose suspend (LGS) and predictive low glucose suspend (PLGS) functions. **LGS systems** stop insulin release for two hours when the sensor glucose drops below a preset threshold and automatically resume insulin release regardless of the sensor glucose levels after two hours. It has been successfully employed in children and teenagers in order to decrease the risk of hypoglycemia. It is known that fear of hypoglycemia is the most important barrier to the achievement of optimum metabolic control in T1D. The ASPIRE study (Automation to Simulate Pancreatic Insulin Response) compared SAP with LGS and SAP without LGS and found a reduction of hypoglycemia and time spent in moderate and severe hypoglycemia in patients with LGS ON, without an increase of hyperglycemia and deterioration in glycemic control in terms of HbA1c (14). The LGS system shows benefits also in patients with proven impaired hypoglycemia awareness reducing both severe hypoglycemia (coma and seizures) and moderate hypoglycemia.

Predictive Low Glucose Suspend systems use sensor glucose concentration trends to predict glucose values into the future (e.g., 30 min) and then suspend insulin delivery when hypoglycemia is predicted, ideally before hypoglycemia occurs. PLGS systems are explored in another chapter.

To conclude, it is important to keep in mind that significant resources are required for education in the implementation of new technologies. Health care providers need to consider the level of patient/caregiver motivation as well as realistic expectations and individualized goals and should provide ongoing support and refresher education to enable effective use of CSII and SAP.

Take Home Messages

- CSII allows the delivery of tiny doses of insulin versus an insulin pen/syringe and is particularly useful for low dose insulin-sensitive children and in childhood. It represents the first therapeutic choice from the onset of T1D in preschool children.

- CSII compared with MDI can improve both metabolic control, particularly in patients poorly controlled, and quality of life. The

improvement of metabolic control, evaluated both as a value of HbA1c and as a reduction in glycemic variability, is not associated with severe and moderate hypoglycemia and is evident when there is associated the use of CGM and advanced functions, such as CHO counting, bolus calculator and temporary basal rates.

- Set failure can occur. It can potentially result in the development of ketosis/diabetic ketoacidosis within a matter of hours. All pump users should be aware of potential set failure and should be able to measure β ketones via a portable meter. Moreover, all CSII users must have the ability to manage multi-injective therapy in case of pump failure.

- Diabetes therapeutic education regarding the use of technologies needs to be continuous.

- SAP therapy is defined as a combination of CSII and rt-CGM and represents the first step on the path toward an artificial pancreas.

- SAP with algorithms refers to devices that modify insulin delivery based on CGM data. It includes low glucose suspend (LGS) and predictive low glucose suspend (PLGS) functions. LGS systems stop insulin delivery for 2 hours when the sensor glucose falls below a preset threshold and automatically resume insulin delivery regardless of sensor glucose levels after two hours.

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SECTION 1 CHAPTER 6

PREDICTIVE LOW GLUCOSE SYSTEM AND HYBRID CLOSED LOOP

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Introduction

Insulin pump therapy (or continuous subcutaneous insulin infusion, CSII) and continuous glucose monitoring (CGM) enhanced metabolic control in diabetic patients. While combined systems (sensor augmented pump therapy, or SAP) showed additional benefits—see Chapter 5—algorithm implementation has resulted in improvements not only to glycated hemoglobin (HbA1c) but also to other metrics, such as time spent in hypoglycemia, glucose variability and disease burden.

In 2006 the Juvenile Diabetes Research Foundation (JDRF), one of the biggest organizations involved in pushing forward research on type 1 diabetes, launched an initiative intended to accelerate progress toward Artificial Pancreas (AP) systems [1]. They created a pathway divided into multiple steps in a crescendo of technical and clinical challenges to reach a fully multi-hormone closed loop, the most automated system (see Figure 1) in 2009.

The aim of this chapter is to describe first generation systems and to record each improvement of insulin therapy with the evolution of devices

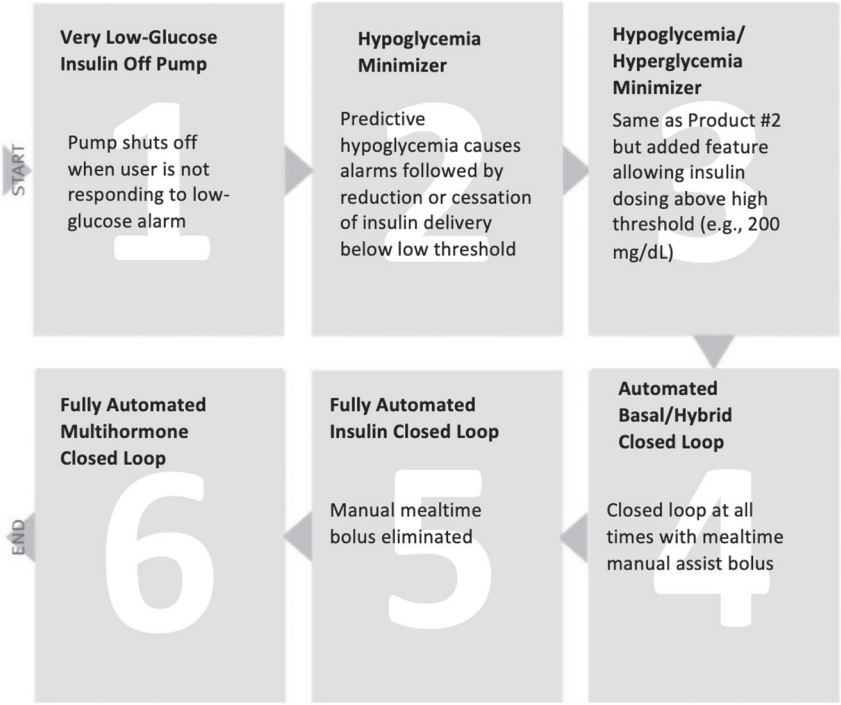

Figure 1. 2009 AP road map, as shown in the paper from Aaron Kowalski from JDRF [1].

First generation devices

What distinguishes first generation devices from SAP is hypoglycemia prevention, called *Low Glucose Suspend* (LGS), in which insulin is stopped when the glucose level falls below a predetermined threshold. The interruption lasts for two hours, after this time basal rate delivery is resumed automatically unless the patient performs "corrective" action before the end of two hours (see Figure 2).

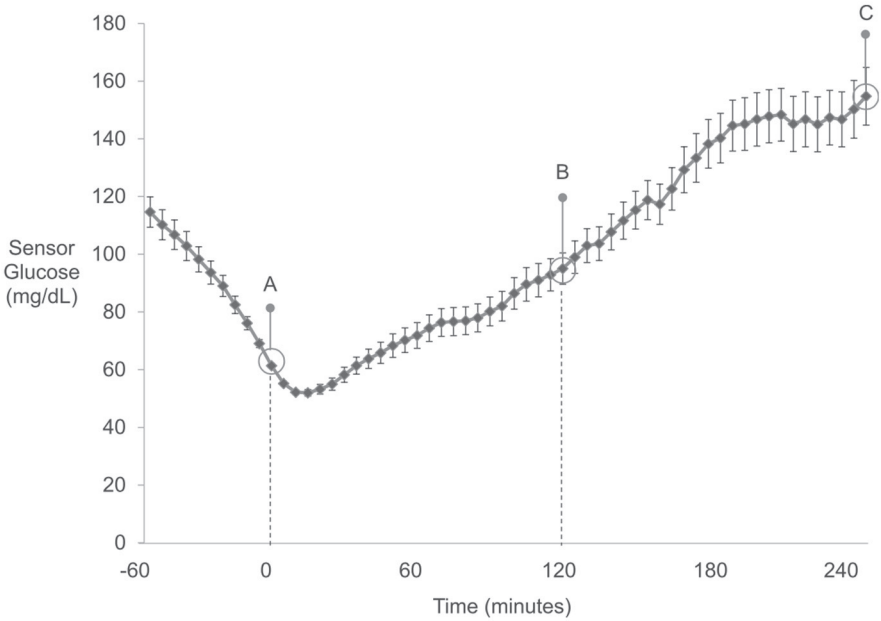

Figure 2. Performance of LGS algorithm from the study of Ly et al [2]. A:Insulin delivery is automatically suspended when the glucose level of the sensor drops below 60 mg/dL. B: Insulin administration restarted automatically after 2 h. At that time, the mean glucose level measured after 2 h of insulin suspension was 99 6 6 mg/dL. (5.5 6 0.3 mmol/L). C: The average glucose level measured after 2 hours of insulin recovery was 155 6 10 mg/dL. (8.6 6 0.6 mmol/L).

Studies analyzing SAP with LGS compared to SAP without LGS usually include adults and children [3]. Most of the studies involved patients who had not previously used a pump (except in those concerning the MiniMed Veo system) [3,4].

The comparison of SAP systems with the LGS active (ON) versus SAP with the LGS not active (OFF) highlights:

- reduction of time spent in hypoglycemia (< 70 mg/dL, 3.9 mmol/L) and < 50 mg/dL (2.8 mmol/L) in patients with LGS ON (-26.8%) with fewer nocturnal events [3,4];
- no increase in hyperglycemia [3,5];
- no difference in HbA1c value [3];
- no change in QoL [3].

These studies highlighted a great improvement in hypoglycemia magnitude, but the reduction in time spent in hypoglycemia was minimal. For this reason, a new generation of devices was designed to prevent hypoglycemia to a greater extent.

Second generation devices

The *Predictive Low Glucose Suspend* (PLGS) features characteristics of second generation AP devices, involving the use of an integrated algorithm capable of "predicting" hypoglycemia, relying on the value and the speed of change in glucose values detected from the sensor. In fact, based on this prediction, the insulin pump interrupts insulin delivery before reaching the hypoglycemia threshold, recovering delivery after glucose stabilization.

The first commercially available PLGS algorithm was SmartGuard™, which interrupted insulin administration when it was expected that the glucose sensor value would reach or fall below a predetermined low glucose level within thirty minutes and systematically resumed basal delivery after a predicted sensor value was above a certain threshold [6] (Figure 3).

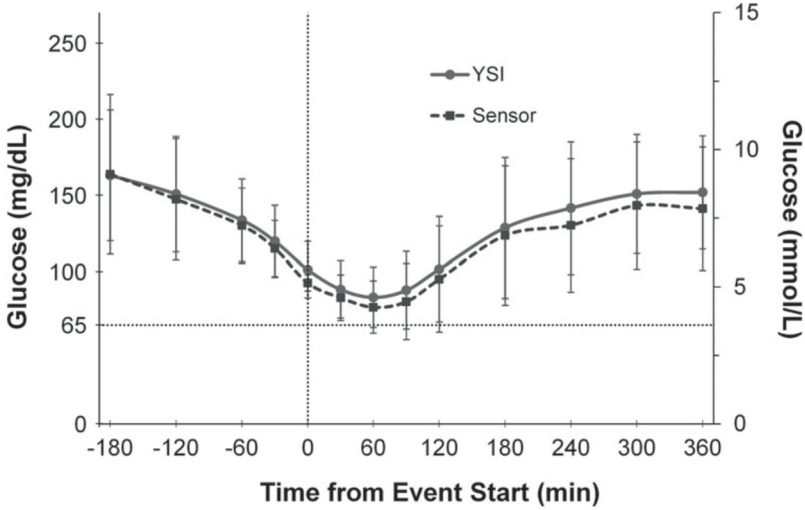

Figure 3. Mean glucose values before, during, and after predictive insulin suspension events, from the paper of Buckingham et al. [7] YSI = Yellow Springs Instruments, used as reference plasma glucose values.

The comparison of SAP with SmartGuard™ ON versus SAP with SmartGuard™ OFF highlights:

- further reduction in the number and duration of hypoglycemia events (value <65 mg/dl, 3.6 mmol/L) at both nighttime and daytime, in children and adolescents (-1.4%) [6-8];
- increased time spent above 140 mg/dL (7.8 mmol/L) (+76 min/day) [6];
- no ketonemia in the morning [6,8];
- increase in time in range (70–180 mg/dl, 3.9–10 mmol/L) if the sensor is used for >70% of the time [9];
- less fear of hypoglycemia, with improved quality of life [10].

After these studies, physicians started using this technology in all those patients more prone to hypoglycemia, to avoid unnecessary severe events, but more hyperglycemia was observed after SmartGuard ™ activation. Using the system, it became clear that a strong education was necessary to minimize hyperglycemic rebound after PLGS activation, especially when patients take carbohydrates to resolve impending hypoglycemia. A paper from Biester et al. clearly demonstrated that taking extra carbohydrates during pump suspension increases glucose after PLGS activation, while no interference with pump operation gives better post-suspension glucose values [11]. For this reason, an educational program for children and adolescents intending to use PLGS was developed to set realistic expectations and to improve glucose outcomes using this technology [12].

Before moving to the next generation, there was space for improving PLGS by reducing the number of operations, with better performance of the system.

A new algorithm was then developed, called Basal-IQ™, which uses the last four sensor glucose values to predict the sensor's glucose concentration 30 minutes in the future. Insulin delivery is interrupted if the predicted glucose is <80 mg/dL (4.4 mmol/L) or if the glucose concentration drops below 70 mg/dL (3.9 mmol/L). Insulin delivery starts again the first time the system receives a higher CGM glucose reading than the previous, if no further drop in glucose is expected below 80 mg/dL (4.4 mmol/L), if no CGM data are accessible for 10 min, or if the insulin suspension surpasses 120 min in a 150-min period [13]. Also, this algorithm showed good results on time spent in hypoglycemia (<70 mg/dL, 3.9 mmol/L) of 0.9–1%, without increased time in hyperglycemia (>180 mg/dL, 10 mmol/L)[14], giving a second option to treat people with diabetes.

Third/fourth generation devices

Given the rapid technological improvement in diabetes research, hyperglycemia minimization was included in *closed-loop devices* (fourth generation), merging third generation in the following step of the pathway elaborated from JDRF [1].

Closed-loop (or closed-loop control, CLC) systems are distinguished by the communication between a glucose sensor and a sophisticated algorithm-controlled insulin pump, which can be on the pump or in a different device (such as a smartphone), able to maintain glucose in the target range. Fourth generation devices are also called *hybrid* closed loop (HCL) because, despite having an automatic basal dose calculator, they need meal announcements to avoid glucose derangement (especially hyperglycemia).

Even if highly sophisticated, numerous studies can be found about CLCs, with the first appearance in the literature in 1977. Different prototypes have been tested since, but only some of them reached the market and are available for children and adolescents with diabetes (Medtronic 670G ™, Tandem Control-IQ ™, CamAPS FX app). Compared to previous generation systems, these CLCs demonstrated:

- increased time in range (70–180 mg/dL, 3.9–10 mmol/L, 71%, + 10%), with less time spent in hypoglycemia (<70 mg/dL, 3.9 mmol/L, -0.88%) compared to SAP [15];
- increased time in range (67%, + 6%) with similar time spent below 70 mg/dL (3.9 mmol/L) compared to PLGS [16];
- more time spent in target range in children and adolescent with suboptimal glucose control (TIR 65%, + 13%) [17];
- reducing the burden of diabetes, reducing the time spent on diabetes management, and improving sleep quality through the closed loop.[18,19]

Studying the devices available on the market, advantages are maintained if HCL usage is elevated, and a TIR of 70% is achieved if HCL is used for at least 75% of time [20]. Technological difficulties (error alerts, non-adequate usage), too much work (calibrations, finger sticks), alarms, disappointment in glycemic control, and expense are leading causes of HCL discontinuation, with a dropout rate of 30% at 6 months after initiation [21].

New algorithms are in the development phase, and we expect new opportunities from devices increasingly similar to a functioning pancreas,

called artificial pancreases, which will be discussed in the following chapter.

Take home messages

- There is a roadmap to develop new devices capable of automatically deliver insulin (and other hormones) based on glucose values.
- First generation (suspend-at-low) and second generation devices (suspend-before-low) are algorithms demonstrated to reduce time spent in hypoglycemia (<70 mg/dL, 3.9 mmol/L) compared to SAP without increasing hyperglycemia in children and adolescents with diabetes.
- Third/fourth generation devices showed increased time in range (70–180 mg/dL, 3.9–10 mmol/L) and reduced burden compared to SAP and PLGS.
- New algorithms are in the development phase, and we expect new opportunities from devices increasingly similar to a functioning pancreas in the following years.

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SECTION 1 CHAPTER 7

ARTIFICIAL PANCREAS

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Introduction

Management of type 1 Diabetes (T1D) necessitates innumerable daily steps and decisions, with a substantial disease burden to patients. The pediatric age adds unique management challenges, given the physiologic and age-linked changes throughout childhood demanding frequent therapy corrections and personalized therapy. Notably, children and adolescents continue to have the highest Hemoglobin A1c (HbA1c) levels across all ages (1), with an augmented risk of developing short- and long-term complications.

Advances in diabetes technology have transformed the treatment of T1D, with insulin pumps systems evolving to provide flexible and accurate insulin delivery and Continuous Glucose Monitoring (CGM) technology, which has added another milestone in the quest for automated insulin delivery (AID). CGM systems provide real-time glucose information with predictive alerts and alarms and allow patients and clinicians to gain a wealth of information previously not available. Most importantly, CGM has allowed researchers to develop multiple algorithms for AID by becoming the liaison for the integration and modulation of insulin delivery through CGM-derived data.

Artificial Pancreas (AP) systems have the potential to meet the peculiar needs of the rapidly changing insulin regimens in the pediatric age, customizing insulin delivery to reduce hypo-, hyperglycemia and the problem of diabetes managing in this generation. This chapter aims to

review the available and future AP systems and describe the evidence for their clinical benefits in the pediatric population with T1D.

Artificial Pancreas Models

AP systems (also known as AID or Closed-Loop [CL]), are a category of diabetes management devices consisting of a CGM system, an insulin pump and an internal control algorithm within the pump or on a separate device (smartphone), all communicating wirelessly. Several AP systems are available and permitted by the US Food and Drug Administration (US-FDA) or by the Conformité Européenne (CE) for use in pediatric patients and are described in Table 1. Many more are in the development stage with feasibility or pivotal clinical trials for FDA or CE registration. AP systems are further categorized in single or multi-hormone systems. In single hormone AP systems, real-time glucose information is gathered by the CGM device and transmitted to the control algorithm which in turn modulates the pump's insulin delivery by automatically increasing, decreasing or suspending insulin infusion rates every 5–10 minutes to a preset glucose level or glucose range; in some models, the algorithm instructs the pump to provide automatic correction insulin boluses to mitigate hyperglycemia. Similarly, dual hormone AP systems deliver insulin and glucagon or another hormone in a CGM-derived glucose-responsive manner. The use of an insulin/glucagon AP system poses challenges relative to the need for stable glucagon. Recently, dasiglucagon (Zealand Pharma, Denmark), a stable-in-solution glucagon analog, has shown promise for potential use in an AP system (2). Although the insulin-glucagon systems offer more protection from hypoglycemia, they present additional challenges for the very young patient, due to the need for two infusion sets in addition to the CGM, which may limit pump site placement. Recently, clinical interest has resurfaced in pramlintide, the stable analog of amylin, which is co-secreted with insulin in the β cells and has been shown to improve post-prandial hyperglycemia in people with T1D. The co-infusion of pramlintide and insulin in AP models has shown promising results with improved Time in Range (TIR) of 70–180 mg/dL (3.9–10.0 mmol/L) without an increase in hypoglycemia in young adults (3).

Table 1. Approved Artificial Pancreas Systems (FDA and/or CE) for pediatric age.

Brand-Model **Algorithm**	**Features**	**FDA** **Approval**	**CE** **Mark**
MiniMed 670G system with Guardian 3 CGM Hybrid Closed Loop PID-IFB* on the pump	300 units reservoir 7-day sensor life 2-4 calibrations/day Requires carbohydrate entry and meal bolus Requires correction bolus No advanced bolus feature **Auto Mode:** replaces pre-programmed basal insulin settings Sensor Glucose (SG) set point: 120 mg/dL Temporary target setpoint: 150 mg/dL Correction bolus target: 150 mg/dL	Yes Age 7 and older	Yes Age 7 and older
MiniMed 780 System with Guardian 3 CGM Advanced Hybrid Closed Loop PID-IFB* with DreaMed technology on the pump	300 units reservoir 7-day sensor life 2-4 calibrations/day Require carbohydrate entry and meal bolus No advanced bolus feature **Auto Mode:** replaces pre-programmed basal insulin settings SG target set point:120 mg/dL OR 100 mg/dL Temporary target setpoint: 150 mg/dL Automatic Correction bolus target: 120 mg/dL	No	Yes Age 14 and older

Tandem t:slim x2 with Control IQ and Dexcom G6 CGM Advanced Hybrid Closed-Loop TypeZero MPC** on the pump	300 units reservoir 10-day sensor life No calibrations Require carbohydrate entry and meal bolus Advanced bolus available up to 2 hours extension Works on pre-programmed basal rate settings (personal profiles) **Control IQ:** Maintains active personal profiles at SG: 112.5-160 mg/dL Increases/Decreases basal if predicted SG >160 mg/dL/ <112.5 mg/dL Stops basal if predicted SG < 70 mg/dL Automatic correction bolus if predicted SG>180 mg/dL **Exercise activity:** Maintains active personal profiles at SG: 140-160 mg/dL Increases/Decreases if predicted SG >160 mg/dL/ <140 mg/dL Stops basal if predicted SG < 80 mg/dL Automatic correction bolus if predicted SG>180 mg/dL **Sleep activity:** Maintains active personal profiles at SG: 112.5-120 mg/dL	Yes Age 6 and older	Yes Age 6 and older

	Increases/Decreases basal if predicted SG >120 mg/dL/ <112.5 mg/dL Stops basal if predicted SG < 70 mg/dL No automatic correction bolus		
CamAPS Fx APP **Dana Diabecare RS insulin pump and** **Dexcom G6 CGM** Hybrid Closed-Loop MPC** APP on Android smartphone	300 units reservoir 10-day sensor life No calibrations Requires carbohydrate entry and meal bolus Personalized meal sizes option **Auto Mode**: replaces pre-programmed basal insulin settings Default glucose target 104 mg/dL (range 104-131 mg/dL) Ease-off for reduced insulin requirements (0-24 hours) Boost Mode: for increased insulin requirements (0-13 hours)	No	Yes EU and UK Age 1 and older (APP)

*PID-IFB= Proportional Integral Derivative controller with insulin feedback;**MPC= Model Predictive Control

Control Algorithms

The algorithm represents the crucial portion of AP systems. Presently, four types of algorithms have been developed and used in AP systems. Proportional Integral Derivative (PID) is a responsive algorithm that responds to the measured glucose levels. In many systems, an insulin feedback (IFB) function is added to identify the insulin on board and help prevent hypoglycaemia. Model Predictive Control (MPC) is a proactive algorithm that predicts blood glucose at a specified time in the near future. In addition, they can "learn" the routine of the user, individualizing insulin delivery. Fuzzy Logic (FL) differs from PID and MPC algorithms by calculating the insulin dose based on how a clinical expert would adjust in real time using the information produced by CGM. Lastly, the bio-inspired algorithm is based on a mathematical model of the way β cells produce insulin in response to changes in glycemia. (4, 5). Most monitoring algorithms include security modules, such as insulin delivery restriction, by restraining the maximum rate of insulin delivery or the amount of insulin on board, and insulin suspension for situations when sensor glucose (SG) levels decrease rapidly. Generally, body weight and insulin demand. are needed to initialize AP systems. Currently, the majority of the AP systems are considered hybrid or advanced hybrid closed-loop systems (HCL or AHCL), in that the users need to enter carbohydrates and insulin dose for their meals. Thus far, fully automated AP systems studies have been associated with early post-prandial hyperglycemia followed by late post-prandial hypoglycemia due to limitations of subcutaneous insulin absorption (4, 5). New studies are exploring the use of ultra-rapid insulin analogs in CL systems (NCT04200313); these studies, combined with the ones exploring multi-hormone systems (NCT03800875) for flatter postprandial excursions and modified algorithms, may allow the loop to be fully closed and eliminate the need for meal announcements in the future.

Evidence of AP Systems Benefits in the Pediatric Population

Clinical trials studying AP systems have multiplied in the last decade. Early feasibility trials were reviewed in a meta-analysis comparing 24 studies (n=585, 219 in adult, 265 in pediatric, and 101 in combined studies). The majority evaluated a single AP hormone, while five evaluated a double hormone (insulin and glucagon) and two evaluated both. TIR was 12.59% higher with insulin-only AP systems ($p<0.0001$), whereas dual hormone AP systems revealed a greater progress in TIR compared with single hormone

systems (19.52% versus 11.06%, p=0.006). However, many dual hormone systems studies were compared to pump therapy alone, whereas most single hormone systems were related to sensor-augmented pumps (SAP) (6).

Great excitement has been building around newer systems, especially in view of their US-FDA approval or the CE mark. The MiniMed 670G system (Medtronic, Northridge, CA, USA) was the first US-FDA approved HCL system in 2016 for T1D individuals >14 years, followed by US-FDA approval (2018) and the CE mark for children over seven years. In the single-arm pivotal trial, this PID-driven system with a 120 mg/dL glucose setpoint (Auto Mode) showed increase in TIR, reduction in HbA1c by 0.5% and hypoglycemia (<70 mg/dL or <3.9 mmol/L) (2). Subsequent real-world analyses of the MiniMed 670G use in pediatric and young adults, however, showed limited time in Auto Mode at 32%, and a 19% discontinuation rate after eight months follow-up. The main reasons were issues with sensor calibrations or errors, skin irritation and frequent Auto Mode exits. (7). A new AHCL, the MiniMed 780G system, has just received the CE mark. The results of the single-arm at-home study were presented at the 80th American Diabetes Association (ADA) Virtual Scientific Sessions. This system offers a target setpoint of 120 mg/dL or 100 mg/dL and gives auto-correction boluses to 120mg/dL every 5 minutes once auto basal has reached the algorithm's maximum doses. The study included 39 adolescents (age 14–21 years) and 118 adults (>22 years) with T1D wearing the system for 45 days at 120 mg/dL and 45 days at 100 mg/dL glucose setpoint. HbA1c was overall reduced by 0.5%; the adolescent group experienced an increase in TIR from 18% at baseline to 59% and 62% at study end at the 120 mg/dL and 100 mg/dL setpoints, respectively. The adolescents reached the highest TIR (82%) overnight (100 mg/dL setpoint). In this age group, Auto Mode time increased from 37% at baseline to 93.8% at study end (NCT03959423).

A very exciting study with T1D subjects aged 14–29 years was also presented at the 80th ADA, the Fuzzy Logic Automated Insulin Regulation (FLAIR) study (NCT03040414). In this randomized, crossover trial, 113 T1D patients, of which 65% were 14–21 years old, used the MiniMed 780G AHCL system versus the MiniMed 670G for two 12 week periods. Time in hyperglycemia decreased from 42% to 34% in the AHCL ($p<0.001$), with minimal time in hypoglycemia (<54 mg/dL or <3 mmol/L) at 0.46%. TIR increased from 57% to 67% ($p<0.001$); TIR >70% in AHCL rose from 12% to 32%, and time in Auto Mode was 86% for AHCL versus 75% for 670G ($p<0.001$).

The Tandem t:slim Control IQ system (Tandem Diabetes, San Diego, CA, USA) has also received US-FDA approval and the CE mark for T1D individuals ≥6 years (Table 1). The system has an MPC-driven algorithm (TypeZero, Dexcom, Charlottesville, VA, USA) embedded in the t:slim x2 pump that communicates wirelessly with the Dexcom G6 (Dexcom, San Diego, CA, USA) CGM system. The algorithm uses pre-programmed pump settings and maintains them at SG 112.5–160 mg/dL (6.25–8.9 mmol/L), with increase, decrease, and suspension features based on predicted SG levels, outlined in Table 1. In addition, it delivers automatic correction boluses if SG is predicted to increase to >180 mg/dL (>10.0 mmol/L). The pivotal trial for the Tandem t:slim x2 with Control IQ enrolled 168 T1D subjects, of which 48 were adolescents (>14 years), who were randomized to use Control IQ (Closed-Loop Control, CLC) versus SAP. After six months, the CLC TIR was 71% (versus 59% SAP, $p<0.001$). TIR increased by 11% in the CLC group ($p <0.001$), the effect of CLC was immediate and persisted for the six months of the study. The impact was strongest at nighttime and was seen across HbA1c ranges. The HbA1c decreased by 0.33% in the CLC group ($p<0.0014$) and time spent in CLC was 92% (8). More recently, the DCLP5 trial results (NCT 03844789) were presented at the 13th International Conference on Advanced Technologies and Treatments for Diabetes (ATTD). The study, which included children aged 6–13 years, showed that the use of Control IQ (CLC) over 16 weeks increased TIR to 67% (versus 53%, SAP, $p<0.001$), with an regulated dissimilarity of 10.8% ($p<0.001$). TIR in the CLC group increased after the first month to 68% and remained stable through the study. Overnight TIR went from 54% to 80% at 16 weeks. Hypoglycemia was very low at <1.6% (<70 mg/dL or <3.9 mmol/L) and <0.2% (<54 mg/dL or <3 mmol/L) and TIR >70% was reached by 47% (CLC) versus 14% (SAP) of participants. Time spent in CLC was 92.8% throughout the study.

One of the most extensively studied AP system is the CamAPS system (Cambridge, UK) (5). The latest UK version, CamAPS FX, consists of a DANA RS insulin pump (Sooil USA, San Diego, CA, USA), a Dexcom G6 CGM system and the CamAPS FX app hosted in an Android smartphone; the CamAPS FX app has recently received the CE mark in the European Union and the United Kingdom for ages ≥1 year. This MPC treat-to-target control algorithm computes a innovative temporary basal insulin infusion rate every ten minutes to reach a default glucose level of 104 mg/dL (5.8 mmol/L) (see Table 1). The US version, Florence M system uses a modified Medtronic 640G pump and Guardian 3 CGM (NCT02925299). In a free-living, randomized controlled trial with 86 participants (50% aged 6–21 years), the CamAPS HCL system versus SAP resulted in a TIR

improvement of 10.8% (65% versus 54%, P< 0.0001) at twelve weeks. In addition, there was a 0.36% greater HbA1c reduction in the HCL group (P< 0.0001), lower time in hypoglycemia <70 mg/dL (3.9 mmol/L) (p= 0.0013) and a 10% reduction of time in hyperglycemia >180 mg/dL (<10 mmol/L) (p< 0.0001) (5). Remarkably, this system was studied in children as young as one year old, showing high TIR (70%), no severe hypoglycemia and no difference between CamAPS HCL with diluted insulin (U20) versus standard strength insulin (U100) (9).

Last, but not least, the Insulet Omnipod 5 system powered by Horizon (Insulet Corporation, Acton, MA, USA) is currently being investigated in the pivotal clinical trial (NCT04196140). The system includes an Omnipod tubeless pump (Pod) with a personalized MPC algorithm built into the Pod, a phone controller (PDM) and wireless Bluetooth communication with the Dexcom G6 CGM system. The PDM is used to start and stop automated mode, deliver boluses, and change settings. Glucose targets are customizable from 110 to 150 mg/dL (6.1–8.3 mmol/L) and are adjustable by the time of day and the algorithm adapts based on the user's insulin delivery history. The HypoProtect feature can be enabled for exercise or elevated hypoglycemia risk. This system has been studied extensively in feasibility studies (2) and it has been tested and "stressed" with missed boluses and/or physical activity, showing considerable flexibility and adaptability in school-age children and even in very young patients (≥2 years old) (NCT03216460). The results of the pre-pivotal portion of the clinical trial (NCT04196140) were presented at the 80th ADA meeting. A 14-day standard therapy period, followed by 14 days HCL at-home period in T1D subjects aged 6–70 years was evaluated at variable glucose targets of 130–150 mg/dL (7.2–8.3 mmol/L) followed by free choice of glucose target (110–150 mg/dL or 6.1–8.3mmol/L). In children aged 6–13 years, TIR for the HCL group was 53.5–64.8% with the higher targets of 130-150 mg/dL (7.2–8.3 mmol/L), and 71.2% with the 110 mg/dL (6.1 mmol/L) target, with minimal hypoglycemia (<54 mg/dL or <3 mmol/L) of 0.1%. Participants spent 97.3% of the time in automated mode. These results underline the flexibility of this future system, especially in school-age children, with adjustable glucose targets to users' various needs, thus providing greater safety for situations where hypoglycemia risk is increased.

Conclusions

The growing wealth of clinical studies showing clinical benefits of various AP systems in children, adolescents and adults, support Dr. Kovatchev' s

quote that the “artificial pancreas is here to stay” (10). Undoubtedly, there are persistent challenges with AP systems, such as post-prandial hyperglycemia and exercise-induced hypoglycemia. More sophisticated algorithms, multiple hormones systems and ultra-rapid acting insulins may flatten post-prandial hyperglycemia. For exercise, several studies have already shown safety and improvement of TIR in adolescents in ski camps and other exercise routines, although the need for carbohydrate intake remains for insulin-only systems, whereas the presence of glucagon may blunt the hypoglycemia caused by physical activity (2). Psychosocial assessments have shown the acceptability and benefits of this treatment approach in children, adolescents and their families; however, the cost of these systems or coverage by health care systems may limit access to these systems in the vulnerable T1D groups that would likely benefit the most. Hope, however, remains high, so that in the near future highly customizable, affordable systems could become available to improve the health, and ultimately reduce the burden, of people living with T1D.

Take Home Message:

AP systems are evolving into highly sophisticated devices, with single, dual and multi-hormone systems being studied and enhanced to reduce disease burden in people living with T1D. AP systems have shown to increase Time in Range (70–180 mg/dL or 3.9–10.0 mmol/L), decrease hypo- and hyperglycemia and reduce HbA1c. The newer systems have shown very high time (>85%) spent in automated mode and high acceptability by patients and their families. AP systems are becoming particularly suitable for children and adolescents in view of their adaptability, automatic correction doses for missed boluses and variable glucose setpoints to further reduce hypo and hyperglycemia. More systems are becoming commercially available in the US and EU, giving patients a variety of options for customizable therapy.

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SECTION 1 CHAPTER 8

NUTRITION IN TYPE 1 DIABETES, CARBOHYDRATE COUNT AND BOLUS CALCULATORS

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Introduction

Individuals with type 1 diabetes (T1D) treated with multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) should receive training by diabetes specialists (diabetologists, dieticians, etc.) on how to follow a healthy and varied diet and on how to quantify carbohydrates (CHO) in foods through CHO counting (CHC) of meals. Moreover, they should know the relationship between insulin intake and the CHO content of the meals [1].

Carbohydrate counting (CHC) is a healthy meal-planning tool that can help patients effectively change the dosage of insulin in order to improve glycemic control [2]. According to ISPAD 2018 guidelines, the estimated energy input and essential nutrients should be distributed as follows:

- CHO 45–50%
- Fats <35%
- Proteins 15–20%

This method, even though providing greater flexibility, does not mean total freedom, but does allow eating freely, following the principles of a healthy and varied diet [3].

The dose of insulin administrated before a meal can be calculated by using the individually calculated ICR (Insulin-to-Carb Ratio) for each patient, taking into consideration the meal's amount of CHO and considering the impact of the glycemic index in the post-prandial period. Though, there's growing evidence of the impact of other macronutrients (especially fats and proteins) on postprandial glycemia after six hours, so these macronutrients should be taken into account in the calculation of the total dosage and time of administration of the insulin bolus [4].

Carbohydrate counting

CHC is a technique for calculating the amount of insulin bolus needed, based on the content of CHO as the main nutrient, to reduce the postprandial glycemic excursion. The use of CHO counting aims to improve the control of glycemia and the flexibility of food choices. [1]. Patients with T1D using an insulin pump, based on the pre-prandial blood sugar levels and the meal's CHO content, can accurately calculate the dosage of insulin required before a meal, to improve postprandial glucose control [5].

CHC can be identified as three levels [6]:

- *Level 1 (basic step)*: patient recognizes that CHOs affect blood sugar; they learn where CHOs are present in food and how to quantify the grams of CHO in different foods.
- *Level 2 (intermediate step)*: the learn how to assess the changes in blood glucose levels in relation to CHO consumption, physical activity, and insulin therapy. These factors introduce the calculation of the insulin-to-carb ratio (ICR).
- *Level 3 (higher step)*: patients learn to use the ICR effectively.

The ICR factor specifies how many grams of CHO will be provided with 1 unit of insulin; it varies according to insulin requirements at mealtimes (based on the CHO intake at that time), blood glucose levels and the amount of physical activity undertaken so that children and adolescents can have a more flexible approach to meals [3]. It varies based on each specific meal (it should be high at breakfast time, low for lunch and high for dinner) and changes during growth periods [1]; for this reason, periodic reevaluation of

the ICR is recommended. Although this tool increases meal flexibility, diet quality remains very important.

In clinical practice, the glycemic index (GI) is a rate index for food, used to define the quality of the diet. The GI is the expression of glycemic response after eating an amount of fixed CHO contained in different foods compared as much carbohydrates contained in white bread. This method is very useful to predict postprandial glucose excursions after meals [3,7].

Another method to control postprandial glycemic variations is the glycemic load (GL) that account for both the quality, therefore the glycemic index, and the quantity of carbohydrate consumed [3]. Thus, the accurate calculation of bolus insulin required should take into account these three factors: *ICR, GI* and *GL.*

Fat–protein counting

In addition to CHO, it has been shown that proteins and fats also have an impact on postprandial blood sugar levels. CHO causes an immediate increase of blood glucose, within two hours of the meal, while fats and proteins cause a late increase, usually around three to six hours after the meal.

Recent studies, conducted in both adults and children/adolescents affected by T1D, have revealed that meals with a high protein/fat content increase blood sugar levels over time [9].

In fact, only counting CHO is an ineffective method to control glycemia; the calculation of fat and protein units (FPU) should also be adopted since this was suggested to cover post-prandial excursions resulting from meals rich in fats and proteins [9].

One FPU is defined as 100 kcal derived from fats and/or proteins present in foods.

FPU was calculated using the equation [10]:

$$FPU = (4 \times Protein\ [kcal] + 9 \times Fat\ [kcal])/100$$

This approach is often used with insulin pumps because it offers several boluses options for different meals:

- *Normal bolus (N):* Insulin is administered rapidly and with a single shot (it acts mainly on CHO);
- *Square-wave bolus (S-W):* Insulin is administered during an extended period of time (it acts mainly on fat and protein);
- *Double-wave bolus (D-W):* Insulin is administered as a combination of N and S-W (for mixed meals).

For meals rich in fats or proteins, the time of S-W should be calculated as follows: up to 3 h for a meal containing 1 FPU, 4 h for 2 FPU, 5 h for 3 FPU and lastly 8 h, when a meal includes more than 3 FPU (300 kcal). A review of the literature has shown that the D-W is more effective in controlling postprandial blood glucose levels in comparison to N for meals with high fat content (pizza, french fries, etc.) [11].

Bolus calculator

The pump also offers the possibility of the use of the bolus calculator. This is a helpful tool used to determine the prandial insulin dose, according to the quantity of CHO at the time of the meal, pre-prandial blood glucose levels, and remaining active insulin.

The combined use of CHC and bolus calculator can help to reduce blood glucose excursions with a limited number of hypoglycemic episodes. It is also associated with improved glycemic control which results in a reduction of HbA1c and improved postprandial glucose excursions, with a greater number of values within the target range [12].

Take-home messages

- A healthy, balanced and varied diet and insulin therapy are cornerstones of the management of T1D.
- CHC and FPU are two advanced tools to manage insulin therapy to improve postprandial glucose control in subjects with T1D.
- CHC and FPU allow patients with T1D to have a more flexible approach to diet and lifestyle.

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SECTION 1 CHAPTER 9

PHYSICAL ACTIVITY AND EXERCISE MANAGEMENT IN TYPE 1 DIABETES

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Physical activity, associated with a balanced diet, is a therapeutic cornerstone for the improvement of health in children and adolescents with type 1 diabetes (T1D). The beneficial effects of regular physical activity in youths with T1D include improvement of body composition, cardiorespiratory fitness, strength and wellbeing on the one hand, and reduction of cardiovascular risk factors, such as insulin resistance, dyslipidemia and endothelial dysfunction, on the other hand. Several systematic reviews and meta-analyses of physical activity intervention studies also demonstrated that the improvement of care and education of an individual with T1D yielded an overall significant effect on the levels of HbA1c.

Contrary to what was thought in the past, excessive weight is frequently described in patients with T1D. Indeed, a rate of 31.8% of overweight and obesity has been recently reported in a large worldwide study of children and adolescents with T1D, in parallel to the prevalence of weight excess in the general pediatric population. Therefore, it is likely that the same determinants of obesity in the general population, such as individual biological factors, socioeconomic status, lifestyle behaviors and pressures from an obesogenic environment, act in youths with T1D as well. Weight gain may occur in T1D patients in spite of nutritional education programs and regular clinical follow-up, which theoretically should contribute to overweight prevention. In addition, it has been also postulated that the intensive regimen of insulin therapy needed to maintain glucose control may contribute to weight gain. Obesity and/or abdominal fat are relevant

co-morbidities of T1D, since they are associated with insulin resistance, poor glucose control, and cardio-metabolic risk factors.

Based on these premises, motivating youths with T1D to perform physical activity and reduce sedentary behaviors is essential. Regrettably, most pediatric patients with T1D do not achieve the recommended 60 minutes per day of moderate/vigorous physical activity and spend much more than the recommended two hours in sedentary behaviors (such as watching television, surfing the internet or playing videogames). Several barriers put T1D patients off physical activity. Besides the reasons reported by non-diabetic adolescents (i.e., time constraints, lack of peer or parental support, lack of resources, or perceived lack of athletic ability), there are disease-related barriers, such as loss of control of diabetes and fear of hypoglycemia, which may limit participation in physical activity in youths with T1D.

As a result, children, adolescents and their families should be made aware of strategies to achieve safe physical activity and improve their physical fitness and health, despite the possible complications of glucose homeostasis and energy needs due to that physical activity.

To have a better understanding of the complex interactions between physical activity and diabetes management, it is essential to clarify the distinction between physical activity, exercise and sport, since these terms should not be used interchangeably. Generally, the term “physical activity” includes several components of daily living. On the contrary, the definition of “exercise” or “sport” is considerably more specific and restrictive, and it is intended for activities that aim to improve or maintain several components of fitness, related to health and/or athletic skills.

In this chapter, we will focus on the impact of the type of exercise on glycemic control and the main education topics about the adjustment of insulin dose and composition of meals/snacks in relation to the type, intensity, and duration of exercise undertaken. Other issues that will be considered are blood glucose monitoring and the new technology-based tools for blood glucose management. Established international exercise guidelines or consensus statements have been recently published for the pediatric population with T1D and may be consulted for an in-depth understanding.

Exercise characteristics and glycemic control

The type of exercise and the pre-exercise glycemic control of the patient may have a significant impact on glucose level during and after exercise, leading to either hypoglycemia or hyperglycemia. The physiological mechanisms intervening to adapt the insulin levels in response to exercise are altered in T1D. A patient in good glycemic control is at high risk of hypoglycemic episodes during aerobic exercise (i.e., jogging, cycling, and swimming), since there is no way to modulate the circulating insulin levels to the glucose-lowering effect of exercise. At the same time, the metabolic pathways of glycogenolysis, gluconeogenesis and lipolysis are inhibited. On the contrary, exercise may induce hyperglycemia, ketosis or ketoacidosis if the patient is in poor glycemic control or is exercising in conditions of under-insulinization. Hyperglycemia can also occur in well-controlled patients as a consequence of sympathetic response to brief and intense anaerobic exercise (e.g., sprinting or weightlifting), or during high intensity interval training or as a result of often competitive stress/anxiety levels. Some team sports are characterized by moments of vigorous activity interspersed with moments of activity of medium-low intensity or rest, which lead to a lower drop in blood glucose levels than prolonged exercise of moderate intensity. The risk of hypoglycemia is high not only shortly after exercise also but persists for at least 24 hours after exercise, especially during the night, for the contraction-induced increase in muscle insulin sensitivity.

Glucose monitoring

Given the high variability in the glycemic response to exercise, the appropriate blood glucose concentration before starting exercise should be individually established. In a recent consensus statement, Riddel et al. suggested that glycemic levels in the range 7.0–10.0 mmol/L 126 mg/dL–180 mg/dL) may be an acceptable target for exercise performance, especially for aerobic exercises of a maximum length of 60 minutes. Special precautions should be taken when glycemia is <5 mmol/L (<90 mg/dL): in this case, carbohydrate ingestion is recommended before exercising, and physical activity is allowed when blood glucose reaches at least 5 mmol/L (>90 mg/dL). When glycemia is >15 mmol/L (>270 mg/dL), a blood ketones test is recommended: exercise must be reduced to a short duration (<30 min) at light intensity; a small supplementary dose of insulin dose might be needed if ketones are below 1.5 mmol/L, whereas exercise is contraindicated if blood ketones exceed this level.

Hypoglycemia occurs with early signs, such as palpitations, sweating, hunger, anxiety, and tremors, that reflect the activation of the autonomic nervous system to the low blood glucose levels. Sometimes, several symptoms, such as tachycardia or sweating, can be misinterpreted as a consequence of exercise, and the prompt correction with carbohydrates can be missed or delayed. For this reason, it is essential to record blood glucose values before and in response to exercise, without relying only on symptoms. Strict glucose monitoring during exercise is especially required in patients with longstanding diabetes (over 10 years), who may present hypoglycemic unawareness due to autonomic neuropathy: they may experience severe hypoglycemia with abnormal behavior, seizures, and coma.

Besides the self-monitoring of blood glucose (SMBG), real-time continuous glycemic monitors (rtCGM) are useful to control glucose levels before starting, during physical activity and after completing the effort, in order to track individual responses to the training sessions. However, under conditions where blood glucose is rapidly decreasing, such as in physical activity during hyper-insulinism, this technology can overestimate blood glucose. Therefore, it may still be advisable to measure glycemic levels through blood glucose meters when there are rapid changes in sensor glucose values or when current values do not correspond to the symptoms. It is also crucial to take into account the direction and rate of change in glycemic levels to guide management decisions. This possibility is now feasible with the use of rtCGM, which may also provide individualized alerts and safety alarms.

Type and timing of insulin delivery

So far, the majority of children and adolescents with T1D are treated with a multi-injection regimen or insulin pump. Both feature excellent flexibility as it is possible to reduce basal insulin and/or bolus rates before, during and after exercise to prevent or limit hypoglycemia.. This adjustment is typically required for exercise of moderate intensity with a duration >30 min. While bolus modification can be applied to both regimens, basal dose adjustment is usually preferable for those patients on insulin pumps, particularly for unplanned exercise. According to the pre-exercise insulin type, the risk of hypoglycemia may occur within 1–1.5 hours after injection with rapid-acting insulin or 2–3 hours with regular insulin. Therefore, the corresponding bolus dose should be reduced to prevent the cumulating hypoglycemic effect of exercise performed during

the insulin peak. Several algorithms have been proposed to manage insulin adjustments for aerobic exercise and mixed aerobic and anaerobic activities in people with T1D.

Exercise accelerates the absorption of subcutaneously administered insulin, leading to relative hyperinsulinemia. This is why it is recommended to avoid injecting insulin at a site that will be strongly involved in muscle activity, such as the legs or arms.

Before starting to practice specific sports, such as contact ones or swimming, youths using a pump should disconnect it for up to 2 hours, in order to limit the risk of impaired glycemic control and ketosis.

Sophisticated systems that integrate CGM and insulin pump (sensor-augmented pump) may be advantageous for preventing hypoglycemia in exercising people. According to the models, these devices are provided with alarms for high and low readings, as well as “Up” and “Down” trend arrows for rapidly fluctuating values; some models allow the glucose readings directly on the pump screen. The latest devices are able to suspend basal insulin delivery with sensor-detected hypoglycemia (low glucose suspend function); furthermore, they can even predict impending hypoglycemia, by suspending basal insulin before the occurrence of hypoglycemia.

Diet management and carbohydrate supplements

Children and adolescents with T1D need the same total energy intake and macronutrient distribution (carbohydrates 50–60%, proteins 12–15%, fat 25–35%) as their non-diabetic peers. Depending on the intensity and duration of exercise, caloric intake may increase from 10 to 30%, while the macronutrient distribution does not change. The variety and the right amount of healthy food choices, including whole-grain products, legumes, lean meat, fish, semi-skimmed milk, cheese, eggs, vegetables and fruit, should guarantee a good quality diet. Unbalanced diets, such as high protein or high fat and low carbohydrate diets should be avoided. Supplements are unnecessary if the nutritional recommendations are fulfilled. Counseling on the risks of supplement intake is essential. Furthermore, education should be provided on healthy nutritional choice for optimizing training adaptions and performance. Likewise, adequate fluid intake is essential to prevent dehydration and heatstroke. Water is the best choice for activities lasting less than 60 minutes, while sports drinks

containing 6% simple carbohydrates may be useful for more prolonged activities, meeting the need of preventing hypoglycemia.

In fact, the glucose-lowering effect of physical activity may require carbohydrate supplements before, during and after exercise. The amount and type of carbohydrates depend on body mass, type, and intensity of physical activity and relate to the insulin circulating levels. Fear of hypoglycemia may often cause overcompensation, by ingesting excessive amounts of carbohydrate or reducing insulin doses more than necessary, leading to hyperglycemia during or after exercise. The consequences are long term poor HbA1c and/or body fat gain. Therefore, young people with T1D should record their physical activity, insulin, food and blood glucose levels in detail. In this way, they are familiar with their blood glucose fluctuations to different physical activities and exercise conditions and manage diabetes control accordingly. Special education is needed about the impact of different kinds of food and drinks on glycemic levels.

Guidance for safe exercise

Youths with T1D can participate in any kind of sport or exercise, either at a recreational or competitive level, provided they are in good glycemic control and free of any diabetes-related complication. Precaution should be taken for any strenuous exercise causing significant elevations in blood pressure (such as heavy weightlifting or high intensity sprints) in patients with nephropathy or proliferative retinopathy. Similarly, running or any exercise that results in excessive load on legs and feet needs careful prevention of blister and ulcers in patients with peripheral neuropathy. Because of potential damage to the retina, sports such as boxing require a proper medical assessment.

It is recommended that patients with T1D wear an identification bracelet or necklace and avoid exercising alone, in order to be adequately assisted in the case of severe hypoglycemia. They should be able to monitor their glucose levels and rely upon immediate access to sources of quickly absorbable carbohydrates. Glucagon should be easily available at schools, gyms or any other sports settings. People with T1D should be advised to dress in breathable clothes and carry bottles of water to prevent heatstroke. Furthermore, they should wear padded, polyester-blend socks and supportive athletic shoes to prevent foot injuries, blisters or wounds.

Conclusions

Physical activity is essential for youths with T1D to counteract the negative effects of a sedentary lifestyle. Any form of organized and non-organized physical activity can be undertaken, with the aim to achieve at least 60 minutes of moderate/vigorous physical activity each day. Diabetes care teams should support a young person with diabetes to achieve this goal and overcome barriers and limitations.

Several factors affect blood glucose levels: therefore, glycemic management during exercise must be individualized, often through a trial and error approach. New technologies, such as continuous subcutaneous infusion of insulin and continuous glucose monitoring, can greatly help manage diabetes during physical activity.

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SECTION 1 CHAPTER 10

MICRO- AND MACROVASCULAR COMPLICATIONS IN TYPE 1 DIABETES: DO THEY EXIST IN THE PEDIATRIC AGE?

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Introduction

People with type 1 diabetes (T1D) still have 2 to 4 times more morbidity and mortality than the general population despite advances in disease management [1].

The long-term complications of T1D are largely caused by its vascular complications, which reduce the quality of life and life expectancy of individuals, placing a significant economic burden on health systems [1]. The risk of developing vascular complications is related to T1D duration and is higher in individuals diagnosed during childhood compared to those diagnosed during adulthood [2,3]. The current increasing incidence of T1D, particularly in younger children, raises concerns about more individuals being at risk of vascular complications during their lifespan.

Vascular complications: definition and epidemiology

Vascular complications of diabetes are classified into microvascular and macrovascular. The former affect small vessels, especially retinal vessels, in the kidneys and nerves, from which diabetic kidney disease (DKD), diabetic retinopathy (DR) and diabetic neuropathy (DNeu). Macrovascular complications, on the other hand, affect larger vessels [1].

DKD affects up to 50% of all people with T1D throughout their lives; it is the leading cause of End-Stage Renal Disease (ESRD) and a key determinant of cardiovascular mortality [1,4]. DKD is the result of renal damage caused by structural and functional changes that occur with progressive increases in albuminuria with an early stage of hyperfiltration followed by a progressive decline in the rate of glomerular filtration. The major renal structural changes are mesangial expansion, glomerular and tubular basement membrane thickening, and glomerular sclerosis [4].

RD is the most frequent eye disease and the leading cause of blindness in people with T1D, with an overall prevalence of 35% [5]. This complication is classified as mild-to-moderate non-proliferative, severe non-proliferative, and proliferative retinopathy. DR is the result of damage in retinal microvascularization, such as thickening of the basal membrane, increased capillary permeability, changes in arterial and venular caliber, vascular tortuosity, retinal hemorrhage, microaneurysms, cotton stains and lipid exudates [5]. These alterations can be clinically silent for many years and then manifest as non-proliferation retinopathy, a cause of blindness. Aggravating retinal microvascular conditions may lead to intravascular coagulation, hypoxic suffering and resultant retinal neovascularisation. Rupture of these vessels leads to retinal hemorrhages that manifest as proliferative retinopathy, vision threatening. In addition, in individuals with T1D macular edema may form, fluid accumulation within the central neural retina, causing visual loss [5].

DNeu refers to a wide spectrum of neurological alterations associated with diabetes and affects 11–50% of patients with T1D. It can be either somatic or autonomic [6]. Sensorimotor polyneuropathy (or peripheral neuropathy) is the most common form of DNeu and is characterized by symmetric damage of peripheral small sensory and large motor nerve fibers [6]. A wide range of symptoms involving the cardiovascular, urogenital or gastrointestinal systems may be due to autonomic neuropathy [6].

Macrovascular complications of T1D primarily include cardiovascular, cerebrovascular, and peripheral vascular disease, and are associated with increased morbidity and mortality [1].

When do vascular complications develop?

A childhood and adolescent diagnosis of T1D is associated with a higher risk of vascular complications than an adult diagnosis. This is mainly due

to the increased duration of diabetes and possibly to a more aggressive disease pathogenesis. [2].

Early signs of vascular complications often show up in adolescence and are potentially reversible.[2].

Vascular complications are virtually always subclinical during childhood and adolescence, and advanced stages, such as ESRD or proliferative retinopathy, are extremely rare.

However, early subclinical manifestations can be common during adolescence and include increases in urinary albumin excretion, glomerular hyperfiltration, changes in retinal microvasculature, and early stages of atherosclerosis, such as arterial stiffness, endothelial dysfunction, and increased carotid intima-medial thickness [2].

The most recent data show that in one third of young people with T1D there is evidence of early manifestations of at least one vascular complication after an average duration of T1D of eight years. The prevalence of the different complications has been reported to be: DKD 5.8%, DR 5.6%, DNeu 8.5%, arterial stiffness 11.6%, hypertension 10.1% and cardiovascular autonomic neuropathy 14.4% [7].

The increased risk of developing vascular complications during adolescence is the result of difficulties in achieving optimal glycemic control in this age group, with data consistently showing an increase in hemoglobin (HbA1c) during this period of life [2,3]. Non-optimal levels of HbA1c in adolescence are mainly due to poor adherence to self-management of diabetes, the presence of eating disorders and psychological problems [2]. However, other factors, such as rapid growth and pubertal hormonal changes can also contribute to the risk of complications, together with the effect of cardio-metabolic risk factors such as obesity, insulin resistance, hypertension, and dyslipidemia [2,3].

About 86% of adolescents have a cardiometabolic risk factor and 14-45% have at least two. In addition, a cluster of these risk factors is associated with high rates of multiple vascular complications [3].

Other risk factors for vascular complications in adolescence include sex (increased risk in females), duration of T1D, genetic predisposition and lifestyle, especially smoking, alcohol, physical inactivity and stress. [2,3].

When to start screening for vascular complications

At the initial stage, vascular complications are often silent. Once Developed, symptoms may be more difficult to reverse. Therefore, identification of subjects at risk and screening for subclinical signs of complications is essential to early implementation of effective preventive and therapeutic strategies.

International guidelines recommend starting screening for vascular complications during adolescence. Based on the International Society for Pediatric and Adolescent Diabetes (ISPAD), screening should begin from the age of 11, with a duration of diabetes of 2-5 years and repeated each year, with a potential variation in frequency according to the risk profile of the individual [8].

DKD screening is based on the detection of abnormal levels of urinary albumin (albuminuria) that can be obtained with 24 hours or collections of timed urine or even by measuring the albumin-creatinine ratio (ACR) on a spot urinary sample [8].

Twenty-four-hour or timed urine collections are often difficult to collect in children and adolescents and therefore ACR is the preferred method in this age group. Assessing ACR in a spot urinary sample is the easiest method to evaluate albuminuria in an office setting. However, first-voided urine in the morning is preferable because of the diurnal variation in albumin excretion and postural effects. Because of biological variability, albuminuria should be found in at least two of three urine samples [8].

Albuminuria (previously described as microalbuminuria) is defined as an ACR between 2.5 and 25 mg/mmol in males and 3.5 to 25 mg/mmol in females. Values above the upper limit of the albuminuria range indicate proteinuria (previously defined as macroalbuminuria) [8].

Annual DR screening is conducted using a dilated eye examination by an ophthalmologist or optometrist. In patients with a duration of diabetes of less than ten years and good glycemic control, screening can be repeated every two years, while it is recommended to repeat it more frequently if they are at high risk [8].

Screening for peripheral DNeu is based on a comprehensive foot examination including inspection, palpation of pulses, assessment of reflexes, determination of proprioception and vibration, and monofilament sensation. This should be complemented by clinical data, such as a history

of pain, paresthesia, and numbness [8]. Currently, there is no recommendation to perform screening for autonomic neuropathy.

For macrovascular disease, current international guidelines recommend regular screening for hypertension and dyslipidemia [8]. Under the age of 13 years, hypertension is defined as systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) ≥95th percentile for age, sex, and height, on three or more occasions**.** Confirmation of hypertension may require 24-hour ambulatory blood pressure monitoring.

Elevated blood pressure (previously defined as prehypertension) is defined as blood pressure in the 90–95th centiles or ≥120–129/80 mmHg in adolescents aged 13 years or older.

Screening for dyslipidemia should be carried out from the age of 11 years regardless of the duration of diabetes. If lipid levels are within normal values, they should be repeated every three to five years. In case of family history of hypercholesterolemia or early cardiovascular disease, screening should be commenced as early as two years of age [8]. Non-fasting lipids are appropriate for screening, but if results are abnormal, they need to be confirmed in the fasting status.

How to treat vascular complications

- **Improving glycemic control**

One of the key objectives to prevent vascular complications is to achieve optimal blood glucose control by intensifying insulin therapy[8]. This is supported by a large number of tests regarding the association between suboptimal glycemic control and the risk of complications, starting from the control of diabetes and complications Trial/ Epidemiology of interventions and complications of diabetes up to more recent observational studies[8].

Significant advances in T1D management technology achieved over the past 10 years, such as the introduction of continuous subcutaneous insulin infusion and continuous glucose monitoring (CGM), up to the latest automated insulin delivery systems, 3 combining real-time CGM with insulin pumps and a control algorithm to direct automated insulin administration, have contributed to better results.

Despite such progress, most adolescents do not achieve the recommended objectives for glycemic control (HbA1c <53 mmol/mol) [2,3]. This testifies to the need for additional new therapies to achieve better glycemic control, along with the management of other metabolic risk factors.

- **Targeting additional risk factors**

Before implementing drug interventions, international guidelines recommend lifestyle interventions as the first steps to control dyslipidemia and hypertension [8].

Initial treatment of elevated blood pressure prevede dietary changes and increased exercise. If the objectives are not reached within 3 to 6 months, pharmacological interventions may be necessary.[8]. Anti-hypertensive drugs should be implemented earlier if hypertension is detected. Recommended anti-hypertensive drugs are angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), long-acting calcium channel blockers, or a thiazide diuretic. ACEIs (or, if not tolerated, ARBs) are recommended for use in adolescents with hypertension and albuminuria [8].

In the case of dyslipidemia, if fasting LDL cholesterol is >2.6 mmol/l (>100 mg/l), it is first necessary to implement lifestyle interventions and improve glycemic control. Statins should be introduced if LDL cholesterol below 3.4 mmol/l is not reached (<130 mg/l)[8].

In adolescence, the use rates of statins and ACE in adolescents with T1D are still low. This is due to limited data and experience with these drugs in this population. In the study Adolescent Type 1 diabetes cardio-renal Intervention Trial (AdDIT), evaluating the use of ACEI and statins during adolescence to protect against vascular complications, statins have been shown to reduce exposure to high lipid levels and ACEI can reduce new cases of albuminuria. This could potentially lead to protection against future complications [9]. Reassuring evidence was also provided about the safety profile of both drugs in this age group [9], which should support doctors in their prescription.

Healthy eating and appropriate physical activity are recommended in adolescents to reduce the risk of overweight and obesity [8], together with preventive measures for additional risk factors, such as smoking and alcohol alcohol [8].

- **Additional interventions**

Vision threatening DR is generally treated with laser photocoagulation or, more recently, with intravitreal injections of anti-vascular endothelial growth factor (VEGF). Anti-VEGF treatment is also used to treat diabetic macular edema with visual loss [8].

Several additional treatments have been studied to improve glycemic control, weight control and other risk factors. These include metformin, sodium-glucose co-transporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 receptor agonist, and dipeptidyl peptidase-4 inhibitors [10].

The potential benefit of adding metformin to insulin treatment in youth with T1D is still a matter of debate. A meta-analysis did not show better glycemic control with the addition of metformin. Its use, however, has been associated with a reduction, although small, of the total daily dose of insulin and BMI[3]. More recent studies have provided preliminary data to support the use of metformin in young people with T1D to improve insulin sensitivity and cardiovascular risk profile. However, more extensive studies are needed to confirm these results [3].

In adults with diabetes, SGLT2-inhibitors are associated with improved HbA1c, weight control, blood pressure, and cardiovascular and renal outcomes. However, there is a paucity of data for youth with T1D [10]. While these drugs hold promise, the risk associated with euglycemic ketoacidosis remains a concern for implementation in the paediatric population. [10].

Take-home messages

- Early subclinical manifestations of vascular complications are common in adolescents with T1D.
- T risk factors are T1D duration, female sex, suboptimal glycemic control, hypertension, dyslipidemia, obesity, smoking and genetic predisposition.
- Adolescence provides important opportunities for prevention and intervention strategies to prevent vascular complications.Targeting glycemic control, blood pressure, and dyslipidemia are the main goals of current treatment strategies, but there is a need for more tailored personalized interventions.

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SECTION 1 CHAPTER 11

COMMON COMORBIDITIES: COELIAC DISEASE

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Introduction

Celiac disease (CD) is defined as an immune-mediated systemic disorder precipitated by exposure to gluten and related prolamines in genetically susceptible patients, where enteropathy is the most common clinical feature [1]. Even though a recognized dietary antigen is considered a trigger agent, CD is classified as an autoimmune disorder (AD) for the following reasons: a common genetic background shared with other ADs, the presence of autoantibodies, and CD8+ T cells mediated tissue damage.

CD patients and their first-degree relatives are estimated to have an overall burden to develope other concomitant ADs of up to 30%. In children, the most frequent AD associated with CD is type 1 diabetes (T1D). It seems that the prevalence of T1D in patients with CD is up to. Otherwise, the prevalence of CD in patients with T1D is even higher, between 2 and 11[3]. The prospective TEDDY cohort study demonstrated that the onset of T1D generally precedes CD diagnosis, with CD developing more often within the next five years. Moreover, T1D and CD are both more frequent in individuals affected by other ADs or genetic syndromes, like Down and Turner syndromes. Surprisingly, female sex is a risk factor for developing CD while for T1D the male sex appears to be more affected. When the two disorders coexist, the female to male ratio becomes 1:1.

CD and type 1 diabetes share common pathogenetic mechanisms

The association of CD and T1D has historically been attributed to a common genetic background. Similar to many autoimmune diseases, the HLA genes represent the strongest genetic risk factor. HLA-DRB1*04-DQA1*03:01-DQB1*03:02 (HLA-DR4-DQ8) and HLA-DRB1*03-DQA1*05:01-DQB1*02:01 (HLA-DR3-DQ2) are HLA haplotypes known as predisposition factors for either conditions. Different combinations of these haplotypes pose different risks for T1D and CD. In particular, homozygosity for HLA-DR3-DQ2 is the most predisposed to CD while for T1D are HLA-DR3-DQ2 and HLA-DR4-DQ8 that give the highest risk. The genotype HLA-DR3-DQ2 and HLA-DR4-DQ8, finally, characterizes most patients with coexistence of T1D and coeliac disease [4]. HLA genes account for almost 50% of the genetic risk. Genome wide association studies have discovered other non-HLA genes with 26 loci found to be associated with both disorders. As might be expected, these genes code especially for proteins involved in the immune response, thus acquiring a protective or predisposing role.

However, the common genetic background cannot explain the rapid increase in the prevalence of CD and T1D during the last 50 years. The common environmental factors, on the other hand, are more likely to play a role. CD and T1D share very similar pathogenic mechanisms. In both conditions, environmental triggers, like changes in intestinal microbiota or seasonal infection or seasonal infections, activate—through different mechanisms—a CD4 specific immune response directed against gluten in CD and different islet cell antigens (ICA) in T1D. Some evidence shows that gluten itself, which is essential for the development of CD, can act as a trigger also for other ADs, including T1D. For example, NOD mice fed a gluten-containing diet developed T1D significantly more than NOD mice fed a lifetime gluten-free diet (GFD). Incidence was 15% versus 64% [5]. This incidence could even be reduced to 8% if NOS mice were fed GFD during pregnancy. Similarly, in humans a high gluten intake by mothers during pregnancy increases (2-fold) the risk of their children developing T1D [6]. However, differently from CD, once T1D appears, gluten withdrawal is not able to stop the progression of the disease.

Antigen-specific CD4 T cells provide "a sign of help" for B cells to produce autoantibodies (anti-TG in CD and anti-ICA in T1D). In clinical practice, autoantibodies are very relevant in both T1D and CD for their diagnostic role. However, they do not appear to play an important

pathogenetic role. Furthermore, the presence of antigen-specific CD4+ T lymphocytes and autoantibodies alone is not enough to cause tissue damage in the absence of activated CD8+ T lymphocytes. The presence of autoantibodies without enteropathy or β cell destruction is a condition named respectively potential celiac disease (PCD) and prediabetes. The effecting phase leading to tissue damage is the activation of cytotoxic cells that, by specific receptor-ligand interactions, infiltrate and destroy the target organ. In untreated CD patients, in particular, enterocytes upregulate their surface stress signals, such as MIC-A and HLA-E molecules, as part of a stress response to gluten, viral infections or other unknown triggers. These molecules bind respectively two NK receptors expressed on activated IELs: NKG2D and CD94. These molecules bind respectively two NK receptors expressed on activated IELs: NKG2D and CD94. Intestinal villous atrophy is determined by this ligand-receptor interaction [7]. In the T1D NOD model, similarly, NKG2D was expressed on intrapancreatic self-reactive CD8+ cells, while MHC-like molecules are expressed on stressed β cells. Unlike T1D, however, where CD4 T cells are found that are specific to an autologous antigen, such as DR4-restricted antinsulin cells found in pancreatic lymph nodes of T1D, in the CD model CD4 T cells are specific to a food antigen. Autoantibodies against TG2 are produced thanks to an intramolecular aid mechanism that exploits the gluten-TG2 complexes [8].

Clinical aspects

Because of the strong mutual association between CD and T1D, the presence of one disorder suggests a screening program for other concomitant ADs. However, the timing and modalities of screening are not uniformly recommended by different guidelines. For pediatric CD, ESPGHAN guidelines [1] do not specify the timing for screening nor the modality. In particular, screening for T1D through the detection of ICA antibodies does not seem to be effective due to their low specificity: the five-year positive predictive value of a single positive autoantibody for T1D is only around 10–25%, but increases when multiple antibodies are positive [9]. In clinical practice, glycemia is routinely checked when laboratory tests for the monitoring of CD are performed, generally every one to two years. For T1D, the NICE, ESPGHAN and NASPGHAN guidelines suggest screening for concomitant CD through the dosage of anti-TG antibodies at diagnosis (total IgA to rule out a shortage of IgA). It will then be carried out again at two and five years if the original screening was negative and the symptoms do not appear before [10-11-12]. However,

since long term complications of asymptomatic CD in T1D have been poorly explored, the American College of Gastroenterology, the American Diabetes Association, and more recently the US Preventive Task Force (primarily directed to adult care) [13-14-15], are more careful and recommend laboratory tests for CD only in case of suggestive clinical symptoms. The tendency to screen asymptomatic T1D children for CD is justified from the beneficial effect of a GFD on growth, nutritional parameters (iron and folic acid), bone mineral density and quality of life [16]. CD could also be an independent risk factor for the early development of diabetic nephropathy, retinopathy and cardiovascular disorders in adulthood. However, it remains to determine the effect of GFD on these complications. Moreover, it would appear that adherence to a GFD is similar in symptomatic and asymptomatic subjects. On the other hand, conflicting results have been found regarding the effect of a GFD on metabolic control. Most literature suggests, however, that there are no differences in hba1c concentration or risk of severe hypoglycaemia [16]. It should also be considered that maintaining a GFD can be heavy, especially in T1D children who must already control their daily carbohydrate intake, and that the use of packaged GFD products can lead to nutritional deficiencies and thus worsen cardiovascular and metabolic complications in T1D. Randomized clinical trials to evaluate the long term effect of GFDs on patients with a double diagnosis of asymptomatic CD and T1D are warranted.

As aforementioned, in T1D screening for CD through IgA anti-TG and total IgA measurement is recommended. Low antibody titres suggest caution and repeat testing (including antiendomysium (EMA) antibody detection) before performing a jejunal biopsy. In fact, screening for CD in T1D can lead to three different clinical scenarios: negative CD serology, positive CD serology with intestinal damage, or positive CD serology without intestinal damage (PCD). This last condition is more frequent in patients with T1D compared to the general population and its management (a GFD versus a gluten-containing diet) is also debated for patients without a concomitant T1D. More recent studies suggest that the progress of the PCD is only in half of cases. A third of patients even stop producing anti-TG2. Also, in T1D patients on a gluten-containing diet, anti-TG can present a temporary positivity and then become negative in 20% of the cases [17.] Unfortunately, we still do not know the chances for these patients to develop CD in future.

Conclusion

CD and T1D are two strongly associated conditions that share many pathogenetic features, including a common genetic HLA and non-HLA background, environmental triggers like virus infections, changes in intestinal microbiota and gluten itself, the presence of antigen specific CD4+ T cells and the presence of autoantibodies associated or not with damage to target organs (overt disease versus potential CD and prediabetes). Despite the long term benefits of a GFD in patients with T1D and asymptomatic CD these have not yet been fully exploited, and screening for CD is generally recommended after T1D is diagnosed.

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SECTION 1 CHAPTER 12

THYROID AUTOIMMUNITY IN TYPE 1 DIABETES MELLITUS

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Introduction

Autoimmune thyroid diseases (AITD), Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the most prevalent autoimmune diseases in children and adolescents with type 1 diabetes mellitus (T1DM). AITD occur more frequently in T1DM children and adults than in the general population (1–3), suggesting a strong shared genetic susceptibility and pathological mechanisms between these diseases. On the other hand, T1DM and coeliac disease are the most frequently associated non-thyroidal autoimmune diseases in pediatric patients with AITD (4, 5).

Epidemiology

Patients with T1DM exhibit an increased risk of developing another autoimmune disorder such as AITD (HT and GD, 15–30%), Addison's disease (0.5%), autoimmune gastritis (5–10%), coeliac disease (4–9%) and vitiligo (2–10%) (3, 6).

Thyroid autoimmunity as evidenced by the presence of autoantibodies varies from 2.9% to 4.6% in the general population and from 12.1% to 23.4% in young patients with type 1 diabetes.

Thyroid antibodies are detected in only 17-25% of patients with T1DM at onset. Their presence is strongly predictive for the development of hypothyroidism (risk ratio of about 25%). (5, 7, 8). However, detections increase with age, duration and long-term persistence of antibodies against glutamic acid decarboxylase (GADAb) (3, 7) . They occur more frequently in 2.5-3 years from the onset of the disease. They are found more often in females than in males and often emerge during puberty (8).

AITD frequency in Europe is estimated at 3% for hypothyroidism and 0.75% for hyperthyroidism. Their incidence in T1DM patients is 2–4-fold higher than in the general population. In the T1DM population, a prevalence of 14–28% and 0.5–7% has been reported for HT and GD, respectively **(**2, 9–11**)**. Moreover, HT was more often diagnosed in white people, whereas GD was more commonly diagnosed in Black people (1).

Pathogenesis

AITD is characterized by lymphocytic infiltration due to the loss of immunological tolerance to specific thyroid autoantigens. This results in the production of autoantibodies against thyroglobulin 1 (TGAb), thyroxine peroxidase (tpoab) and thyroid stimulating hormone receptor (TSH) (Trab), causing alteration in thyroid function to varying degree (3).

Almost 25% of young patients (<21 years) diagnosed with T1DM present positivity to at least one other organ-specific autoantibody (2, 12).

To date, it is not yet completely clear the pathogenic mechanism that explains the high percentage of multiple autoimmunities in young T1DM patients. A common genetic substrate and a defect in the immune regulation common to several autoimmune diseases could explain this observation. It is widely known that some HLA haplotypes predispose to both T1DM and other autoimmune diseases. It is well known that some HLA haplotypes predispose at the same time to T1DM and other autoimmune diseases; for instance, the DR3-DQ2/DR3-DQ2 genotype is associated with TPOAb production (2). In addition, in patients with T1DM, haplotypes HLA-DQA1 0301, DQB 0301 and DQB1 0201 are associated with the development of hyperthyroidism, whereas haplotype DQA1 0501 is related to hypothyroidism. The haplotype HLADQB1 05 apparently plays a protective role in the development of AITD. (2, 3).

Specific diabetic autoimmunity such as GADAb, that reduces the conversion of glutamic acid into gamma-aminobutyric acid (GABA) in

patients with T1DM, may not only indicate an increased risk for a more severe course of T1DM but can also be considered a biomarker of other endocrine autoimmune diseases, as well as thyroid autoimmune ones (1, 12). A significant association between GADAb positivity, female sex and an increased rate of thyroid autoimmunity was reported (1, 12). GABA is located in follicular thyroid cells and is also involved in regulating the release of thyroid hormones. Therefore, the autoimmune response directed against this self-antigen in β cells could also affect thyrocytes (1, 12).

Moreover, there was a strong correlation between thyroid autoantibody and the female sex. In animal models as in T1DM patients, estradiol accelerates the progression of autoimmune diseases by disturbing the action of type 2 T-helper lymphocytes (Th2). Androgens, on the other hand, have a protective effect. The effect of female sex hormones on the development and progression of the autoimmune process can be confirmed by the increased incidence of autoantibodies in adolescents (3). It was reported that female adolescents with T1DM are three times more likely to develop positive TPOAb compared to males (1). Furthermore, in T1DM patients thyroid autoantibodies prevalence rises with age increasing and diabetes duration (7).

The highest prevalence of thyroid antibody positivity was observed around puberty (14–15 years) and after 3.5–4 years of diabetes (1).

The immune mechanisms involved in the pathogenesis of autoimmune diseases are complex and still not completely understood. An altered gut microbiota homeostasis due to antibiotics or probiotics may influence the development of T1DM, as well as other autoimmune diseases (3).

New evidence suggests a role of adipokines in increasing autoimmune disease incidence in obesity; in particular, the pro-inflammatory activity related to these adipokines would appear to be the link between the adipose tissue and the immune system (1). Even infections, although not directly responsible for the induction of autoimmunity, can amplify autoimmune processes through inflammation (1).

Otherwise, many genes play an important role in modulating the immune system. Their polymorphisms in genes such as those encoding the alpha subunit of the Interleukin 2 receptor (IL2RA), interferon induced with helicase C domain 1 (IFIH1) and cytotoxic cell antigen T 4 (CTLA-4) may affect the development of autoimmune disease. These data can confirm a strong genetic susceptibility to autoimmune diseases (4).

Thyroid hormones physiologically determine increased intestinal uptake of glucose, glycogenolysis and insulin catabolism in the liver, causing a hyperglycemic effect. Even a slight decrease in their levels can thus cause an increased risk of hypoglycemia. This explains why hypothyroidism in T1DM children is often associated with hypoglycemia as a result of heightened insulin sensitivity. In these patients is predictable the presence of impaired growth due to both chronic hypoglycemia and thyroid hormone deficiency (3).

Family history

There is some evidence that T1DM and TDI share a common genetic background, as they often concur with patients and families. It is recognised that the risk of autoimmune disorder increases in first-degree relatives of T1DM subjects: 8% of first-degree relatives have AITD (9). In comparison to the general population, family members of children with T1DM are more likely to have autoantibodies and other forms of autoimmune disease. (8).

Clinical course

Most patients with T1DM have no symptoms of thyroid dysfunction despite the presence of anti-thyroid antibodies in the blood serum. However, the presence of thyroid antibodies, common among new patients with T1DM onset, may be significant to predict the possible clinical manifestation of AITD (9).

Literature shows that as many as 30% of patients with T1DM may have hypothyroidism. Overt hypothyroidism is diagnosed in 4-18% of patients with T1DM, while subclinical hypothyroidism in 40-55%. These are higher prevalences than the general population (3).

Clinical features include the presence of painless goiter, weight gain, retarded growth, tiredness, lethargy, cold intolerance, bradycardia and dyslipidemia (6), while glycemic control may not be significantly impacted in the first stage of thyroid disease. Thyroid hypofunction due to progressive thyrocyte damage, as primary hypothyroidism or subclinical hypothyroidism (SH), is found in about 3-8% of young people with T1DM. (8). About 90% of T1DM patients TPOAb-positive, initially euthyroid, may progress to SH within 5 years (2).

Chronic hypoglycemia and thyroid hormone deficiency can cause growth disorders in T1DM children. On the contrary, no effects of SH on growth, BMI and glycemic control have been reported till now (3).

Confirmation of hypothyroidism occurs by demonstrating low free thyroxine (FT4) and increased concentration of TSH. Subclinical hypothyroidism, on the other hand, can be detected in an asymptomatic individual with a normal thyroxine level and slightly increased TSH. Hyperthyroidism should be suspected in case of unexplained difficulty in maintaining glycemic control, weight loss without loss of appetite, agitation, tachycardia, tremor, heat intolerance, thyroid enlargement, or characteristic eye signs (6, 8).

Hyperthyroidism in children T1DM has been shown to be associated primarily with acute complications of diabetes such as ketoacidosis, hypoglycemia or arterial hypertension. However, long-term metabolic control effects and the demand for insulin were not always reported. (10, 11). However, hyperthyroidism, by enhancing the metabolism of the entire body, could lead to an increase in glucose demand, stimulating gluconeogenesis and glycogenolysis, decreasing insulin sensitivity, increasing the absorption of muscle glucose and lipolysis. This can lead to deterioration of metabolic control (3). Hyperthyroidism is confirmed by observation of a reduced level of TSH and increased levels of FT4 and FT3, as well as the presence of Trab in GD.

Treatment

As well as in the general population, treatment of AITD in pediatric T1DM patients is based on replacement therapy with oral L-thyroxine in cases of hypothyroidism. This is enough to normalize TSH levels and, if a goitee is present, may allow its regression. (8).

Nevertheless, since dyslipidemia may be present in T1DM patients with SH, early L-thyroxine treatment should be considered in these patients to positively affect the risk of developing hyperlipidemia and atherosclerotic cardiovascular diseases (1).

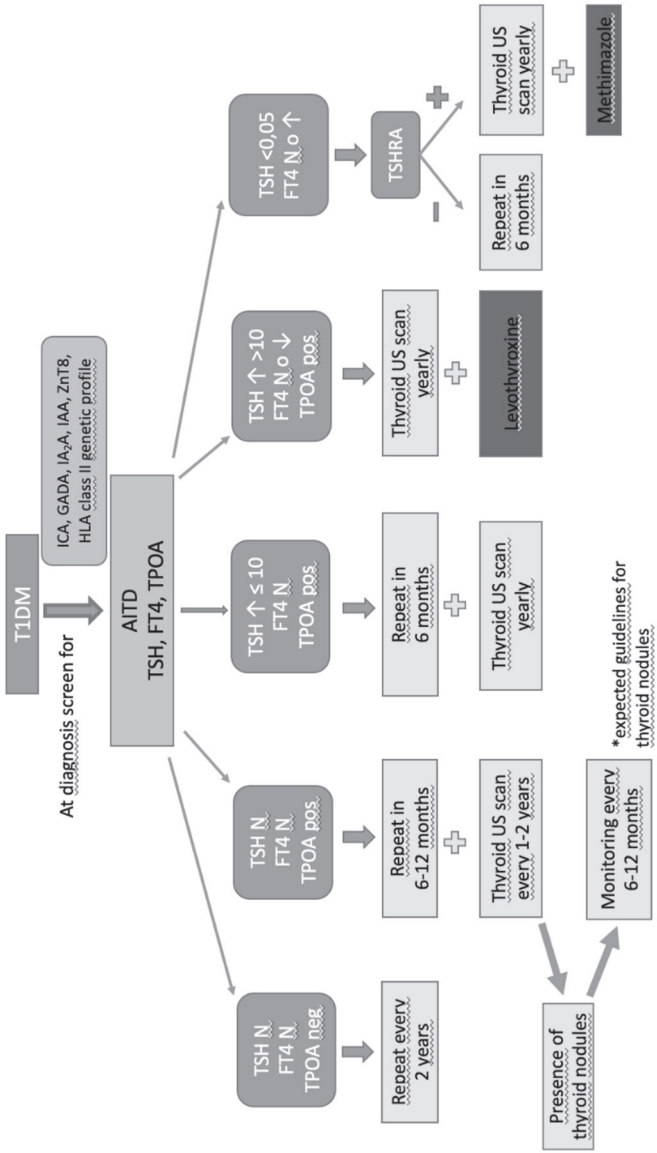
The most common antithyroid medications used are carbimazole and propiltiouracil; the former is the preferred treatment for children because propiltiouracil increases the risk of liver failure. Beta-adrenergic blocking drugs are usually indicated in the case of an acute phase of hyperthyroidism, such as thyrotoxicosis, to control tachycardia and

agitation. Surgery or radioactive iodine represent the main treatment options for persistent or recurrent hyperthyroidism (8). Preclinical diagnosis and early treatment of GD were not associated with better responsiveness to therapy (10).

Screening for AITD in T1DM patients

The observed increase in the incidence of other autoimmune diseases in patients suffering from T1DM necessitated the introduction of early screening of these diseases.: in accordance with the guidelines of the American Diabetes Association (ADA) and the International Society for Pediatric and Adolescent Diabetes (ISPAD), thyroid function and thyroid autoantibody should be evaluated in the diagnosis of diabetes in children with T1DM.(Figure 1). If thyroid screening shows no changes, the tests should be repeated every two years. A higher frequency is recommended if AITD symptoms occur (1, 3, 8, 13). In the case of positivity to TPOAb and TGAb, thyroid function tests are suggested every 6–12 months. In the presence of thyroid antibody positivity or goiter, a thyroid ultrasound should be performed at least once a year (Figure 1).

Figure 1. Screening algorithm for thyroid autoimmunity in type 1 diabetes mellitus.



AITD in T1DM and thyroid cancer

The relationship between thyroid malignancy and T1DM has been poorly investigated. An increased thyroid cancer prevalence has not been detected. Furthermore, the potential link of HT with thyroid carcinoma must also be considered. Recently, papillary thyroid cancer has been described in two young T1DM and HT females (14 and 20 years old) (1). These reports confirmed the usefulness of thyroid ultrasound scans in routine diagnostics in T1DM patients with suspected thyroid disease.

Conclusions

1. Patients with T1DM exhibit an increased risk of other autoimmune disorders such as AITD (HT and GD).
2. Screening for AITD is strongly recommended in all cases of new-onset T1DM in children and during the follow-up.
3. Early diagnosis of AITD can improve thyroid diseases and T1DM management.

Take Home Messages

1. AITD (HT and GD) are autoimmune diseases most frequently found in children with T1DM..
2. AITD occur more frequently in children and adults with T1DM than in the general population.
3. Screening of thyroid function, including TPOAb and TGAb evaluation, is recommended for diagnosis of T1DM and subsequently every 2 years in asymptomatic people without goiter and in the absence of thyroid autoantibodies. A more frequent assessment is indicated otherwise.
4. In the case of T1DM patients with positive thyroid antibodies, goiter or nodules, ultrasound of the thyroid gland is introduced into the routine diagnostic method
5. HLA class II genetic profiling in T1DM patients may be useful in identifying those at risk of multiple autoimmunities.

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SECTION 2:

GENETIC FORMS OF DIABETES

SECTION 2 CHAPTER 1

MONOGENIC DIABETES OF THE YOUNG

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Introduction

Until the end of the last millennium, it was falsely believed that the only pathogenesis of pediatric diabetes was autoimmune and that any child with diabetes had “Type 1 Diabetes Mellitus.”

In the 90s the Italian Study Group on Diabetes of the Italian Society of Pediatric Endocrinology and Diabetology (ISPED) conducted a polycentric study looking for children with pre-diabetes type 1. All centers had to report all children and adolescents with fasting glycemia above 100 mg/dl in the presumption that they were about to develop autoimmune diabetes. Disbelief was born when it was noted that very few children selected with blood sugar above 100 mg/dl fasting had positive antibodies and that, instead, they all had a disease that was believed to be very rare: monogenic diabetes. This is probably the reason why in Italy this form of diabetes seems to be so frequent in the pediatric age. As the *discovery of America* was “*accidental*” because Christopher Columbus was looking for a shorter way to reach the Indies, so Italian researchers were looking for *pre-diabetes type 1* and they came across *monogenic diabetes*.

Monogenic diabetes is due to mutation of single genes comprising a large spectrum of phenotypes, namely Neonatal Diabetes Mellitus (NDM), Maturity Onset Diabetes of the Young (MODY), Maternally Inherited Diabetes with Deafness (MIDD) and, rarer, diabetes-associated syndromic diseases. Various studies suggest a prevalence of MD from 1% to 6% among pediatric patients with diabetes and MODY is the commonest form of MD (~45%), followed by NDM. Most forms of MD are caused by a reduced ability to produce or secrete insulin, but rare variants result in insulin resistance.

Over the last decades, there have been extraordinary advances in the identification of the molecular genetic basis of MD and, to date, mutations in more than 14 different genes have been associated with the onset of MODY (Table 1). Functional analysis has revealed the implication of these genes in the pancreatic development and/or in the functional processes of the β cells involved in insulin secretion.

The most frequent forms of MODY are *GCK*-MODY2 and *HNF1α*-MODY3, which represent ~70% of all patients with MODY. While *GCK*-MODY2 is the most common subtype in Spain, Italy, France, Germany and the Czech Republic, *HNF1α*-MODY3 seems to be predominant in North Europe. Unfortunately, MODY is often incorrectly diagnosed as type 1 or type 2 diabetes, so patients are unnecessarily subjected to insulin therapy. However, if correctly diagnosed, they can be managed more appropriately with low-dose of sulphonylureas or, in some cases, no pharmacological intervention. Since treatment options can vary significantly between MODY subtypes, a precise molecular diagnosis is critically important to direct the most appropriate therapeutic choice for these patients, leading to improvement in their quality of life. In addition, once the mutation has been defined, family members at risk may be screened and, after genetic counselling, appropriate genetic tests may be proposed.

HNF4α - MODY 1

MODY1 is caused by mutations in the hepatocyte nuclear factor 4α *(HNF4α)* gene on chromosome 20q12-q13.1. HNF4α regulates gene expression of hepatic nd pancreatic β cells, embryogenesis and development in utero, amino acid metabolism and glucose and lipid homeostasis. It plays a different role in fetal and adult β cells. MODY1 patients share similar clinical and metabolic features to MODY3 patients, probably due to the connection between HNF-4α and HNF-1α. HNF-1α and HNF-4α, infact, interact functionally within pancreatic cells and regulate common targets.

Heterozygous mutations in the *HNF4α* gene are associated with macrosomia (56%) and neonatal hypoglycemia (37%) due to hyperinsulinemia in utero. Over time, apoptosis of β cells switches to defective insulin secretion later in life until developing diabetes at puberty or in the young adult. In the same families, it is possible to find cases of hyperinsulinism and cases of diabetes. As MODY3, MODY1 patients are particularly sensitive to sulfonylureas, even if, in some cases, the administration of insulin may be necessary.

GCK - MODY2

MODY2 is due to a glucokinase (*GCK*) gene mutation which encodes the glucokinase enzyme. Glucokinase acts as the "glucose sensor" in the β cell by regulating glucose metabolism with insulin secretion. Heterozygous loss-of-function *GCK* mutations have negative effects on the kinetic parameters of the enzyme leading to glucose-sensing defect and, therefore, an increase in the blood glucose threshold that triggers insulin secretion.

Clinically, a MODY2 patient is characterized by a mild increase of fasting glucose and HbA1c levels, usually with neither micro- nor macro-vascular complications and without the need for pharmacological intervention. Increasing evidence supports the idea that patients treated either with insulin or oral antidiabetic drugs, could stop therapy after the molecular diagnosis of *GCK*-MODY2 without a deterioration in their metabolic state. In some cases, improper treatment with insulin may lead to iatrogenic weight gain and insulin resistance. Due to the mild phenotype, it is evident that MODY2 patients require fewer clinical controls than other diabetic patients. Taken together, these elements explain the importance of a correct MODY2 diagnosis, especially in pediatric age, granting better clinical management and lower expenses for health care systems. Within this context, a simple and easy-to-implement 7-item flowchart (7-iF) was designed in order to identify those patients more likely affected by *CGK*-MODY2. Most updated criteria for etiological diagnosis of diabetes (i.e pancreatic antibodies, HbA1c values) are taken into account by this flowchart, which has been validated in one of the largest Italian pediatric diabetes cohorts.

HNF1α - MODY 3

MODY3 is caused by mutation in the hepatocyte nuclear factor 1α *(HNF1α)* gene, located on chromosome 12q24.2. HNF1α is a transcriptional factor that binds to DNA in the form of a homodimer or heterodimer with another transcription factor, HNF1β. It regulates the expression of genes involved

in glucose metabolism and genes involved in cell proliferation and apoptosis. It is expressed in the pancreas, kidney and liver, being involved in the regulation of the development and control of cellular maturation of these tissues. *HNF1α* mutations lead to reduced β cell mass or impaired function that causes a progressive reduction of insulin secretion and consequently hyperglycemia requiring drug therapy, with the tendency to develop micro- and macro-vascular complications. The onset of the disease usually is in adolescence with initial post-prandial hyperglycemia, and then generalized. Since HNF1α is also expressed in renal tubular cells, in MODY3 there is a low renal threshold for glucose uptake, due to an impaired expression of the tubular glucose transporter secondary to the HNF1α defect, therefore MODY3 patients could have glycosuria even if glycemia is lower than 180 mg/dL. Given the severity of the phenotype, many MODY3 patients are misdiagnosed either as type 1 diabetic and treated immediately with insulin, or as type 2 diabetic because obesity is widely increasing among the younger population. However, unlike other types of diabetes, in which the treatment is based on the use of metformin and insulin, the main therapy in MODY3 is sulfonylureas, oral hypoglycemic agents which bind to the SUR1 receptor associated with the K^+ ATP-dependent (K-ATP) channel, located on the β-cell membrane, in order to stimulate insulin secretion.

PDX1/IPF1 - MODY4

MODY4 is a very rare MODY subtype. It is due to mutations in the pancreatic and duodenal homeobox1 *(PDX1/IPF1)* gene, located on chromosome 13q12.1, which encodes a transcriptional factor necessary for pancreatic development and β cell maturation. Homozygous mutations are associated with pancreatic agenesis and permanent neonatal diabetes that requires insulin since birth, whereas heterozygous mutations lead to β cell impairment and consequently hyperglycemia, including permanent neonatal diabetes.

HNF1β - MODY5

MODY5 is caused by mutations of the hepatocyte nuclear factor 1 β *(HNF1β)* gene, located on chromosome 17q12. The protein is an active transcription factor in the form of a homodimer or heterodimer with HNF1α and is essential for renal development and the differentiation of the nephron, as well as being a critical factor in the regulation of pancreas development. When this gene is mutated, diabetes, congenital anomalies of the urogenital

tract and renal dysfunction can occur, therefore it has been suggested to replace the term MODY5 with the term "syndrome with renal cysts and diabetes" (RCAD) because of the elevated prevalence of the renal phenotype associated with early-onset diabetes. Diabetes is often diagnosed in early adulthood and insulin therapy is necessary. Hattersley et al. stated that patients with MODY5 are those with the lowest birth weight if they inherit the mutation from the father or if the mother is made euglycemic by insulin therapy compared to patients with MODY2 or MODY3, confirming that insulin, which is one of the most important hormones for fetal growth, is already deficient before birth.

NEUROD1 - MODY6

MODY6 is caused by mutations in the Neurogenic differentiation factor 1 (*NEUROD1*) gene, located on chromosome 2q31.3, which encodes a transcription factor of the basic-helix-loop-helix family, involved in pancreatic and neuronal development. *NEUROD1* heterozygous mutations lead to diabetes in children or young adults, while homozygous mutations cause neonatal diabetes and neurological anomalies.

KLF11 - MODY7

MODY7 is due to mutations in the Krueppel-like factor 11 *(KLF11)* gene, located on chromosome 2p25. which control the pancreatic exocrine cells growth and acts as a tumor suppressor for pancreatic cancers. This gene plays an important role in glucose signaling in pancreatic β cells and gene variants are associated with MODY-like diabetes.

CEL - MODY8

MODY8, also called “diabetes-pancreatic exocrine dysfunction syndrome”, is caused by mutations of the Carboxyl-ester lipase (*CEL*) gene, located on chromosome 9q34.3, which controls the endocrine and exocrine functions of the pancreas. MODY8 is characterized by early-onset diabetes in adolescence or young adulthood, generally before the age of 25 years, and a slowly progressive pancreatic exocrine insufficiency, associated with development of pancreatic cysts and lipomatosis (fatty replacement of pancreatic parenchyma). Mutations are frameshift deletions in regions rich in tandem repetitions (VNTR) of the gene.

PAX4 - MODY9

MODY9 is due to mutations in the paired box gene 4 (*PAX4*) gene, which is found on chromosome 7q32. This gene belongs to a family of PAX transcription factors (Paired Box), so-called because they are provided with particular paired box domains. It has a key role in fetal development, the carcinogenic process and the development of pancreatic cells.

INS - MODY10

MODY10 is due to mutations in the *INS* gene, located on chromosome 11p15.5, which codes for the precursor of proinsulin. Heterozygous mutations have already been previously associated with permanent neonatal diabetes. However, subsequent studies have associated mutations in *INS* with the clinical phenotype of MODY.

BLK - MODY11

MODY11 is caused by mutations of the *BLK* gene, located on chromosome 8p23-p22, which codes for a tyrosine kinase of B lymphocytes of the SRC proto-oncogene family. It is expressed in pancreatic β cells, where it stimulates the synthesis and secretion of glucose-induced insulin, through an activating action against some transcription factors such as IPF1, which has been defined as a primary switch for pancreatic endocrine and exocrine functions.

ABCC8 - MODY12 and *KCNJ11* - MODY13

MODY12 and 13 forms are attributable respectively to mutations in the *ABCC8* and *KCNJ11* genes, both found on chromosome 11p15.1. *KCNJ11* is responsible for the production of the KIR 6.2 subunit of the ATPase K^+ pump, in β cells. The dependent ATP potassium pump (K-ATP) is a hetero octamer consisting of 4 KIR 6.2 subunits and 4 SUR1 subunits encoded by the *ABCC8* gene. The channel is involved in the electrical activity of the β cell membrane, which is why it plays a fundamental role in insulin secretion.

APPL1 - MODY14

The recently discovered MODY14 is caused by heterozygous mutation in the adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (*APPL1*) gene on chromosome 3p14. APPL1 is an anchor

protein consisting of 709 amino acids that, as an adaptor protein, interacts with several proteins including critical components of the insulin signaling pathway. APPL1 expression has a strong correlation with glucose-induced insulin secretion, suggesting that the APPL1's positive in reported secretion in rodents also works in humans and strengthen the hypothesis that human mutations impairing APPL1 expression levels or its work in insulin signaling could decrease not only insulin sensitivity but also insulin secretion. Therefore, APPL1 mutations could be responsible for familiar forms of diabetes. This result is consistent with animal studies in which a very important regulatory role of APPL1 in glucose metabolism has been demonstrated; therefore this molecule could represent a possible target for future treatments aimed at maintaining glucose homeostasis.

Mitochondrial Diabetes

Maternally Inherited Diabetes and Deafness (MIDD) is a mitochondrial disorder characterized by maternally transmitted diabetes and sensorineural deafness. MIDD accounts for up to 1% of all diabetes cases in Europeans and is due to defects in mitochondrial DNA (mtDNA). In addition to diabetes and deafness, the clinical features of MIDD are myopathies and macular dystrophy. Diagnosis of MIDD is often made in early adulthood but first clinical manifestations can occur at any age. More often, MIDD is caused by a 3243A>G point mutation in the mitochondrial gene MT-TL1, codifying the mitochondrial tRNA for leucine and, in rare cases, by other mtDNA variants in MT-TE and MT-TK genes, correspondly encoding the mitochondrial tRNAs for glutamic acid, and lysine.

Diabetes is treated with oral antidiabetic agents and/or insulin therapy.

Take home messages

Why is it important to diagnose monogenic diabetes in pediatrics?

- to clarify the pathophysiology of diabetes mellitus
- to define the clinical evolution and the risk of complications
- to offer patients appropriate treatment based on the etiology of diabetes avoiding unnecessary insulin therapy with improvements in their quality of life
- to identify family members affected and make an early diagnosis even in the absence of symptoms

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SECTION 2 CHAPTER 2

NEONATAL DIABETES

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Introduction.

The concept of "neonatal diabetes" as it is known today emerged in 1995 when a paper that reviewed the clinical data of 57 patients with onset of diabetes within the first months of life identified two main clinical forms: a transient (transient neonatal diabetes mellitus, TNDM) and a permanent (PNDM) subtype. The authors defined NDM as “hyperglycemia occurring within the first month of life that lasts for at least two weeks and requires insulin therapy,” in order to distinguish it from occasional, short-lived hyperglycemia. While TNDM would, by definition, go into remission and not necessitate further insulin therapy, they also observed that it could

relapse later in life. Forty-seven patients met the authors' criteria for NDM, while ten additional patients diagnosed with diabetes within 3 months of birth were also considered for the study, widening the narrow boundaries of the neonatal period as they are defined in the pediatric world. The authors concluded that NDM was different from type 1 autoimmune diabetes (1), a concept later confirmed by Iafusco, who suggested that patients with diabetes onset within the first six months of life have clinical features that are different from type 1 diabetes (2). As a sign that NDM was ready for prime time, a paper describing the first genetic defect associated with TNDM, paternal uniparental isodisomy of chromosome 6 **(**UPD6**)**, was also published in the same year (3). As of 2020, more than 30 NDM genes have been identified (4,5) and it is now well established that NDM can be caused by mutations in single genes that can be dominant, recessive, X-linked or involve a double dose of imprinted genes. In addition, NDM can be either isolated or syndromic and, in most cases, is found in subjects without signs of type 1 diabetes autoimmunity. However, NDM can also be caused by mutations in genes involved in immune regulation (6) and these patients may show autoimmunity against multiple organs. In general, hyperglycemia in patients with NDM is treated with insulin (7), with the important exception of individuals bearing mutations that activate *KCNJ11* or *ABCC8* genes, who can be safely treated with oral hypoglycemic agents of the sulfonylureas class (see below).

NDM can be caused by different mechanisms that lead to pancreatic β cell dysfunction. In the brief dissertation that follows, we have tried to group these into four broad categories: 1) mutations causing defects in insulin production, secretion and/or β cell apoptosis, 2) mutations altering pancreas development or the maintenance of β cell identity, 3) mutations causing autoimmunity against β cells, 4) chromosomal aberrations leading to doubling the dosage of imprinted genes and related defects.

PNDM or TNDM caused by mutations in genes involved in insulin production, secretion and pancreatic β cell apoptosis: *INS*, *GCK*, *KCNJ11*, *ABCC8*, *SLC2A2*, *SLC19A2*, *EIF2AK3*, *EIF2S3, EIF2B1, IER3IP1.*

INS. Heterozygous, the gain of function mutations of the insulin gene **(***INS***)** is the second cause of PNDM in terms of frequency (4,5,8). These mutations cause insulin misfolding that results in reduced insulin content, with a dominant negative effect on the secretion of the normal allele product and, ultimately, the induction of endoplasmic reticulum (ER) stress that leads to

β cell apoptosis (4,8). Most patients with proteotoxic *INS* mutations present with diabetes between two and six months of age, but some individuals may have a later onset, even if they belong to the same family as an already confirmed PNDM proband. These mutations are usually located in the *INS* gene coding sequence but intronic mutations with an effect on splicing, leading to the production of altered proteotoxic insulin, have also been described (4). More rarely, biallelic loss-of-function *INS* mutations are found to be the cause of PNDM (e.g., mutations of the start codon). In this case, insulin is not produced at all and the patient presents with diabetes in the very first days of life (4). Finally, mutations in the *INS* gene promoter have been associated with exceedingly rare cases of TNDM (4). Patients with *INS* gene mutations must be treated with insulin.

GCK. Heterozygous loss-of-function mutations of the glucokinase gene (*GCK*) are probably the most frequent genetic defect leading to a (mild) glucose metabolism alteration, known as GCK/MODY (Maturity Onset Diabetes of the Young). On the other hand, biallelic *GCK* mutations cause PNDM (with very rare exceptions), with onset within 1–2 days from birth. In these cases, PNDM ensues as a consequence of the complete failure of glucokinase to phosphorylate glucose in the β cell, leading to a lack of ATP production and a blocked insulin secretion. These cases are rare and mainly found in populations with a high consanguinity rate (4). These cases are also treated with insulin, though in principle they should respond favorably to sulfonylureas (SU). However, attempts to treat these patients with SU have been unsuccessful so far.

KCNJ11*, *ABCC8. Heterozygous, activating mutations of these two genes, which encode for the two subunits of the ATP-dependent potassium channel (K_{ATP}), are by far the most frequent causes of PNDM and TNDM. *KCNJ11* (encoding for KIR6.2) mutations are more commonly found to be the cause of PNDM, while *ABCC8* (encoding for SUR1A) mutations are a common cause of TNDM. Heterozygous, activating mutations in *KCNJ11* and *ABCC8* have an impact on insulin secretion by altering the ATP-dependent closure of the K_{ATP} channel, which is needed to trigger β cell membrane depolarization and the subsequent influx of Ca^{2+} ions. Interestingly, though rarely, PNDM can also be caused by a combination of activating and inactivating mutations of ABCC8 (9). More than 90% of PNDM cases associated with activating mutations of *KCNJ11* and *ABCC8* respond to treatment with sulfonylureas (SU), with long-lasting (>10 years) efficacy (10, and F. Barbetti et al., unpublished observations). In 81 patients with *KCNJ11*/PNDM, the median sulfonylurea dose at one year after the switch from insulin was 0.30 mg/kg/d (10). In addition to PNDM, severe *KCNJ11*

and *ABCC8* mutations may cause developmental delays with (DEND) or without (intermediate/incomplete DEND, iDEND) epilepsy. In patients with DEND, glibenclamide doses at the time of switchover from insulin may exceed 2 mg/kg/d and may stay as high as 0.87 mg/kg/d after five years of therapy (e.g., mutation *KCNJ11*/Val59Ala) (11). The quite vast neurologic symptomatology associated with *KCNJ11* and *ABCC8* mutations may partially benefit from SU treatment as well (10). However, cases with DEND may fail to respond to SU and often require insulin theraphy. While cases with K_{ATP}/PNDM (severe mutations) are usually sporadic, it is not uncommon to find family members of patients with K_{ATP}/TNDM (mutations with moderate effect) who are themselves mutation carriers but have onset of diabetes or other glucose metabolism disturbances later in life. This phenotypic heterogeneity may, in some cases, be due to a TNDM-phase that has been mild enough to be overlooked (F Barbetti et al., submitted for publication).

SLC2A2. Biallelic mutations of *SLC2A2* codifying for GLUT2, a glucose transporter, cause Fanconi-Bickel syndrome (FBS), but they have very rarely been associated with TNDM with affected patients developing features of FBS only later in life (4,8).

SLC19A2. Biallelic mutations of *SLC19A2* (solute carrier family 19 member 2) cause a rare syndrome (less than 90 cases reported) composed of megaloblastic anemia, insulin-deficient diabetes (with onset during the neonatal or infancy period) and sensorineural deafness called TRMA (Thiamine-responsive megaloblastic anemia, or Rogers' syndrome). It is known that the *SLC19A2* gene product is the only thiamine transporter of the pancreatic β cell (in addition to bone marrow and a subgroup of cochlear cells), but the pathophysiology of impaired insulin secretion and/or β cell dysfunction is presently unclear. Of note, patients may respond to high dose thiamine supplementation with the resolution of their megaloblastic anemia and an improvement of glucose metabolism (12).

EIF2AK3. Homozygous or compound heterozygous mutations in *EIF2AK3* cause Wolcott-Rallison syndrome (WRS), which is characterized by neonatal/infancy onset diabetes, short stature and recurrent liver dysfunction that may result in sudden hepatic failure and death. WRS is the first cause of neonatal diabetes in countries where consanguineous marriages are common. The pathophysiology of diabetes in WRS had first been linked to ER stress and β cell apoptosis due to uncontrolled protein synthesis. More recently, however, alternative mechanisms of disease have been proposed that involve impaired fetal β cell development resulting in reduced β cell

mass and impaired insulin secretion (13). Of interest, a simultaneous pancreas-liver-kidney transplantation has been performed successfully in a WRS patient with life-threatening liver failure (14).

EIF2S3. *EIF2S3* encodes for eIF2γ**,** one of the components of the heterotrimeric eIF2 complex that is crucial for translation initiation. Mutations of *EIF2S3*, which maps at Xp22.13-p21, give rise to MEHMO (Mental delay, Epileptic seizures, Hypogonadism, Microcephaly, Obesity) syndrome. Recently, patients with an *EIF2S3* mutation have been described who, in addition to typical MEHMO syndrome, showed early-onset diabetes or alternating hypo- and hyperglycemia.

EIF2B1. Very recently de novo mutations of *EIF2B1* have been identified in five individuals with neonatal diabetes. *EIF2B1* encodes the α subunit (eIF2Bα) of the heterodecameric EIF2B complex which is involved in the regulation of eukaryotic Translation Initiation Factor 2 (eIF2) (15). eIF2Bα**,** by binding phosphorylated eIF2α, modulates protein synthesis and the integrated stress response. The eIF2Bα mutations that have been found affect the region coding for the part of the molecule that interacts with eIF2α, probably leading to an unregulated unfolded protein response and β cell death (15).

IER3IP1. Mutations of *IER3IP1* were first described in patients with neonatal diabetes, microcephaly and epilepsy in 2011. The *IER3IP1* gene product is an endoplasmic reticulum protein and biallelic loss-of-function mutations likely determine a severely reduced ability of neurons and pancreatic β cells to cope with ER stress, which in turn triggers cell apoptosis.

Mutations in other genes involved in the integrated stress response and β cell apoptosis**,** such as *DNAJC3* and *PPP1R15B,* cause diabetes at an age outside the neonatal period and are not treated here.

PNDM caused by mutations in the genes involved in endocrine pancreas development and maintenance of β cell identity: *CNOT1*, *GATA6, GATA4, GLIS3, HNF1B, MNX1, NEUROD1, NEUROG3, NKX2-2*, *PDX1*, *PTF1A, RFX*

A gene causing isolated pancreatic agenesis: ***PDX1*.** *PDX1* gene defects are the archetype of neonatal diabetes due to pancreatic agenesis**,** resulting in neonatal diabetes and complete exocrine pancreas insufficiency. *PDX1*

(also known as *IPF1*) is specifically expressed in the developing pancreas and, for this reason, the effects of loss-of-function biallelic mutations are confined to this organ. Interestingly, hypomorphic mutations of *PDX1* can give rise to isolated neonatal diabetes, without exocrine deficits. Differently, all other genes involved in pancreas development are also expressed in other tissues and give rise to complex syndromes that include neonatal diabetes and defects in other organs. *PDX1* is also expressed in the fully differentiated β cell, where it is involved in the regulation of insulin gene transcription and in maintaining β cell identity.

Genes causing PNDM and defects of the central nervous system: ***CNOT1***, ***MNX1, NEUROD1***, ***NKX2-2***, ***PTF1A***. Recessive mutations in four of these genes cause PNDM associated with CNS defects spanning from cerebellar agenesis (*PTF1A*) to cerebellar hypoplasia and sensorineural deafness (*NEUROD1*) to severe developmental delay (*NKX2-2*, *MNX1*) (15). Of note, mutations in a distal enhancer of *PTF1A* lead to isolated pancreatic agenesis. A syndrome that includes pancreatic agenesis and holoprosencephaly linked to a specific heterozygous mutation of *CNOT1* has recently been described by two research groups in four unrelated subjects (5).

Genes causing PNDM/TNDM and cardiac defects: *GATA6*, *GATA4*. Heterozygous mutations of *GATA6* and *GATA4* give rise to PNDM due to pancreatic agenesis (*GATA6*, *GATA4*) or TNDM with pancreatic hypoplasia (*GATA4*), paired with congenital cardiac defects such as atrial and ventral septal defects (*GATA6*) or septal defects plus pulmonary stenosis (*GATA4*).

Genes causing PNDM and gastrointestinal defects: *RFX6*, *NEUROG3*. Biallelic mutations of *RFX6* cause a complex syndrome with typical findings as bowel atresia, gallbladder agenesis and neonatal diabetes due to the absence or severely reduced numbers of β, α, and γ cells (Mitchell-Riley s.) (17). The *RFX6* gene is also crucial for maintaining the functional identity of adult pancreatic β cells. *NEUROG3* biallelic mutations cause neonatal diabetes and enteric anendocrinosis; patients with this syndrome suffer from severe malabsorptive diarrhea in addition to hyperglycemia.

A gene causing PNDM and congenital hypothyroidism: *GLIS3*. A syndrome including PNDM and congenital hypothyroidism due to recessive mutations of *GLIS3* was described in 2006 in six patients. As expected, this transcription factor is highly expressed in the pancreas and thyroid. A reappraisal of clinical data obtained in 11 patients has recently expanded the GLIS3 clinical phenotype to liver and renal dysfunction as well.

A gene causing TNDM/PNDM and renal defects: *HNF1B*. Rarely, heterozygous mutations of *HNF1B*, usually associated with autosomal dominant diabetes with onset in adolescence or adulthood (*HNF1B*/MODY), cause severe pancreatic hypoplasia and neonatal diabetes mellitus (both transient/relapsing and permanent). In these individuals**,** renal abnormalities (e.g., renal cysts, other) are also found.

PNDM due to mutations in genes of autoimmunity: *FOXP3*, *IL2RA, LRBA.* *FOXP3* mutations were identified in 2001 as the cause of a syndrome consisting of autoimmune neonatal diabetes (with positive type 1 diabetes autoantibodies), polyendocrinopathy and enteropathy (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked, IPEX). *FOXP3* codify for a transcription factor that, in quiescent conditions, is mainly expressed in T regulatory (T_{REG}) cells. *FOXP3* mutations lead to a functional impairment of the suppressive activity of T_{REG} cells which in turn impinges on B cell regulation. IPEX phenotypes are highly variable**,** and the severe clinical expression of the disease can often require hematopoietic stem cell transplantation as a therapeutic tool (6).

A single case of autoimmune PNDM (diabetes onset: six weeks) associated with compound heterozygous, loss-of-function mutations of *IL2RA* (CD-25) has been described in a patient with IPEX-like features (6).

Recessive mutations with the complete loss of Lipopolysaccharide-responsive Beige-like Anchor (LRBA) protein can rarely cause autoimmune diabetes during the neonatal period. Interestingly, however, type 1 diabetes autoantibodies are negative in most patients with *LRBA*/PNDM. Additional features of patients with *LRBA* mutations include hematological disorders (e.g., autoimmune proliferative disease), gastrointestinal disorders (autoimmune enteropathy) and recurrent infections (6). Previously, *LRBA* mutations have been implicated in the pathogenesis of Common Variable Immunodeficiency-8 (CVID-8).

These three genetic defects, together with those deriving from mutations in CTL4, BACH2, IL10 and STAT3, are collectively called "Tregopathies" (18).

Other genes, such as *STAT1*, *STAT3*, *SIRT1*, *ITCH* and *AIRE,* which are implicated in monogenic autoimmune diabetes with onset in infancy (STAT1; STAT3)/childhood/adulthood, are not treated here.

TNDM due to 6q24 aberrations and *ZFP57* mutations

Paternal uniparental disomy (UDP) of chromosome 6 (UDP6) (3) usually causes about 60–70% of TNDM cases with onset in the first week of life. In this disease, because partial or complete uniparental disomy of the paternal chromosome 6, there is transcription of imprinted, unmethylated genes on the two paternal copies with a "double dose" gene expression (4). Differently from TNDM patients affected by *ABCC8* and *KCNJ11* mutations, patients with UDP6 may present minor dysmorphic features such as umbilical hernia and macroglossia. Furthermore, while most chromosome 6q24 defects are sporadic, an autosomal dominant pattern is often observed in *ABCC8*/TNDM or *KCNJ11*/TNDM.

In 2008 recessive mutations of the *ZFP57* gene have been identified as a rare cause of TNDM. Individuals with *ZFP57*/TNDM may show developmental delay with normal intelligence and cardiac defects in addition to the malformations found in UDP6 (19).

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SECTION 3:

HYPOGLICEMIA

SECTION 3 CHAPTER 1

CONGENITAL HYPERINSULINISM

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List of Abbreviations *(Italics for genes):*

ABCC8: gene codifying for ATP-Binding Cassette, subfamily C, member 8
CACNA1D: gene codifying for Calcium Channel, Voltage-Dependent, L-Type, Alpha-1D Subunit
CDKN1C: gene codifying for Cyclin-Dependent Kinase Inhibitor 1C
CH: Congenital Hyperinsulinism
ChD: Channel Defects
DZX: Diazoxide
FOXA2: gene codifying for Forkhead Box A2 Transcriptional Factor
GCK: gene codifying for Glucokinase enzyme
GLP-1: Glucagon-like peptide-1
GLUD1: gene codifying for Glutamate Dehydrogenase enzyme
H19: imprinted maternally expressed noncoding transcript
HADH: gene codifying for 3-Hydroxyacyl-CoA Dehydrogenase enzyme
HK1: gene codifying for Hexokinase 1 enzyme
HNF1 α: gene codifying for Hepatocyte Nuclear Factor-1-Alpha
HNF4 α: gene codifying for Hepatocyte Nuclear Factor-4-Alpha
HY: hypoglycemia
IGF2: Insulin Like Growth Factor 2
K_{ATP}: ATP-sensitive membrane potassium channels
KCNJ11: gene codifying for Potassium Channel, Inwardly Rectifying, Subfamily J, member 11

KCNQ: gene codifying for voltage-gated potassium channel Kv7.1, KQT-like Subfamily, member 1
Kir6.2: Inwardly Rectifying Potassium Channel, subunit of K_{ATP}
PG: plasma glucose
PGM1: gene codifying for Phospho-Gluco-Mutase 1 enzyme
PMM2: gene codifying for Phospho-Manno-Mutase 2 enzyme
SLC16A1: gene codifying for Solute Carrier Family 16 (Monocarboxylic Acid Transporter)
SUR1: Sulfonylurea Receptor, subunit of K_{ATP}
UCP2: gene codifying for Uncoupling protein 2

Background: Neonatal Hypoglycemia

Neonatal hypoglycemia (HY) can be defined as the concentration of plasma glucose (PG) low enough to cause symptoms and/or signs of impaired cognitive function in the newborn. This concentration could be unique for each individual, also varying during the first 48 hours of adaptation to extrauterine life, when PG physiologically decreases. Unfortunately, many infants can also be asymptomatic as a reactive hormone response and neural distress occur. So, close glucose monitoring is crucial for "at risk" neonates, identifiable on the basis of phenotypic and anamnestic findings (large for gestational age, small for gestational age, fetal/perinatal distress, diabetic mother). Those showing persistent HY above the first 48–72 hours of adaptation to extrauterine life, should be investigated for congenital endocrine/metabolic disorders[1]. Official guidelines recommend investigation and treatment of asymptomatic newborns with PG lower than specific threshold levels for age (25 mg/dL: first 4 hours of life; 35 mg/dL: 4–24 hours; 50 mg/dL: 24–48 hours; 60 mg/dL: >48 hours). Some long-term follow-up studies have identified a PG of 45–47 mg/dL as a safe target to prevent a neurological deficit in infancy[2].

Definition and Epidemiology

Congenital hyperinsulinism (CH) is the most common cause of persistent HY in the pediatric population. Its incidence is approximately 1: 50,000 in the general population and up to 1: 2,500 in cases of consanguinity. CH encompasses a heterogeneous group of disorders characterized by dysregulation of insulin secretion from the pancreatic β cell[3]. In addition to the classic forms with neonatal onset, there are also late forms (0.5-5.0% of cases) with onset over twelve years, characterized by glycemic fluctuations [3].

Etiology and Pathogenesis

In healthy subjects, insulin secretion is strictly linked to PG level and glucose metabolism in the pancreatic β cell: after its uptake, the intracellular ATP/ADP ratio raises and ATP- sensitive membrane potassium channels (K_{ATP}) close, with consequent slow intracellular depolarization and opening of voltage-dependent calcium channels; this results in calcium-induced insulin exocytosis. In CH, one or more of these steps could be disrupted for a genetic defect, thus insulin release can become continuous and independent of serum PG levels or may be released following events such as meals and exercise[3].

Molecular basis and classification

In addition to the syndromic forms, about 14 genes are currently known which are responsible for the monogenic forms. Based on the known pathogens, CH can be divided into four categories[4].

I. *Channel Defects* (ChD; genes *ABCC8*, *KCNJ11, KCNQ1, CACNA1D*)

ABCC8 and KCNJ11 codify for K_{ATP} channel subunits Kir6.2 and SUR1, respectively. Their mutations cause the most prevalent (about 50%) and severe forms of CH, in which dysfunctional or low expressed (altered synthesis or maturation) K_{ATP} channels cause persistent membrane depolarization. They also show wide phenotypic variability, depending on the mutation type: double recessive mutations (homozygous or compound heterozygous) cause generally severe forms while dominant inactivating mutations are responsible for milder forms and even for late onset cases, sometimes characterized by hypo- and hyperglycemia, or just diabetes[5-7]; finally, paternally inherited monoallelic mutations can cause focal adenomatous hyperplasia of β cells as better explained below. Mutations in KCNQ1, a voltage-gated potassium channel (Kv7.1), cause cardiac arrhythmias, deafness and defects of the gastrointestinal system, but have also been reported to cause increased insulin release during oral glucose tolerance tests; CACNA1D mutations cause the activation of L-type voltage-sensitive calcium channel and, besides neuromuscular abnormalities, provoke continuous calcium-induced insulin release from β cells[4]. Some authors also include mutations of SLC16A1 in this first class, encoding for the monocarboxylate transporter 1 and responsible for pyruvate and lactate cellular uptake, physiologically low expressed on β cells. Its mutations lead to over-expression of the transporter and insulin over-release after

exercise (exercise induced CH) when pyruvate and lactate metabolism augment ATP concentration in the β-cell[4].

II. *Metabolic Defects* (MeD; genes *GLUD1, GCK, HADH, UCP2, HK1, PMM2, PGM1*)

This category includes enzymatic defects that cause alteration in the concentration of metabolites that trigger insulin release, mainly by intracellular increase in ATP. GLUD1 activating mutations represent the second most common cause of CH. They promote a decrease of glutamate dehydrogenase enzyme (GDH) susceptibility to the allosteric inhibition by GTP: in β cells this results in the augmented synthesis of α-ketoglutarate and consequent increased intracellular ATP/ADP ratio, and in hepatocytes it results in increased ammonia production. So, CH linked to GLUD1 mutations is also defined as Hyperinsulinism/Hyperammonemia syndrome. GDH is allosterically strengthened by leucine, so protein loading can result in HY with asymptomatic hyperammonaemia. It is sometimes associated with epilepsy, possibly due to reduced synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the brain, all this is due to the hyperactivity of GDH4.

Glucokinase is a key enzyme regulating the glucose threshold for insulin secretion. GCK activating mutations cause augmented affinity of this enzyme for glucose in β cells, resulting in augmented glucose metabolism and ATP production. Clinical presentations are variable for severity and age of onset (even adulthood) and a dominant inheritance is often detectable by familial anamnesis.

HADH recessive mutations are responsible for the most frequent CH form in the case of consanguineous parents. They cause defective L-3-hydroxyacylCoA-dehydrogenase activity and the loss of its inhibitory effect on GDH; consequently, also these forms show Leucine sensitivity, but not hyperammonemia since HADH is not expressed in the liver[4].

UCP2, HK1, PMM2, and PGM1 codify for enzymes involved in β cell glucose metabolism and their mutations enhance glucose oxidation and increase intracellular ATP synthesis.

III. *Transcription factors - defects* (TfD; genes *HNF1 α, HNF4 α, FOXA2*)

This subclass includes defects in the transcriptional factors that rules insulin secretion (HNF1α and HNF4α, hepatocyte nuclear factors) and the

embryogenesis and organogenesis of endoderm-derived tissues including the pancreas (FOXA2). The exact mechanisms by which these mutations cause insulin over-release have not been understood yet. A peculiar feature of HNFs forms is the switch from HY into maturity-onset diabetes of the young (MODY), because of progressive β cell failure.

IV. *Syndromic conditions*

CH could be evidence of various syndromic conditions like overgrowth syndromes (Beckwith- Wiedemann, Simpson Golabi-Behmel, Sotos, Costello), congenital disorders of glycosylation, postnatal growth failure syndromes (Kabuki, Costello) and other complex disorders in which the causative mechanism is still not clear.

Morphological classification

From the histopathological viewpoint, CH is classified into three variants[3,5]:

a) In diffuse forms, all β cells have the same defect at the molecular side (autosomal recessively or more rarely dominantly inherited) and show the same morphology: very abundant cytoplasm and highly abnormal nuclei (3–4 fold bigger than normal).
b) In focal forms, a β cell cluster develops as nodular adenomatous hyperplasia because of a confined molecular defect in the 11p15.1–11p15.5 imprinted region, which involves the ABCC8 or KCNJ11 genes. It sometimes develops in a patient with a recessive ABCC8/KCNJ11 paternally inherited mutation when a somatic loss of the maternal allele shows after. This "double hit" pathogenic process reveals the recessive ABCC8/KCNJ11 paternal mutation and causes the uncontrolled replication of a double mutated cell, due to the imbalance of growth factors/suppressor located in the 11p15 region (maternally expressed tumor suppressor genes H19 and CDKN1C; paternally expressed growth factor IGF2). The focal lesion is made of endocrine cells with large cytoplasm and nuclei, 3–5 fold bigger than nearby acinar nuclei.
c) In atypical forms, different molecular defects affect multiple foci throughout the entire pancreas and their cells show nuclear enlargement.

Clinical and Laboratory Findings

CH usually becomes clinically evident in the neonatal period with nonspecific symptoms like poor feeding, hypothermia, irritability, lethargy, apnea, seizures and even coma. Certain clinical features and metabolic alterations may suggest a specific molecular cause and guide genetic testing in order to rapidly achieve the best targeted management. Among the clinical features, macrosomia is frequently found in ABCC8/KCNJ11, GCK and HNFs forms; epilepsy independent from HY could be found in GLUD1 forms. Among the metabolic findings, hyperammonemia is a marker of GLUD1 mutations, while the elevation of plasmatic C4-OH-carnitine and urinary 3-OH-glutarate indicates a HADH double mutation. Neurologic impairment could progressively develop as a consequence of misdiagnosed/untreated hypoglycemic neonatal crisis and is strictly related to their recurrence and severity. Forms with late onset are mostly associated with peculiar dominant ABCC8/KCNJ11 or HNFs mutations. In both cases, they could appear with a switch from HY to hyperglycemia or even with impaired fasting glucose or diabetes. In these cases, a history of mild recurrent HY in infancy and/or gestational diabetes (especially for ChD) and diabetes appearing in adolescence (especially for HNFs) should be investigated. Also, GCK forms can be found in adulthood for a misdiagnosis of mild HY in infancy. Hypoglycemic symptoms in children and adolescents could be more specific and clearly recognized by the patients. They are classifiable as neurogenic, like tremors and sweating (due to the activation of the autonomic nervous system as a result of the stimulation of the counter regulatory response), and neuroglycopenic, like weakness, difficulty thinking, confusion, tiredness (due to the reduced availability of glucose for nerve cells). The occurrence of these symptoms with PG lower than 60 mg/dl and their prompt solution with glucose administration define the HY according to the “Whipple’s triad.”

Diagnosis and Management

It is essential to identify and treat neonatal HY through the screening of an at-risk newborn or when unspecific symptoms appear. Parenteral glucose should be promptly administered in case of symptoms: a bolus of 200 mg/kg, followed by infusion of 10% glucose at a velocity to be adapted on the basis of PG (6–8 mg/Kg/min)[8,9]. It is also fundamental to collect the “critical blood-urine sample” which allows an etiological diagnosis of persistent HY to be made, in order to prevent further crisis and neurologic sequelae. Any detectable level of insulin in a hypoglycemic plasma sample

is diagnostic of CH[8,9]. Indirect manifestations of this phenomenon are the lack of ketonemia and fatty acidemia. Urine must be free of ketones (excepted for HADH deficit). Additional diagnostic criteria for CH are: an increase in glucose levels > 1.5 mmol/L in response to glucagon or octreotide injection and the request of more than 8 mg/kg/min of intravenous glucose to keep euglycemia[5,6,8,9,10]. Once the diagnosis of CH is made, it is necessary to proceed with a genetic test; ABCC8/KCNJ11 mutations must be investigated first, except for cases in which metabolic and clinical features orient toward other forms. Concurrent to genetic tests, timely therapy should be started with diazoxide (DZX) and then, in the case of failure, with octreotide[5,6,9,10]. DZX binds the SUR1 subunit of KATP channels and, by their opening, inhibits insulin secretion. Its dose ranges from 5 to 25 mg/Kg/day given orally in three administrations. Side effects include hypertrichosis, fluid retention, cardiac failure, pulmonary hypertension and electrolytic imbalance. In some cases, chlorothiazide therapy may be necessary to prevent fluid retention[5,8,9,10]. Many ABCC8/KCNJ11 mutations cause DZX failure. These cases need an urgent 18-Fluoro-DOPA-PET to find out possible focal forms and must be treated with octreotide until definitive surgical resolution (focal adenoma excision). Octreotide is a long acting somatostatin analog that inhibits insulin secretion by decreasing insulin gene promoter activity and calcium mobilization. Its dosage ranges from 5 to 35 mcg/kg/day given by subcutaneous injection every 6–8 hours and requires continuous dosage adjustment for a possible tachyphylaxis. Side effects are mostly abdominal inconvenience, diarrhea (seldom necrotizing enterocolitis) and later, bile sludge/gallstones and suppression of pituitary hormones. Long acting analogs administrated every 28 days have also been successful for on children[5,8,9]. Other treatment options of DZX non-responsive cases include Nifedipine (a calcium channel blocker), Sirolimus (an immunosuppressive agent that blocks the mammalian target of rapamycin which appears to be over-expressed in β cells), Glucagon-like peptide-1 (GLP-1) receptor antagonist “Exendin” that opposes GLP-1 action to enhance insulin release[5,8,9]. According to the most recent evidence, 18-Fluoro-DOPA PET should be offered already when no mutation is identified or in the case of focal forms, which could be due to a single recessive paternal inherited mutation in ABCC8/KCNJ11, even if responsive to DZX. Sub-total pancreatectomy should be an option for diffuse forms, after drugs failure; but this could induce iatrogenic diabetes[6,10].

Take home messages

- Congenital hyperinsulinism (CH) is the main cause of neonatal prolonged hypoglycemia and is associated with high incidence of neurodevelopmental deficits.
- Any inappropriate measurable insulin level during hypoglycemia allows the diagnosis of hyperinsulinemic hypoglycemia that could be transient or persistent and so genetic (CH).
- CH shows high genetic, histopathological and phenotypic variability and could even appear in adolescence/adulthood.
- As soon as the diagnosis of CH is certain, a molecular investigation is necessary, together with a DZX trial in neonatal cases. Unresponsive cases could benefit from octreotide or other experimental drugs.
- An imaging study with 18F-DOPA-PET is required when a focal or atypical form is hypothesized on the basis of the molecular analysis to reach the definitive surgical solution.
- Surgery could be offered as a last option for diffuse forms not sufficiently controlled by available drugs but likely to lead to diabetes.

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SECTION 3 CHAPTER 2

METABOLIC HYPOGLYCEMIC DISEASES

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Introduction

Hypoglycemia is the result of a defect in one or several of the mechanisms that normally integrate glucose homeostasis during feeding and fasting (e.g., glycogenolysis, fatty acid oxidation, hormonal response). As such, it is a feature of a variety of different disorders [1]. This chapter focuses on the most common inborn metabolic disorders (IMD) causing hypoglycemia. Several mechanisms cooperate in order to maintain satisfactory glucose concentrations in humans (Figure 1). These mechanisms are finely regulated by hormonal signals (i.e., insulin, glucagon, cortisol, adrenaline, growth hormone, thyroxine) [2]. After a meal, increased insulin concentrations lead to glucose uptake into cells (GLUT2 transporter). Glucose can be either catabolized to produce ATP (via glycolysis and the Krebs cycle) or stored as glycogen in the liver. Besides glucose, several dietary carbohydrates (e.g., fructose, galactose) can enter glycolysis after being metabolized by specific enzymes (e.g., fructose-bisphosphate aldolase B, galactose-1-phosphate uridyltransferase). In the post-absorptive phase, glycogenolysis occurs. Hepatic glycogenolysis is the main source of glucose during short to medium fasting. Shortly after (5–6 hours fasting), gluconeogenesis (hepatic and renal) provides an increasing amount of glucose and progressively becomes the main glucoregulatory mechanism when glycogen stores are depleted. Fatty acid oxidation (FAO) and ketogenesis start after 12 hours of fasting (glucose sparing effect) and become predominant during long to very long fasting. Acyl-CoA esters requirethe carnitine shuttle to cross the inner mitochondrial membrane in order to be

oxidized; reducing equivalents (to be transferred to the electron transport chain) or ketone bodies (mainly acetoacetate and 3- hydroxybutyrate) are generated. During very long fasting the brain can switch to ketone bodies as the main energy source instead of glucose [1]. Notably, the above-mentioned responses can come earlier under circumstances associated with increased glucose requirements (e.g., younger patients, intercurrent disease, exercise) [2].

Definition and Classification

The estimated incidence of symptomatic hypoglycemia in newborns is 1–3/1,000 live births. The overall prevalence of IMD causing hypoglycemia is around 3/10,000 in children [3]. Hypoglycemia is often defined as a glucose level low enough to induce signs and/or symptoms of cognitive dysfunction. Children are at high risk of developing hypoglycemia, having less glycogen storage in their liver and (overall) less muscle protein together with a higher glucose demand. Currently, there is no consensus on the glucose level at which a person is considered to have hypoglycemia. This also reflects differences based on the etiology and the patient's age. Variable thresholds have been proposed for glucose concentration: < 45 mg/dl (2.5 mmol/L) at all ages, < 50 mg/dl in older infants and children, < 40 mg/dl on the first day of life, < 45 after 24 hours of age, < 25 mg/dl in preterm infants [3,4].

Depending on the focus, different classifications for hypoglycemia have been developed [1]:

- *Pathophysiology*: underproduction of glucose, overutilization of glucose, a combination of the two (Table 1)
- *Timing*: Fasting, postprandial, hectic
- *Clinical*: With hepatomegaly, without hepatomegaly

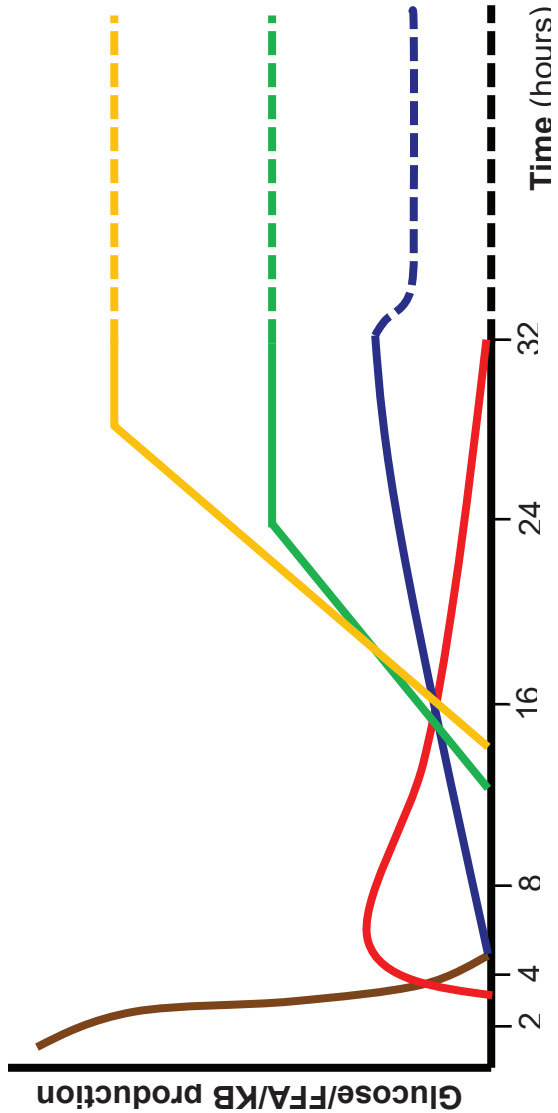


Figure 1. Main metabolic pathways involved in glucose homeostasis during the absorptive and fasting phases including exogenous carbohydrates (brown), glycogenolysis (red), gluconeogenesis (blue), fatty acid oxidation (green), ketogenesis and ketolysis (yellow).

Major groups of IMD associated with hypoglycemia are summarized in Appendix 1.

Ketotic hypoglycemia is the most common cause of childhood hypoglycemia. Usually onset occurs between 18 months and 5 years of age and remits before 9 years. It includes a group of disorders presenting with hypoglycemia together with high amounts of ketones in which the exact genetic cause is unknown (impaired gluconeogenesis secondary to decreased alanine availability has been postulated). Usually, the patient presents with symptomatic hypoglycemia and (massive) ketosis after a long fast often precipitated by an intercurrent illness. Metabolic acidosis can occur too. The clinical condition of the child significantly improves with the infusion of dextrose; conversely, the administration of glucagon does not greatly increase glucose concentration. Since there are no specific tests for the diagnosis of ketotic hypoglycemia, the diagnosis is made following the exclusion of the remaining causes of hypoglycemia. Some patients previously diagnosed with ketotic hypoglycemia are now being diagnosed with a specific IMD with the increasing availability of innovative diagnostic techniques (e.g., next- generation sequencing).

Glycogen storage diseases (GSD) are a group of IMD due to the defect of enzymes and transporters used in the breakdown and synthesis of glycogen More than 12 types are recognized. All GSD are inherited in an autosomal recessive pattern, except type IXa and IXd showing X-linked recessive pattern. Traditionally, they are classified as hepatic GSD and muscle GSD (e.g., GSDV, GSDII). Hypoglycemia and hepatomegaly are the primary manifestations of the hepatic GSD (GSD0a, GSDI, GSDIII, GSDVI, GSDIX, GSDXI). GSDI is the most common and severe GSD; it is due to a defect of either the catalytic (GSDIa, 80%) or the microsomal glucose 6-phosphate transporter (GSDIb, 20%) subunits of the glucose 6-phosphatase system. GSDI usually presents at age 3–6 months with, lactic acidosis and hypoketosis, fasting hypoglycaemia, hepatomegaly, a doll-like face, failure to thrive, hyperlipidemia and hyperuricemia. In addition,GSDIb patients show severe neutropenia and frequent infections. GSDIII is caused by glycogen debrancher enzyme deficiency. Patients show less severe hypoglycemia and hyperlipidemia and higher transaminases with prominent ketosis (without lactic acidosis) compared to GSDI. Liver fibrosis can develop into cirrhosis and eventually malignancies. GSDIIIa patients (85% of the cases) also show progressive (cardio)myopathy. GSDVI and GSIX are secondary to liver glycogen phosphorylase and glycogen phosphorylase kinase defect, respectively. They are relatively mild disordersand have a tendency to improve with age. However, hyperlipidemia, increased

transaminases and growth retardation can also be found. Other hepatic GSD include GSDXI (Fanconi-Bickel syndrome: hypoglycemia and Fanconi syndrome) and GSD0a (fasting-induced ketotic hypoglycemia and postprandial hyperglycemia) [5]. Hypoglycemia can also develop after the ingestion of specific carbohydrates.

Table 1. Pathophysiological classification of hypoglycemia (simplified)
A. Underproduction of glucose
1.Glycogen shortage (prematurity, dysmaturity)
2. Insufficient supply of nutrients and/or increased losses (vomiting, diarrhea)
3. Gluconeogenesis defect (e.g., GSDI)
4. Glycogenolysis defect (e.g., GSD III, VI, IX)
5. Fatty acid oxidation disorders
6. Hepatic impairment (e.g., secondary to galactosemia or mitochondrial respiratory chain defect)
7. Primary disturbed hormonal regulation (growth hormone, cortisol glucan, adrenaline) or secondary to exogenous cause (e.g., drugs)
8. Alcohol abuse
9. Heart failure
B. Overutilization of glucose
1.Hyperinsulinism (maternal diabetes mellitus, syndromic, monogenic, iatrogenic or metabolic)
2. Ketogenesis defect
3. Hyperviscosity
C. Combination
1. Hypoxemia
2. Hypothermia
3. Sepsis, shock

Hereditary fructose intolerance (HFI) is caused by a reduced concentration of aldolase B, which causes arrest of gluconeogenesis and glycogenolysis. Symptoms usually occurs with the introduction of fructose, sucrose or sorbitol in the diet, and include vomiting, pallor, sweating, lethargy, failure to thrive, convulsions and eventually coma. Acute liver failure, hypoglycemia, metabolic acidosis and renal dysfunction can be also observed. Most

patients develop a dislike for to fruit, sweets and other foods containing fructose.

Classical galactosemia is caused by mutations in the galactose-1-phosphate uridyltransferase (GALT) gene. Symptoms usually occur at short distance after the ingestion of breast or formula milk and include vomiting, diarrhea, poor feeding, nuclear cataract, jaundice, liver failure and renal failure; E. Coli sepsis can also occur. Long-term complications include intellectual disability and hypergonadotropic hypogonadism. Less common galactose metabolism disorders include galactokinase deficiency and UDP-galactose epimerase deficiency [1].

Fructose 1,6 bisphosphatase (FBPase) deficiency is a disorder of gluconeogenesis characterized by acute crisis of hypoglycemia, lactic acidosis and ketosis presenting with hyperventilation, apneic spells, hepatomegaly, seizures, coma. The crises typically occur when glycogen reserves are limited (e.g., newborns, high dietary fructose intake) or exhausted (e.g., fasting, intercurrent illness). The frequency of the episodes decreases with age; in between attacks patients are usually well [1,6].

Pyruvate carboxylase (PC) deficiency is a defect of both gluconeogenesis and the Krebs cycle. Although fasting hypoglycemia can occur, it usually presents with severe encephalopathy, developmental delay, seizures, movement disorders, failure to thrive and metabolic acidosis [1,2]

Organic acidemias (OA) are due to defects of enzymes involved in branched-chain amino acid catabolism. In OA, mitochondrial accumulation of CoA metabolites causes ketotic hypoglycemia, metabolic acidosis, increased lactate and ammonium. Methylmalonic acidemia (MMA), Propionic acidemia (PA) and Isovaleric acidemia (IVA) are the most common forms of OA.

Three different clinical scenarios are recognized:

- Neonatal: lethargy, poor feeding, encephalopathy, myoclonic jerks, multi-organ failure.
- Chronic intermittent: episodes of ketoacidosis, lethargy, cerebral involvement.
- Chronic progressive: vomiting, failure to thrive, psychomotor retardation, renal disease.

OA are included in newborn screening (NBS) programs in several countries with an increasing number of patients diagnosed pre-symptomatically [1].

Fatty acid oxidation disorders (FAOD) constitute a group of recessively inheriteddefects characterized by hypoketotic hypoglycemia and presenting with great variability. Four processes are required for mitochondrial FAO: 1) carnitine uptake through the carnitine transporter; 2) entry of fatty acids into the mitochondria through the specific carriers carnitine palmitoyltransferase 1 (CPTI) and II (CPTII) and carnitine acylcarnitine transferase; 3) beta oxidation via a spiral pathway (enzymes with different chain length specificity); and 4) transfer of electrons to the respiratory chain. Defects in riboflavin metabolism canshow the same symptoms as FAOD (riboflavin is the precursor of the respiratory complex I).

Three typical presentations are known for FAOD:

1. Acute hypoketotic hypoglycemia with lactic acidosis, encephalopathy, liver dysfunction (including hyperammonemia); symptoms usually present under catabolic circumstances (e.g., newborns, prolonged fasting, intercurrent illness).
2. (Hypertrophic) cardiomyopathy and arrhythmias
3. Myopathy presenting with weakness and/or acute rhabdomyolysis; symptoms can be precipitated by exercise or intercurrent illness.

Before NBS was introduced, phenotype 1 was the most common (potentially causing sudden death). Ever since, the number of patients presenting with muscle symptoms has increased. Currently, CPTII deficiency is the most common lipid disorder affecting skeletal muscle. Patients with FAOD can remain asymptomatic throughout their life if they have mild defects [1,2].

Disorders of ketone body metabolism can present either in the first days of life or later in childhood. Catabolic circumstances are triggers to metabolic decompensation. Ketogenesis defects show hypoketotic hypoglycemia (with or without hyperammonemia), metabolic acidosis and liver disease, causing encephalopathy, vomiting and coma. Ketolysis defects present with episodes ofhyperketotic hypoglycemia and severe ketoacidosis; patients are healthy between episodes [1,6].

Disorders of oxidative phosphorylation (OHPHOS) are clinically, biochemically and genetically heterogeneous. OHPHOS are caused by mutations in the nuclear genes that code for the subunits of respiratory complexes. These disorders can occur at any age with a wide range of symptoms. Children often suffer from encephalomyopathic disease. The suspicion usually arises based on: 1) complex multisystem involvement (including the muscle and/or central nervous system); or 2) a combination

of typical symptoms falling within a recognized mitochondrial syndrome; or 3) the presence of lactic acidosis or other suggestive biochemical/imaging data (e.g., stroke-like episodes, sideroblastic anemia) [1.3].

Congenital disorders of glycosylation (CDG) are due to defects in glycoprotein synthesis. Around 90 CDG types are recognized. A broad spectrum of symptoms including psychomotor retardation, deafness, bleeding tendency, cerebral hemorrhage, cardiomyopathy, hypogonadism and hypoglycemia (hyper- or normoinsulinemic) is known. CDG should be suspected in any child with intellectual disability and/or neurological signs and multi-organ disease [1].

Since hypoglycemia is a sign of end-stage liver disease it can also occur in all IMD leading to liver failure (e.g., GSDIV, tyrosinemia type I, maple syrup urine disease). Factitious hypoglycemia should be considered when hypoglycemic attacks occur without any recognizable pattern [1,7]. Notably, several IMD causing hypoglycemia (e.g., FAOD, OA, galactosemia) are currently included in NBS programs worldwide allowing diagnosis before symptoms onset.

Clinical Features

The classic symptoms of acute hypoglycemia can be classified into two categories: neurogenic and neuroglycopenic. Neurogenic symptoms (due to the activation of the autonomic nervous system and adrenaline release) appear first: sweating, pallor, tachycardia, hunger, weakness, tremulousness, nausea, paresthesias. Neuroglycopenic symptoms are secondary to decreased cerebral glucose availability (blood glucose concentrations < 50 mg/dL): headache, confusion, lethargy, visual disturbances, altered behavior, dizziness, incoordination. Seizures, encephalopathy and coma can develop if hypoglycemia is not promptly treated. The symptoms in newborns may be subtler, including hypothermia, hypotonia, poor feeding, cyanosis, apnea and seizures. As the brain can adapt to use alternative energy substrates (i.e., lactate, ketone bodies) over time, sudden drops in glucose concentrations are more likely to induce symptoms than those achieved slowly. In this respect, repeated and prolonged periods of hypoglycemia may lower the threshold for neurogenic symptoms ("hypoglycemia unawareness") in children with IMD (e.g., GSD). In addition, the symptoms of hypoglycemia may be influenced by medications (e.g., autonomic symptoms are inhibited by beta-blockers) [3,8].

Diagnosis

The diagnosis of hypoglycemia results from the combination of clinical, dietary and biochemical data. A systematic approach is necessary to collect relevant information (Table 2). Relation to the last meal, nutritional history, (un)detectable ketones and metabolic acidosis are the cornerstone of diagnostics.

History taking is central to a patient with hypoglycemia and can provide information on the presence of symptoms related to hypoglycemia, fasting tolerance (how long a person can fast), presence of ketosis (strong breath smell in the early morning, vomiting). It should include:

- Age of onset (neonate, infant, child)
- Associated conditions (e.g., intercurrent disease, small for gestational age, maternal diabetes)
- Relation to the last meal (fasting hypoglycemia, postprandial hypoglycemia, random)
- Relation to/avoidance of food (e.g., protein, fruit, fruit juice, lactose)
- (Overnight) fasting tolerance (e.g., feeding frequency, night snack, morning ketosis)
- Glucose requirements (e.g., >10 mg/Kg/min)

The timing of hypoglycemia is of paramount importance and can help to focus on specific groups of disorders (e.g., hypoglycemia after short fasting suggests disorders of gluconeogenesis or hepatic GSD; FAODs are typically present with hypoglycemia after medium to long fasting). Nutritional history can be assessed through either a three-day food record, 24h recall or food frequency diary to check overall food intake and any restricted/exceeded nutrient.

Information on growth and developmental milestones should also be collected. Family history should inquire about any siblings with symptoms suggestive of fasting intolerance and any previous miscarriages or deaths and consanguinity [6,9]. Physical examination can identify hepatomegaly, splenomegaly and signs of liver disease (e.g., jaundice, itching, bruising, spider angiomas). As the liver and spleen size become larger with age, patients age and height should be considered for adequate assessment [10]. An absence of dental caries can reveal an aversion to sweets/sugar-sweetened beverages (as in HFI). Additional clinical features can be found pointing to a specific diagnosis: short stature in GSD, cataract in galactosemia, arrhythmias and/or a heart murmur in FAOD, multisystem

involvement (e.g., dysmorphic features, inverted nipples, bleeding tendency) in mitochondrial disorders and CDG (Table 2).

Laboratory investigations can provide essential information by measuring metabolites and hormones involved in glucose homeostasis. Samples must be collected during hypoglycemia, otherwise the diagnosis may be missed. Essential blood investigations include lactate, blood gases, ketones (3-hydroxybutyrate), free fatty acids (FFA), acylcarnitines, insulin, cortisol, GH as well as urine organic acids. One spare tube should also be collected for any additional investigations to be performed afterwards. Based on the information available and/or clinical suspicion additional tests can be considered, including blood count, C-reactive protein, electrolytes, liver/renal function tests, CK, uric acid, cholesterol, triglycerides, ammonia, and amino acids. Urine reducing substances test can be helpful to assess the presence of reducing sugars (e.g., fructose, galactose). Transferrin electrophoresis/isoelectric focusing should be required if CDG is suspected [7].

Continuous glucose monitoring systems (CGMS) can provide additional information on the extent, timing and duration of fluctuations of blood glucose. The (combination of) results from these investigations can clarify whether all components of the hormonal and metabolic regulation are functioning adequately to prevent hypoglycemia. However, alternative causes of abnormal test results should always be considered (e.g., lactate elevation secondary to laborious exercise or postprandial blood collection in some GSD patients or increased pCO2 secondary to apnea during blood collection). A specific biochemical pattern can point to a definite diagnosis. For example, low ketones together with increased FFA are usually observed in FAOD (however few exceptions have been reported); FFA/Ketone bodies < 0.3 suggests a defect of ketolysis. Similarly, GSDI is characterized by increased lactate and low ketones concentrations. A simplified diagnostic algorithm can allow the diagnosis of the most common IMD causing hypoglycemia (Figure 2). As the response to fasting is altered in many IMD, assessment of metabolic fasting effects may behelpful to achieve the diagnosis and assess patients fasting tolerance to tailor therapy. Controlled fasting is performed in a hospital under supervision. However, in some patients fasting can lead to the accumulation of toxic metabolites and sometimes fatal complications. Therefore, fasting tests should only be performed in specialized metabolic units after the least risky diagnostic tests have already been performed and an FAOD has been ruled out. As new diagnostic tests are rapidly available, fasting testing is no longer routinely performed but can be useful in selected cases. The glucagon test explores

Table 2. Timing, biochemical and clinical features of most common IMD causing hypoglycemia. ***M.A.: metabolic acidosis, BCAA; branched-chain amino acids, UOA: urine organic acids, FFA: free fatty acids, KB: ketone bodies.***

Disorder	Timing	Lactate	Ketones	Additional biochemical abnormalities	Clinical features
Ketotic hypoglycemia	Fasting > 6 hours	+/- M.A.	+	Low alanine	Fever Vomiting, diarrhea
GSDI	Fasting (2-4 hours)	+ M.A.	(-)	Elevated lipids, uric acid Elevated transaminases Neutropenia (GSDIb)	Hepatomegaly Doll-like face
GSDIII/VI/IX	Fasting (2-6 hours)	-	+	Elevated lipids Elevated transaminases Elevated CK (GSDIIIa)	Hepatomegaly Cardiomyopathy (GSDIIIa)
HFI	1-2 hours after the ingestion of fructose, sucrose, sorbitol	+ M.A.	+/-	Elevated transaminases	Vomiting, diarrhea Hepatomegaly Liver failure, fatty liver

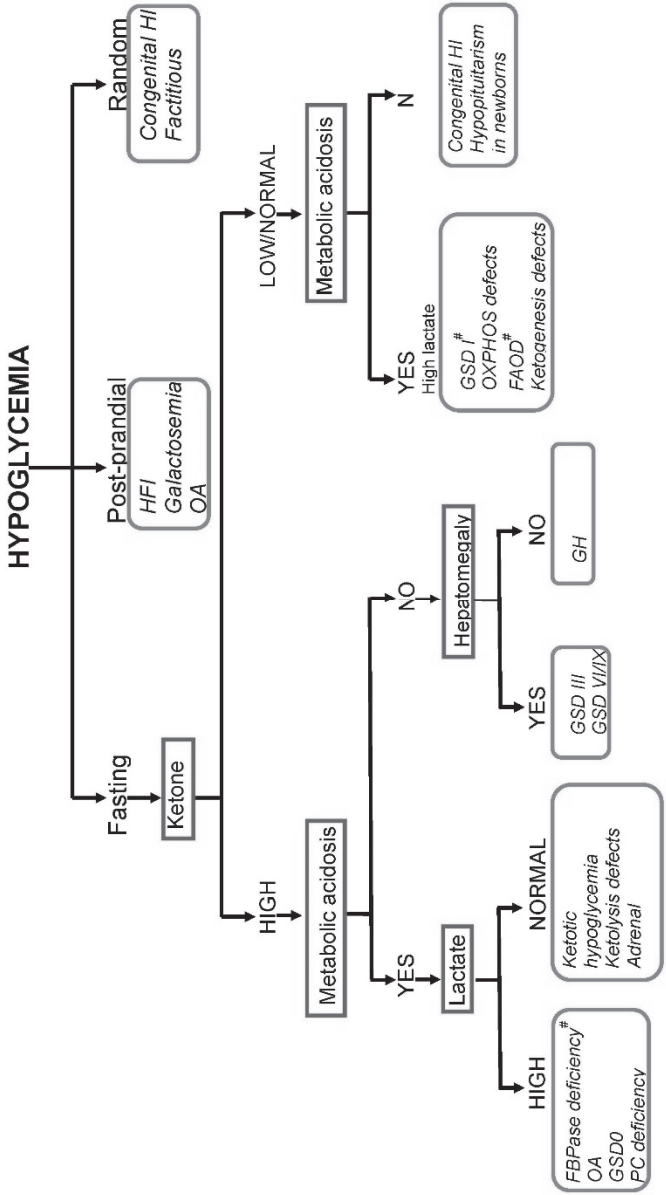
Galactosemia	1-2 hours after the ingestion of galactose, lactose	- M.A.	+/-	Elevated bilirubin Elevated transaminases Abnormal clotting tests	Vomiting, Hepatomegaly, liver failure Cataract, sepsis
FBPase deficiency	Fasting > 6 hours	+ M.A.	+	Elevated alanine Elevated pyruvate and glycerol 3-phosphate	Intercurrent disease Hepatomegaly
Pyruvate carboxylase deficiency	Variable	+ M.A.	+	Hyperammonemia Elevated citrulline	Severe encephalopathy Seizures Movement disorders
Organic acidemias	Prolonged fasting or after an initial symptom-free period (neonatal)	+ M.A.	+	Hyperammonemia Elevated BCAA and glycine Elevated acylcarnitines UOA abnormalities	Encephalopathy Movement disorders Renal disease Cardiomyopathy
Fatty acid oxidation disorders	Fasting > 8 hours	+ M.A.	(-) FFA/KB > 2.5	Elevated acylcarnitines Dicarboxylic aciduria Hyperammonemia	Exercise intolerance Cardiomyopathy Arrhythmias

Ketogenesis defects	Prolonged fasting or after an initial symptom-free period (neonatal)	+ M.A.	- FFA/KB > 2.5	Hyperammonemia Dicarboxylic aciduria UOA abnormalities	Hepatomegaly Seizures Cardiomyopathy
Ketolysis defects	Prolonged fasting	-	+ FFA/KB < 0.3	UOA abnormalities	Intercurrent disease Hepatomegaly
OXPHOS disorders	Variable	+ M.A.	+/-	UOA abnormalities	Multisystem involvement
CDG	Variable	+/-	+/-	High insulin (mostly)	Psychomotor retardation Dysmorphic features Multisystem involvement
Congenital hyperinsulinism	Hectic	-	-	High insulin	Macrosomia Dysmorphic features

the response to aglucagon injection during hypoglycemia to assess the availability of glycogen for the compensation of low blood glucose. Typically, hypoglycemia due to GSDI and GSDXI does not benefit from a glucagon injection. Due to its possible risks (prolonged hypoglycemia) it has been largely superseded by enzyme or mutation analysis [7].

Imaging tests can provide additional information. Abdominal ultrasound and magnetic resonance imaging/computed tomography scan can define liver, spleen, kidneys morphology and structure (e.g., liver steatosis, liver adenomas). Hand X-rays can be helpful in patients with growth retardation. Specific tests can be required based on the accompanying clinical features (Table 2).

The working diagnosis can be confirmed through enzymatic and/or molecular testing. Enzyme diagnostics is generally performed on blood cells or a skin fibroblast (e.g., debranching enzyme or very-long-chain acetyl-CoA dehydrogenase activity).However, some enzymes (e.g., glucose 6-phosphatase)are not expressed in these mediums and require a liver biopsy. As a liver biopsy isinvasive, it has been largely superseded by DNA analysis. In recent years, DNA analysis has become increasingly accurate and rapidly available. Various techniques can search for mutations in IMD genes: single gene analysis (Sanger sequencing), gene panels (e.g., “hypoglycemia panel”) (Next-generation sequencing), whole-exome sequencing (Next-generation sequencing) [1,2].



Treatment

Recognizing and promptly treating acute hypoglycemia helps prevent complications (i.e. brain damage). Treatment of acute symptomatic neonatal or infant hypoglycemia includes intravenous bolus administration of dextrose (200 mg/Kg in newborns, 2 ml/Kg 10% dextrose in children), followed by a continuous infusion of glucose at 6–10 mg/kg/min, adjusting the rate to maintain blood glucose levels in the normal range. Glucose dose may vary depending on child age (0–12 months: 8–10 mg/Kg/min, 1–3 years: 7–8 mg/Kg/min, 4–6 years: 6–7 mg/Kg/min, 7–12 years: 5–6 mg/Kg/min, adolescents: 4–5 mg/Kg/min). Children with congenital hyperinsulinism may require up to 10–15 mg/Kg/min of glucose or more [1,7]. Oral glucose administration (0.3 g/Kg) can be considered in conscious patients with mild hypoglycemia. A bicarbonate infusion could be necessary to counteract metabolic acidosis as well as ammonia scavengers (sodium benzoate, N-carbamylglutamate) and hemodialysis in the case of hyperammonemia (e.g., OA, FAOD). Additional treatments may be required depending on the underlying cause. If HFI or galactosemia are suspected, any source of fructose, sucrose, sorbitol and galactose, or lactose should be stopped, respectively. Carnitine supplementation should be provided in FAOD (except long-chain FAOD) and OA (and glycine in IVA); thiamine can be helpful in case of severehyperlactacidemia (e.g., OA, mitochondrial disorders). Protein intake can be stopped (up to 48 hours) if disorders of aminoacid catabolism are suspected (e.g., OA). Severe cases can require liver transplantation (e.g., mitochondrial disorders, tyrosinemia type 1) [1,7,8]. Resolved acute hypoglycemia, pending a diagnosis, precautions must be applied to avoid new hypoglycemia. The general recommendation is always to avoid fasting and not to lack carbohydrates in the diet, especially in times of metabolic stress. The most common therapies are tailor-made diets with frequent feedings, raw corn starch, sometimes a tube is needed; the goal of such diets is to keep glucose concentrations at acceptable levels. Such schemes are adequate in patients with ketotic hypoglycemia, hepatic GSD and metabolic disorders of the ketone body. Additionally, patients with long-chain FAOD may require supplementation with medium-chain triglycerides. A specific life-long fructose-, sucrose-, and sorbitol-restricted diet regimen should be set in HFI as well as a lactose- and galactose-restricted diet in galactosemia. A low-protein dietary scheme should be provided to patients with OA; supplementation with a small amount of synthetic amino acids can be considered to meet the patient requirements. No curative treatment exists for most mitochondrial disorders. Supportive treatments are the mainstay of management including

vitamins and cofactor supplementation (e.g., riboflavin, Coenzyme Q10, thiamine, biotin) [3,7,9]. Whatever their final diagnosis, in stressful situations such as intercurrent illnesses, heat waves, prolonged fasting, patients may present an increased catabolism due to high fever, higher consumption or reduced intake. Therefore an emergency protocol was designed. Patients (and caregivers) should be encouraged to carry an emergency protocol with them at all times and to implement it when needed

KEY POINTS

- Hypoglycemia is a feature of a number of IMD
- The timing of hypoglycemia in different disorders reflects the physiological response to fasting (hypoglycemia after a short fast suggests carbohydrate disorder, hypoglycemia after a long fast suggests fatty acid oxidation disorder)
- Currently there is no consensus on the threshold to define hypoglycemia
- Nutritional history can provide key clues to the diagnosis of hypoglycemia
- Samples for laboratory investigations must be collected during hypoglycemia (critical samples)
- Once acute hypoglycemia has been managed, tailored dietary treatment plans and emergency protocol should be provided in order to ensure adequate glucose concentrations

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Appendix 1

Major IMD groups associated with hypoglycemia. The most common glycogen storage diseases, fatty acid oxidation disorders and congenital disorders of glycosylation are included.

Disorder	Gene(s)	Protein	Inheritance
GSD0a	*GYS2*	Liver glycogen synthase	Autosomal recessive
GSD0b	*GYS1*	Muscle glycogen synthase	Autosomal recessive
GSDIa	*G6PC*	Glucose 6-phosphatase α	Autosomal recessive
GSDIb	*SLC37A4*	Glucose 6-phosphate transporter	Autosomal recessive
GSDII (Pompe disease)	*GAA*	lysosomal α-glucosidase	Autosomal recessive
GSDIII	*AGL*	Glycogen debrancher enzyme	Autosomal recessive
GSDIV	*GBE1*	Glycogen brancher enzyme	Autosomal recessive
GSDV	*PYGM*	Muscle glycogen phosphorylase	Autosomal recessive
GSDVI	*PYGL*	Liver glycogen phosphorylase	Autosomal recessive
GSDVII	*PFKM*	Muscle phosphofructokinase	Autosomal recessive
GSDIXa	*PHKA2*	Glycogen phosphorylase kinase α2	X-linked recessive
GSDIXb	*PHKB*	Glycogen phosphorylase kinase β	Autosomal recessive
GSDIXc	*PHKG2*	Glycogen phosphorylase kinase γ	Autosomal recessive
GSDIXd	*PHKA1*	Glycogen phosphorylase kinase α1	X-linked recessive
GSDX	*PGAM2*	Muscle phosphglycerate mutase	Autosomal recessive
GSDXI (Fanconi-Bickel)	*SLC2A2*	GLUT2	Autosomal recessive
GSDXII	*ALDOA*	Aldolase A	Autosomal recessive
GSDXIII	*ENO3*	β-enolase	Autosomal recessive

GSDXIV	*PGM1*	Phospoglucomutase	Autosomal recessive
GSDXV	*GYG1*	Muscle glycogenin deficiency	Autosomal recessive
Hereditary fructose intolerance	*ALDOB*	Aldolase B	Autosomal recessive
Classical galactosemia	*GALT*	Galactose-1p uridyltransferase	Autosomal recessive
Fructose 1,6-Bisphosphatase deficiency	*FBP1*	Fructose 1,6-Bisphosphatase	Autosomal recessive
Pyruvate carboxylase deficiency	*PC*	Pyruvate carboxylase	Autosomal recessive
Methylmalonic acidemia	*MMUT, MMAA,MMAB, MMADHC, MCEE*	Methylmalonil-CoA mutase, Methylmalonil-CoA epimerase	Autosomal recessive
Propionic acidemia	*PCCA, PCCB*	Propionyl-CoA carboxylase	Autosomal recessive
Isovaleric acidemia	*IVD*	Isovaleryl-CoA dehydrogenase	Autosomal recessive
Carnitine transporter deficiency	*SLC22A5*	Carnitine transporter	Autosomal recessive
Carnitine palmitoyltransferase I (CPT I) deficiency	*CPT1*	Carnitine palmitoyltransferase I	Autosomal recessive
Carnitine acylcarnitine translocase (CACT) deficiency	*SLC25A20*	Carnitine acylcarnitine translocase	Autosomal recessive
Carnitine palmitoyltransferase II (CPT II) deficiency	*CPT2*	Carnitine palmitoyltransferase II	Autosomal recessive
Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	*ACADVL*	Very-long-chain acyl-CoA dehydrogenase	Autosomal recessive

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and mitochondrial trifunctional protein (MTP) deficiency	*HADHA, HADHB*	Long-chain 3-hydroxyacyl-CoA dehydrogenase, Long-chain enoyl-CoA hydratase, Long-chain ketoacylCoA thiolase	Autosomal recessive
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	*ACADM*	Medium-chain acyl-CoA dehydrogenase	Autosomal recessive
Short-chain acyl-CoA dehydrogenase (SCAD) deficiency	*ACADS*	Short-chain acyl-CoA dehydrogenase	Autosomal recessive
3-Hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency	*HADH*	3-hydroxyacyl-CoA dehydrogenase	Autosomal recessive
Multiple acyl-CoA dehydrogenase (MAD) deficiency	*ETFA, ETFB, ETFDH*	Electron transfer flavoproteins	Autosomal recessive
Riboflavin metabolism defects	*SLC52A1, SLC52A2, SLC52A3, FLAD1, SLC25A32*	RFVT1, RFVT2, RFVT3, FAD synthase, FAD transporter	Autosomal recessive
3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Synthase Deficiency	*HMGCS2*	3-Hydroxy-3-Methylglutaryl-CoA synthase	Autosomal recessive
HMG-CoA Lyase Deficiency	*HMGC2*	3-Hydroxy-3-Methylglutaryl-CoA lyase	Autosomal recessive

Succinyl-CoA:3-oxoacid CoA transferase (SCOT) deficiency	*OXCT1*	Succinyl-CoA:3-oxoacid CoA transferase	Autosomal recessive
Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency	*ACAT1*	Acetoacetyl-CoA thiolase	Autosomal recessive
Monocarboxylate transporter 1 (MCT1) deficiency	*SLC16A1*	Monocarboxylate transporter 1	Autosomal recessive
OXPHOS disorders	*Multiple genes*	Respiratory complexes subuinits	Variable
Phosphomannose isomerase (MPI-CDG) deficiency	*PMI*	Phosphomannose isomerase	Autosomal recessive
Phosphomannomutase 2 (PMM2-CDG) deficiency	*PMM2*	Phosphomannomutase 2	Autosomal recessive

SECTION 4:

TYPE 2 DIABETES

SECTION 4 CHAPTER 1

DM2 IN YOUNG PEOPLE: DISEASE MANAGEMENT

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Epidemiology of T2DM and prediabetes in youth

During the last three decades, the global prevalence of type 2 diabetes (T2DM) in youth has risen substantially, mirroring the massive increase in overweight and obese individuals. First reported in ethnic minorities of the USA (Native American, Afro American, Asian/Pacific Islander, and Latino youths), T2DM is increasingly diagnosed in adolescents and young adults from the emergent economies of Arabia, Asia and the Western Pacific regions. In European youth, as well as in non-Hispanic white Americans, incidence rates appear to be much lower than in USA ethnic minorities, ranging from 0.2 to 1 per 100,000 person-year (**1**). In an Italian multicenter report on a large cohort of young patients referred to 15 pediatric diabetes clinics with a diabetes diagnosis, T1D accounted for 92.4% cases, monogenic diabetes for 6.3% and T2D for 1% (**2**).

In adults, the transition from normoglycemia to T2DM is a gradual phenomenon that occurs through an intermediate slow period of altered glucose metabolism (taking around 5–20 years). Normoglycemia and the abnormalities of glucose metabolism (also called prediabetes) are defined according to American Diabetes Association (ADA) diagnostic criteria, as:

- NGT (Normal Glucose Tolerance) as fasting plasma glucose levels below 5.6 mmol/liter (<100 mg/dL) and Hemoglobin A1c (HbA1c) <5.7% (<39 mmol/mol);

- IFG (Impaired Fasting Glycemia) as fasting plasma glucose levels between 5.6 and 6.9 mmol/liter (100–126 mg/dL) and HbA1c 5.7% to 6.4% (40–46 mmol/mol);
- IGT (Impaired Glucose Tolerance) plasma glucose levels between 7.8 and11 mmol/liter (>140–199mg/dL) at 120 minutes during the oral glucose tolerance test (OGTT) and HbA1c 5.7% to 6.4% (40–46 mmol/mol);
- T2D fasting plasma glucose levels higher than 7.0 mmol/liter (>126 mg/dL) and HbA1c >6.4% (>46 mmol/mol).

Various studies report a high prevalence of prediabetes, ranging from 21–45% in obese adolescents of ethnic minorities to 5–15% in those of European descent. A 5% IFG prevalence rate is already present in obese prepubertal children aged less than 10 years. In severely obese youths the progression from IFG to T2D appears to be much faster than in adults (1–2 years). However, regression from prediabetes to NGT has been observed in 45–81% of children and adolescents within a follow-up period of one to four years, suggesting that the path from NGT to T2D is bidirectional and that the progression from IGT to frank diabetes may be delayed or prevented with appropriate changes in lifestyle and/or pharmacologic intervention **(3-5).**

Pathophysiology and risk factors for T2DM in youth

In healthy subjects, β cell insulin secretion and the insulin sensitivity of peripheral tissue regulate the blood glucose metabolic pathway: an alteration of this balance is the most important determinant in the development of T2D in adults. It has been supposed that insulin resistance, by placing an increased demand on the β cell to hypersecrete insulin, influences the progressive β cell failure of T2D. More recently, a primary role of the impairment in the prompt and slow insulin secretion (first and second phase) in response to nutrient stimulation, has been reported in a number of studies, showing that ongoing decline in β cell function occurs without a change in insulin sensitivity in adults as well as in younger people. In a multiethnic cohort of obese adolescents, compared to NGT obese adolescents, those with IFG had similar degrees of insulin resistance, but reduced first-phase insulin secretion; the IGT group evidenced a more severe impairment of these abnormalities and IFG/IGT showed further worsening of insulin resistance with an additional defect of the second-phase insulin secretion (**6**). These findings indicate that insulin resistance, β

cell dysfunction and glucose intolerance should be considered a continuum that leads to T2DM development in obese youths.

Obesity and genetic predisposition are the major drivers of the evolution of IGT and T2DM in youth. Although excess total body fat is associated with variances in insulin susceptibility in obese children and teens, the demonstration of a direct relationship between the degree of obesity and insulin resistance is still lacking (**4**). The partitioning of fat mass is another relevant factor in the genesis of insulin resistance and β cell failure; indeed, obese adolescents with IGT have significantly reduced subcutaneous (SAT) and increased visceral (VAT) adipose tissue associated with excessive accumulation of intramyocellular lipid content in the muscle and liver. Excessive deposition of fat in the liver increases circulating free fatty acids and down-regulates the β cell function and response to stimuli (**3,4**). Moreover, as the degree of obesity increases, adipose tissue (especially VAT), is infiltrated by macrophages, which are the source of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, IFNγ, and fetuin A. These worsen insulin resistance and impair β cell insulin secretion (**7**).

The high prevalence of T2DM/obesity in first-degree family members of young people affected by T2DM and peculiar ethnic groups, together with a strong concordance of the disease in monozygotic twins, stresses the importance of the hereditary component in the pathogenesis of T2DM. Linkage studies in adults identified many variants of genes that are associated with a defect in insulin secretion and/or abnormalities of glucose metabolism. Whole-genome association studies identified a large number of T2DM susceptibility loci near or inside genes coding proteins involved in insulin secretion and/or insulin resistance. Cumulative genetic risk scores (GRSs) obtained by combining risk alleles of single nucleotide polymorphisms (SNPs) have been proposed to define the risk of T2DM development in adults as well as in obese adolescents (**8**). *Puberty*, with its temporary increased secretion of GH, sex steroid, androgens and gonadotropin that induce a specular reduction of insulin sensitivity; *early life determinants* connected with the duration of pregnancy, neonatal birth weight and maternal health conditions; *an excess in total caloric, unsaturated fat and sugar sweetened beverages intake*, which may alter intestinal microbiota; *reduction of physical activity*; *urbanization*; *use of antipsychotic medications*; *sleep disturbances*; *and persistent organic pollution* are other risk factors which, operating at different stages of child development, may trigger the onset of T2DM in genetically predisposed youths (**3,4**).

T2DM at onset in youth When presenting in youth aged <20 years, T2DM at diagnosis has similar clinical characteristics, such as:

- It occurs in the second decade of life, during the pubertal spurt, at a median age of 13.5 years;
- It presents in nearly all youth with a mean BMI >85th percentile for age and sex (usually above the 90th percentile), with the only exception of children from South Asia and Taiwan who are normal-weight or thin;
- It is more prevalent in girls than in boys (1:4 – 1:6);
- It has a higher prevalence rate in certain ethnic groups than in non-Hispanic white children;
- It is associated with a strong family history of T2DM and obesity in first- and second-degree relations;
- It is often asymptomatic, in the pre-diabetic stages as well as at diagnosis, with hyperglycemia observed occasionally during routine tests;
- It occurs rarely with diabetic ketoacidosis or hyperosmolar non-ketonic crisis, that can sometimes be deadly;
- It tests negative for HLA haplotypes and autoantibodies specific for T1DM;
- It is frequently associated with risk profiles for the development of CVD (hypertension- dyslipidemia, renal impairment) and with symptoms of insulin resistance such as acanthosis nigricans (AN), polycystic ovary syndrome (PCOS) and non-alcoholic fatty liver disease (NAFLD).

These features help to distinguish T2DM from T1DM and monogenic forms of diabetes (TAB 1). According to the ISPAD and ADA guidelines, the diagnosis of T2DM is made by measurements of blood glucose in the absence of any acute physiological stress, and in the presence of one of the four following features:

- fasting plasma glucose of ≥7.0 mmol/L;
- plasma glucose of ≥11.1 mmol/L post-OGTT with 1.75 g/Kg (max 75 g) of anhydrous glucose dissolute in water;
- symptoms of diabetes (polyuria, polydipsia, weight loss) with a casual plasma glucose of 11.1 mmol/L;
- HbA1c >6.5% (>39 mmol/mol) performed in a qualified laboratory using a method certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial (DCCT) assay (**3,4**).

When a random blood glucose level above the limits is found in an obese symptomless adolescent, the test should be repeated and eventually confirmed on the following day. If diabetes autoantibodies testing is available, the diagnostic algorithm proposed by the Diabetes Study Group of the Italian Society of Pediatric Endocrinology and Diabetes helps the differential diagnosis of T1DM, monogenic MODY and T2DM (**2**).

At the time of diagnosis of type 2 diabetes in developmental age, microvascular and risk factors for macrovascular complications are often already present. In the TODAY study, at the time of enrollment, there were the following data: 14% of enrolled had blood pressure $\geq$ 95th percentile; 13% microalbuminuria; 80% low HDL cholesterol levels; 10% high triglycerides.

The rapid progression of complications has been also observed, particularly in ethnic groups (**9**). Most adolescents with T2DM show some degree of increased carotid intimal media thickness (IMT), endothelial dysfunction, left ventricular hypertrophy, cardiac dysfunction and remodeling, that increase the risk of early cardiovascular morbidity and mortality. A premature decline in cognitive function may occur in young adults when T2DM develops in youth. Moreover, a high prevalence of anxiety, eating disorders, social isolation, self-reported depression and worse quality-of-life scores characterize youths with T2DM. Screening of micro-and macrovascular complications should be performed at diagnosis and yearly thereafter, while hypertension is to be evaluated as part of the routine physical examination at every outpatient encounter (**4**).

Screening for risk T2DM in youth

The early identification of youths at increased risk of T2DM has been proposed in obese children and adolescents. Population screening is loaded by high economic cost and, currently, there is no single test that is sensitive, specific and accurate for the classification of the subject at risk. Guidelines issued by ADA and ISPAD recommended *target screening* that has to be performed in the clinical research setting focusing on children that are severely overweight (BMI >85th percentile for age and sex, weight for height >85th percentile, or weight >120% of ideal for height) and with other two risk factors for T2DM:

- positive familiar anamnesis for type 2 diabetes in a first- or second-degree relative

- race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)
- clinical findings of insulin resistance or conditions associated with insulin resistance, or small-for-gestational-age birth weight
- maternal history of diabetes or gestational diabetes mellitus during pregnancy (**3**)

Screening for T2DM in children should start at ten years or when puberty occurs at if its onset is at a younger age. Retesting should be periodically performed every three years unless rapid weight gain or development of cardio-metabolic complications occurs.

Fasting plasma glucose (FPG) is the recommended routine screening test for children, although some high-risk patients with normal fasting glucose may have IGT or diabetes by OGTT criteria (4). ADA introduced *HBA1c* into the diagnostic criteria for diabetes in adults: two repeated measures of A1c are suggested prior to making a diagnosis of diabetes or prediabetes. Indeed, the HbA1c measure has some problems: several non-glycemic factors (red blood cell turnover, hemoglobinopathies, medications, ethnicity, and age) can affect its values; variation in methodologies may lead to inconsistent classification and possible under- or over-treatment depending on the assay used; HbA1c has lower sensitivity for prediabetes and has lower efficacy at a higher cost in children compared with adults, when compared with tests of glycemia. Therefore, HbA1c values in obese youth should be interpreted with caution **(10)**.

The *hyperinsulinemic euglycemic clamp and frequently sampled intravenous glucose tolerance tests (FSIVGT)* are considered the best methods to evaluate insulin resistance and β cell function. However, they are labor intensive, costly, invasive and not suitable for large-scale studies. In recent years, the *OGTT-derived insulin resistance indices (whole body insulin sensitivity index WBISI; the ratio of glucose and insulin areas under the curve)* have been thought to correlate with clamp-derived insulin resistance indices. The homeostasis model assessment of insulin resistance *(HOMA-IR),* which utilizes the values of fasting insulin and glucose (according to the ratio fI (μU/ml) x fG (mmol/L) / 22.5) has been widely used. HOMA-IR changes throughout childhood and, therefore, age-based pediatric HOMA-IR must be obtained from large national cohorts. It has to be noted that all fasting, OGTT- and IVGTT-derived parameters for assessing insulin resistance become invalid once insulin secretion becomes defective (**11**).

The 2 h OGTT is recommended in obese youths because it allows the identification of physiologically distinct groups of individuals with abnormalities in insulin secretion and insulin sensitivity. Besides the fasting, 1 h and 2 h glucose concentrations during the OGTT, it has recently proposed that the shape of the glucose response curve also matters. In healthy adults and obese adolescents three different shapes of glucose and insulin curves have been observed: the *biphasic response curve* with a gradual increase in glucose and insulin concentrations between 30 and 90 min, followed by a decline in glucose and a subsequent second rise in glucose concentration of 4.5 mg/dl and then a slow decline in glucose during the second hour of the test; the *monophasic response curve* in which the absence of a second rise in glucose identifies subjects with low insulin sensitivity and decreased β cell function at increased risk of future IFG and T2DM; the *incessant increase curve* defined by a continuous gradual increase in blood glucose during the two hours OGTT, which foretells an increased risk of the rapid evolution toward frank hyperglycemia. These data were confirmed in a cohort of obese adolescents with recent onset T2DM, who were randomized to three different therapeutic protocols. Compared with the other two groups, the incessant increase group showed similar insulin sensitivity but a higher rate of glycemic failure in response to the treatment (**12**).

One hour post-load hyperglycemia has been proposed as another independent predictor of the risk of IGT and T2DM. In a longitudinal study of obese children, with 1h PG ≥7.4 mmol/L (or 133 mg/dL) exhibited higher BMI, plasma triglycerides, lower whole body insulin sensitivity, β cell function and insulin clearance than children with 1h PG <7.4 mmol/L. Moreover, adolescents with 1h PG ≥7.4 mmol/L were three times more likely to develop prediabetes (**13**).

Clinical-based parameters have been suggested in order to reduce the cost of screening in a large population of obese children. In a cohort of Italian overweight youths aged 5–15 years, with waist circumferences >95th percentile and waist/height ratios of >0.5, *children* with a greater chance of having metabolic and cardiovascular risks were detected. In a further study, TG/HDL-C ratios of ≥2.2 and the combination of TG/HDL-C ≥2.2 and WTHR >0.60 were significantly associated with IGT. The discriminative property of TG > 1.17 mmol/L, as a parameter for IGT screening, were observed in a Canadian cohort; in a group of Italian obese children, TG ≥1.13 mmol/L plus FPG ≥4.44 mmol/L improved the discrimination accuracy, leading to the detection of IGT in more than 66% of obese

children and limiting the need to perform OGTT to only 25% of all obese children (**14**).

The accuracy of the above reported parameters, as screening tools in a general population, needs to be verified in wide multiethnic populations.

Initial treatment at diagnosis depends on the severity of symptoms, metabolic derangement, and presence of ketosis:

1. In asymptomatic metabolically stable (HbA1c <7%) patients education and the adoption of healthy lifestyle changes may be the first-line therapeutic approach for a period of two months; less than 10% of youths with T2DM will reach their glycemic targets with lifestyle modifications alone.
2. If education is unsuccessful or in symptomatic patients with metabolic decompensation (HbA1c 7–8.5%) metformin is the first line drug, with the reinforcement of healthy lifestyle education.
3. Insulin will be initially used when patients present with ketosis/ketoacidosis and/or HbA1c >8.5% (69.4 mmol/mol).

No significant advantages have been observed between the various insulin regimens available. Once a stable metabolic balance is reached, a transition from insulin onto metformin can usually be achieved over a period of two to six weeks during which 90% of youths with T2DM can be successfully weaned off insulin and treated with metformin alone.

The aim of the initial treatment is to achieve fasting glucose levels under 126 mg/dL and an HbA1c level under 6.5% within three to four months. At the time of diagnosis, clinicians should initiate an educational program aiming to achieve permanent changes in the eating habits and lifestyle of the adolescent and their family. Education should be given by the members of the healthcare team (pediatric endocrinologist or pediatrician with diabetes expertise, dietitian nutritionist, psychologist, and/ or social worker) and involve the entire family. Young patients with T2DM should also be motivated to practice moderate-to-vigorous exercise for at least 60 minutes every day through negotiated and enjoyable exercise proposals. Dietary suggestions could start with eliminating soft drinks and juices rich in sugar, increasing fruit and vegetable consumption and portion control. Various diet strategies (balanced and varied diet, very low-carbohydrate diet, lower-glycemic-index diet, intermittent fasting diet…) that try to mirror family, local or national habits have been prescribed. However, currently there are no randomized controlled trials on the effect of these nutritional approaches.

The rate of drop out from healthy lifestyle behavior, however, is quite high; only a minority (<20%) of youths are able to maintain adequate glycaemic control with lifestyle therapy alone (**3,4**).

Long-term treatment

The therapeutic goals of T2DM are weight loss, an increase in exercise capacity, and normalization of blood glucose, as well as the control of comorbidities/complications. HbA1c target of <7% is achievable in most adolescents with T2DM, because there are lower chances of hypoglycemia in T2DM. HbA1c is recommended to be performed at least twice a year. The dangers of smoking and alcohol misuse have to be stressed at each visit. Contraceptive counseling should be included to avoid unplanned pregnancy in diabetes.

Self Monitoring Blood Glucose (SMBG) should be tailored, combining fasting and postprandial glucose measurements with a frequency based on the drug(s) used, and the target of glycemic control required. Once glycemic goals have been achieved, a few fasting and postprandial glucose determination a week are generally enough. More frequent testing is requested in the presence of worsening of metabolic control, if early complications are found, during acute illness, or in the occurrence of hyper- or hypoglycemic events. Patients treated with insulin or sulfonylureas need to use SMBG more often. The potential benefit of continuous glucose monitoring in T2DM youths is a matter of debate.

The pharmacological treatments metformin and insulin remain the only drugs approved by the Food and Drug Administration (FDA) and EMA for the treatment of young people with type 2 diabetes. *Biguanide metformin* should be started at 500 to 1000 mg daily for 7–14 days, augmented by 500 to 1000 mg every 1 to 2 weeks, depending on patient tolerability, until the maximal dose of 1000 mg twice a day or 850 mg three times a day is reached. Metformin was shown to be safe in randomized controlled trials, reducing BMI, blood pressure, A1C by 1.0% to 2.0% and lowering FPG, LDL-C, and triglycerides. Side effects (transient abdominal pain, diarrhea, nausea) can be reduced by slowly titling the dose over 3–4 weeks and with suggestions to take the drug with food. However**,** metformin monotherapy is ineffective in achieving glycemic targets for about 50% of patients within one year of treatment. The association of metformin with other glucose-lowering drugs such as glimepiride or glyburide didn't show superiority to the monotherapies. Metformin in association with insulin glargine has been used in pubertal obese 10–19-year-old youths with IGT or T2DM of <6

months duration without significant effect on the progressive deterioration of b-cell function (**4**).

Various *insulin* regimens, for example basal insulin (0.25–0.5 units/kg starting dose) or short-acting prandial insulin, can effectively achieve glycemic control. The dose should be adapted to the patient's needs. Early insulin therapy and the switching from oral antihyperglycemic drugs to insulin may lead to poor compliance with treatment and poor diabetes control in the long term. The choice of initiating insulin should therefore be evaluated carefully (**3,4**).

Liraglutide (a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist), *Exenatide* (another GLP-1 agonist) and the *sodium-glucose co-transporter-2 (SGLT2) inhibitors,* which inhibit subtype 2 of the sodium-glucose transport proteins (SGLT2) and significantly reduce glucose reabsorption in the kidney, are new drugs which have been used off-label in small studies on adolescents with T2DM: no serious adverse effects were observed. Apart from the difficulty in enlisting a sufficiently large number of young subjects with T2DM, trials that utilize these new drugs are limited by the poor knowledge of pharmacokinetics in youth (**15**).

Few studies are available on the benefits and risks of bariatric surgery in teenagers with T2DM. Thus, bariatric surgery in youth should be considered only in academic settings after interdisciplinary evaluation and should be limited to selected adolescents with severe obesity at risk of micro-and macroangiopathic complications (**3**).

A *structured transition program* from pediatric to adult care providers must be organized, as it is well-known that the rate of drop out during the follow-up is very high among adolescents and young adults with chronic diseases. This fact is of great importance in youth with T2DM who are poorly self-motivated and supported by their families, with a concomitant risk of the early development of complications (**3**).

Conclusions

T2DM in youth is a still now growing health concern, especially in ethnic minorities, in emerging countries and, to a lesser extent, in populations of European ancestry, in which an epidemic of severe overweight has occurred.

Genetic predisposition and obesity, together with the contribution of other personal and environmental factors, play a fundamental role in the pathogenesis of severe insulin resistance and impairment of β cell function.

In comparison to adults, T2DM in youths is a more aggressive form with a faster progression from prediabetes to overt hyperglycemia and earlier development of micro- and macrovascular complications.

Target screening has been proposed in severely obese children with a family history of T2DM, specific ethnicity, signs of insulin resistance and/or maternal history of diabetes and gestational diabetes during the child's gestation. However, no single test appears to be sufficiently sensitive, specific and accurate.

Metformin and insulin, together with continuous educational programs aimed to improve the lifestyle of the young patients and their family, are the only actual therapeutic resource which may improve the prognosis of youth with T2DM.

Prevention of T2DM in youth lies essentially in national health programs aimed to improve healthy lifestyle habits and the education of young people and their families which may reduce the growing impact of obesity on the general population.

	Type 1 DM	Monogenic Diabetes	Type 2 DM
Age at onset	Any age (from 6 months to adult)	Any age (from newborn to adult)	Peripubertal, pubertal
Ethnic distribution	All groups	All groups	Increased in ethnic groups
Family history of DM	In 3-5%	In 90- 100%	In 85-95%
Genetics	Polygenic, HLA haplotypes	Family clustering with autosomic dominant trait	Polygenic
Obesity	As in general population	Rare	Up to 95%
Onset modality	Acute	Variable, according to the type	Insidious, rarely severe
Ketosis	More likely present (up to 80%)	Variable, according to the type	Less likely present (up to 25%)
Signs of insulin resistance	More likely absent	More likely absent	Present
Complications at onset	More likely absent	More likely absent	More likely present

Table 1: Differences between Type 1 DM, Monogenic DM and T2DM in youth.

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SECTION 4 CHAPTER 2

METABOLIC SYNDROME

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Abbreviations:
MetS, metabolic syndrome
HDL-c, high-density lipoprotein cholesterol
T2DM, type 2 diabetes mellitus
CVD, cardiovascular disease
WHtR, waist-to-height-ratio
BMI, body mass index
ADA, American Diabetes Association
NAFLD, non-alcoholic fatty liver disease
PCOS, polycystic ovary syndrome
LDL-c, low-density lipoprotein-cholesterol

Introduction

Metabolic syndrome (MetS) is a clinical entity characterized by the coexistence of a cluster of cardiometabolic risk factors including central obesity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-c), and high fasting glucose. The first understanding of the concept of MetS dates back to the 1920s. MetS is a predictor of the future risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). CVD is the most common cause of mortality in adults worldwide and several meta-analyses agree that MetS is associated with a two-fold increase in cardiovascular events and a 1.5-fold increase in overall mortality in adulthood. In 1989, N.M. Kaplan captured the essence of this syndrome

by renaming it “the deadly quartet” for the co-occurrence of upper body obesity, glucose intolerance, hypertriglyceridemia, and hypertension, and the increased risk of mortality that characterized the affected subjects. The 2001 National Cholesterol Education Program (NCEP) definition of “metabolic syndrome,” is based on the presence of at least three of the following five cardiometabolic risk factors: central obesity, hyperglycemia, hypertriglyceridemia, low-HDL-c, and hypertension.

The roots of this clinical entity have distant origins that can go back to childhood. Metabolic programming occurs since the pre- and peri-conceptional period, influenced by genetic, epigenetic, and environmental factors, affecting long-term metabolic health. Over the past three decades, the prevalence of obesity in childhood and adolescence has grown by 47.1%, becoming one of the most serious public health challenges of our century. Child and adolescent obesity, in addition to being a risk factor for the persistence of obesity in adulthood, is an important cardiometabolic risk factor strongly associated with the development of MetS comorbidities, such as dyslipidemia, hypertension, and T2DM. The childhood obesity epidemic has been responsible for the onset of MetS since childhood, although it was previously recognized as an issue limited to adulthood. This is the reason why prevention strategies applied at the early stages of life are crucial to prevent the metabolic alterations of young adults and the subsequent cardiovascular complications.

Physiopathology

MetS is characterized by dysregulated cellular metabolism. Central obesity, which consists of an altered body composition characterized by increased visceral adiposity, is a key driver of the process of metabolic derangement. In fact, visceral adipose tissue has a pivotal role in the risk of developing MetS due to the release of non-esterified fatty acids and a multitude of other mediators and chemo-attractants into the circulation, leading to an overall increase in systemic inflammation and consequently to insulin resistance.

More specifically, white adipose tissue is a full-fledged endocrine organ that produces a great variety of hormones, enzymes, growth factors, inflammatory mediators, and metabolites, collectively termed adipocytokines or adipokines. The hyperplastic and hypertrophic growth of adipocytes, which occurs in obesity, makes them prone to local hypoxia. Hypoxia determines a cascade of reactions that lead to adipocyte dysfunction with altered adipokine secretion profile, infiltration of macrophages and upregulation of inflammatory factors and the release of cytokines. In particular, the

consequent dysfunction of the adipocytes causes a reduction in the production of adiponectin, an adipokine that exerts anti-atherogenic, anti-diabetogenic, anti-inflammatory, and anti-proliferative actions and produces an increased release of free fatty acids in peripheral tissues. This can alter mitochondrial function, increase the degree of oxidative stress and activate proinflammatory responses in vascular endothelial cells, contributing to the maintaining of low-grade systemic inflammation and endothelial dysfunction observed in obesity. The arising oxidative stress is the major putative causative path of insulin resistance because it acts by causing an overall effect by reducing the insulin ability to stimulate glucose transport to cell surfaces. Peripheral insulin resistance triggers negative feedback that induces a higher compensative insulin production. This mechanism is compensatory until needs exceed the pancreatic beta cell's ability to produce insulin, and plasma glucose levels increase, ultimately contributing to the development of T2DM.

Epidemiology

It is difficult to estimate the prevalence of MetS in childhood due to the variability of the diagnostic criteria used in the different studies. In the United States, an overall prevalence ranging from 1.2% to 9.8% is reported, however, evaluations among school-aged children reported lower prevalence (0.2%–1.2%). This is possibly due to the fact that puberty has a great effect on insulin resistance. In fact, the increase in the secretion of growth hormone, which occurs simultaneously with the pubertal growth spurt, has strong effects on the increase in insulin resistance. Physicians must consider that puberty itself may be involved with the progression of abnormal metabolic processes in children.

As obesity is a central driver for MetS, the prevalence of MetS is higher in obese children, ranging from 10% to 66%, depending on different studies. This great variability is not only due to the use of different diagnostic criteria but also to ethnic differences. A higher prevalence of MetS has been reported in East Asians, Asian Indians, Native Americans, Hispanics, and Japanese Americans. In addition, the prevalence of MetS varies significantly by gender, with male adolescents having a higher risk than females. Although MetS has traditionally been considered a problem of developed countries, the increase in pediatric obesity worldwide has made MetS a concern in developing countries as well, due to the changing diet patterns with high energy and low nutrient density foods increasingly available.

Diagnosis

Most definitions of MetS in children have been adapted from those in adults, considering the anthropometric, metabolic, and cardiovascular parameters related to age and sex, using the specific age and gender cut-off as threshold values. In recent years the scientific community has endeavored to define consistent and internationally validated diagnostic criteria for MetS in the pediatric population, but many different and conflicting definitions have been proposed. However, all diagnostic criteria agreed on the essential components of this clinical entity: central obesity, glucose intolerance, hypertension, and dyslipidemia. ***Table 1*** reports the pediatric and adolescent MetS criteria adapted from the National Cholesterol Education Program Adult Treatment Panel III.

Table 1. Pediatric and adolescent MetS criteria adapted from the National Cholesterol Education Program Adult Treatment Panel III*.

Criteria	Values
Central Obesity (WC)	WC $\geq 90^{th}$ percentile for age, sex, height
High BP (mmHg)	Systolic or diastolic BP $\geq 90^{th}$ percentile for age, sex, height
High Triglycerides (mg/dL)	TG $\geq$110 mg/dL ($\geq$1.24 mmol/L)
Low HDL-c (mg/dL)	HDL-c $\leq$40 mg/dL ($\leq$1.03 mmol/L)
High Fasting Glucose	$\geq$100 mg/dL (5.6 mmol/L) or known T2DM

* Individuals need to have at least three abnormalities in MetS components to be classified as having MetS.
Abbreviations: MetS, Metabolic syndrome; WC, waist circumference; BP, blood pressure; TG triglycerides; HDL-c, high-density lipoprotein-cholesterol; T2DM, type 2 diabetes mellitus.

A detailed medical history and clinical examination are essential to define cardiometabolic risk in children and adolescents. Family history and medical history should be carefully collected to highlight early life metabolic risk factors, such as the presence of T2DM or CVD in first or second degree relatives, maternal history of gestational diabetes, small for gestational age at birth, and major risk race/ethnicity (i.e., African American, Latino, Native American, Asian American, or Pacific Islander). The anthropometric evaluation should include the standardized determination of height, weight, body mass index (BMI) (applying age and sex-specific centiles), and pubertal development according to Tanner stage. The clinical examination should exclude the presence of clinical signs associated with

insulin resistance such as acanthosis nigricans and hirsutism. Since central obesity was considered an independent predictor of insulin resistance, lipid levels, and blood pressure and the main component of MetS, waist-to-height ratio (WHtR) and waist circumference provide a useful tool to define abdominal adiposity in childhood.

Blood pressure measurement is recommended in all children with overweight or obesity from the age of three. Blood pressure should be measured at rest and the mean value of at least two or three measurements should be considered. Systolic or diastolic blood pressure above the 90th percentile is indicative of high blood pressure, but the diagnosis of hypertension in children requires a systolic or diastolic blood pressure ≥95th, or >130/80 mmHg in adolescents ≥13 years old.

Measurement of fasting blood glucose, total cholesterol, HDL-c, and triglycerides is recommended in all children and adolescents with obesity from the age of six years, as the first step for screening for prediabetes, T2DM, and dyslipidemia. Whereas an oral glucose tolerance test with 1.75 g glucose per kg to a maximum of 75 g is indicated only in obese children with specific risk factors, according to the American Diabetes Association (ADA). Diagnostic workup in children and adolescents at risk of MetS is summarized in ***Table 2.***

Additional components of MetS

MetS has been linked to other metabolic and obesity-related diseases, whose pathophysiological mechanisms are also attributable to the metabolic effects of insulin resistance and obesity on different organs and systems: non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), hyperuricemia, and sleep disturbances. Although not yet considered in the definition of MetS, they have been described as strong risk factors for metabolic deterioration and as early signs of CVD in children, as well as in adults.

NAFLD and MetS are strongly associated such that NAFLD has also been described as the hepatic manifestation of MetS. Pediatric NAFLD is defined as chronic liver steatosis in children, which is not secondary to genetic diseases, infections, use of steatogenic medications, ethanol consumption, or malnutrition. Hepatic steatosis is histologically defined as the infiltration of fat into the liver of more than 5%. In recent decades, in parallel with the increase in the prevalence of childhood obesity, the prevalence of NAFLD has also increased, doubling over the past 20 years and becoming the most

Table 2: Diagnostic workup in children and adolescents at risk of MetS and diagnostic criteria for each component of MetS

Adapted from Weihe, P. & Weihrauch-Blüher, S. Current obesity reports (2019) and Valerio, G. et al. Ital. J. Pediatr. (2018).

Steps		**Diagnostic criteria**
1) **Patient and family risk factors**	**Patient's history:** Chronic disease, medication, small for gestational age, oligo-amenorrhea; **Family history**: Gestational diabetes, first- or second-degree relatives with obesity, T2DM or other features of the MetS; African American, Latino, Native American, Asian American race/ethnicity.	
2) **Clinical examination and anthropometric data**	a) body height, weight, BMI, waist circumferences (age- and sex-specific centiles), and WHtR. b) Signs or conditions associated with insulin resistance (acanthosis nigricans, hirsutism) *[1] the 85th, 97th and 99th percentiles approximate z-scores of + 1, + 2 and + 3, respectively	- BMI (*2–5 years*) > 85th percentile: at risk of overweight; >97th: overweight; >99th: Obesity - BMI (*5–18 years*) >85th percentile: overweight; >97th: obesity; >99th: sever obesity *[1] - WHtR >/= 0.5 (index of abdominal obesity)

3) **Arterial blood pressure**	Recommended in all children with overweight or obesity from the age of 3 years. *[2] should be considered the mean value of at least 2–3 measurements. Percentile measured are considered by gender, age and height	- Normal BP: SBP and DBP < 90th percentile; - High normal BP: SBP and/or DBP ≥90th but <95th or BP >120/80 mmHg (even <90th); - Hypertension (Stage I): SBP and/or DBP ≥95th <99th percentile + 5mmHg or BP >130/80 mmHg ≥13 years of age; - Hypertension (Stage II): SBP and/or DBP ≥99th percentile + 5 mmHg*[2] or BP >140/90 mmHg ≥13 years of age
3) **Glucose tolerance and diabetes**	a) **Prediabetes conditions** - Impaired fasting glucose - High HbA1c - Impaired glucose tolerance *[3] Indications for OGTT in children and adolescents with overweight or obesity according to ADA: - Children with fasting plasma glucose ≥100 mg/dl - Adolescents (>10 years of age, or at onset of puberty) with BMI >85th percentile and at least one of the risk factors at point 1)	- Fasting plasma glucose ≥100 mg/dL (5.6 mmol/L)–125 mg/dL (6.9 mmol/L); - HbA1c ≥5.7–6.4% - Plasma glucose after 2 h of the OGTT ≥140 mg/dL (7.8 mmol/L) - 199 mg/dL (11.1 mmol/L) *[3]

	b) **T2DM (non-symptomatic)** *[4] If one test is positive, the diagnosis must be confirmed by a second test in asymptomatic children.	- Fasting glycemia ≥126 mg/dL after 8 h of fasting; - Glycemia ≥200 mg/dl after 2 h of the OGTT; - HbA1c ≥6.5% or ≥48 mmol/l *[4].
4) **Dyslipidemia**	Total cholesterol, HDL-c, triglycerides *[5]LDL-c is calculated by Friedewald's formula; *[6] References values according to the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents.	- TC (mg/dL): borderline 170–199, high ≥200; - LDL-c (mg/dL) *[5]: borderline 110–129, high ≥130; - TG (mg/dL) (*0–9 years*): borderline 75–99, high ≥100; - TG (mg/dL) (*10–19 years*): borderline 90–129, high ≥130; - HDL-c (mg/dL): borderline 40–45, low <40 *[6].
5) **Additional components of MetS**	- ALT, AST; uric acid - Abdominal ultrasound (NAFLD) - Pelvic ultrasound (PCOS)	

Abbreviations: T2DM, type 2 diabetes mellitus; MetS, Metabolic syndrome; BMI, body mass index; WHtR, waist-to-height-ratio; WC, waist circumference; BP, blood pressure; OGTT, Oral glucose tolerance test; ADA, American diabetes association; HbA1c, Hemoglobin glycosylated A1c; TG triglycerides; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, Non-alcoholic fatty liver disease; PCOS: Polycystic ovary syndrome.

common form of childhood liver disease. The prevalence in obese children and adolescents is up to 40%, with a greater risk in males and some ethnic groups (e.g., Caucasian, Asian and Hispanic children). NAFLD could evolve into NASH, defined as the presence of hepatic steatosis with necroinflammation and hepatocellular injury with or without fibrosis. NASH may degenerate into liver cirrhosis which in turn is related to the risk of worse complications (i.e., hepatocellular carcinoma and portal hypertension). Identifying fibrosis in children with NAFLD and NASH is important because these phenotypes are expected to be more likely to progress to cirrhosis. Transaminases and liver ultrasound should be assessed in all children and adolescents with obesity from the age of six years. The finding of a bright liver on ultrasound examination, with or without transaminases elevation, suggests NAFLD.

Polycystic ovary syndrome is characterized by hyperandrogenism (acne, hirsutism, and alopecia) and ovary dysfunction (oligomenorrhea). It is directly associated with an increased risk of infertility, T2DM, MetS, and CVD in adolescent and adult females. Girls with PCOS should be monitored and screened frequently for MetS and, vice versa, girls with MetS must be followed for the possibility of presenting reproductive/endocrine trait of PCOS. The prevention and treatment of MetS, NAFLD, and PCOS are similar and have in common the treatment of excess adiposity and insulin resistance.

Treatment

Since central obesity is the nodal driver of insulin resistance and MetS, most of the preventive and therapeutic interventions for MetS aim to control obesity. The prevention and primary management for MetS is the promotion of a healthy lifestyle, which must involve the whole family, and includes the following three cornerstones: 1) moderate calorie restriction (to achieve a 5–10% body weight loss in the first year); 2) change in dietary composition; 3) increase in physical activity. The most effective interventions include a combined approach to reduce calorie intake and increase energy expenditure. It is important to consider that even apparently small goals, such as a decrease in the BMI standard deviations of 0.125, have an effective impact, being able to reduce multiple cardiovascular risk factors, such as blood pressure and triglycerides, and to increase glucose tolerance and HDL-c. The primary goal in dietary intervention programs should focus on reduced total energy intake. In fact, a low-energy diet, regardless of macronutrients distribution, has been proved effective in improving weight

in overweight and obese children and adolescents. As regards changes in dietary composition, the American Academy of Pediatrics, the American Heart Association, and the World Health Organization have recommended children over two years of age and adolescents increase their consumption of vegetables and fruit, reduce the intake of saturated fats by favoring unsaturated ones (e.g., olive oil and other vegetable oils) and reduce sugar intake. A good model to suggest is the Mediterranean diet as well as the elimination of sugary drinks and the reduced consumption of fast food and processed food high in sodium. Additionally, portion control education and regular meal consumption (five meals per day: three meals and no more than two snacks) are encouraged to avoid grazing. For any dietary intervention and calorie reduction in children, supervision by a dietician experienced in the growth needs of children is recommended.

Increasing the level of physical activity can improve all components of MetS in both adults and children. It reduces body weight and abdominal fat, improves glucose tolerance, and lipid profile by increasing HDL-c concentration and decreasing both LDL-c and triglycerides concentrations. In addition, exercise can lead to an improvement in endothelial function with a reduction in systolic and diastolic blood pressure. The World Health Organization recommends 60 minutes or more of physical activity per day for children and adolescents and suggests that most of this should be of moderate or vigorous intensity. In clinical practice, it has been shown that an effective way to increase energy expenditure in children and adolescents is to encourage them to integrate physical activities into a normal routine, e.g., cycling to school, negotiating a daily goal for total steps using pedometers, or promoting group play activities in the case of school-age children. It is extremely important to provide multidisciplinary care to these patients involving all family members, without forgetting the importance of psychological support and the promotion of good mental health (i.e., self-esteem, correct attitudes towards food and body image, quality health-related life).

In case of failure of multidisciplinary lifestyle interventions, the use of drugs is recommended after 6 months in the case of high-risk children and adolescents, providing lifestyle interventions are not abandoned. Orlistat (tetra-hydro-lipstinate) is the only anti-obesity drug approved for use in adolescents over the age of twelve. It is a gastrointestinal lipase inhibitor that reduces the absorption of triglycerides and cholesterol with known side effects including gastrointestinal symptoms and possible malabsorption of fat-soluble vitamins.

As regards the treatment of complications, to date, only two hypoglycemic drugs have been approved by the Food and Drug Administration to be used in children from the age of ten for the treatment of T2DM, i.e., metformin and liraglutide. This latter, a glucagon-like peptide-1 receptor agonist, was approved in 2019 for pediatric use as monotherapy when the use of metformin is inappropriate due to intolerance or contraindications. For obese children and adolescents with insulin resistance or prediabetes conditions, the use of metformin is still off-label and clinical trials have shown limited effects on weight reduction and insulin sensitivity.

Alterations in all other components of MetS should also be treated, if present, particularly when lifestyle intervention programs are insufficient. In obese children and adolescents with dyslipidemia and lifestyle modification failure, statin treatment may be considered, as well as in children with high blood pressure second-line therapy involves starting treatment with ACE inhibitors.

Bariatric surgery is indicated in adolescents with long-lasting severe obesity resistant to all other treatments (after at least twelve months of intensive treatment), especially in the presence of serious complications.

Conclusions

Non-communicable diseases represent a major challenge of our time, as they are an important cause of death worldwide. Longitudinal studies supported the strong links between childhood obesity, T2DM, MetS, and subclinical CVD risk markers, such as a greater thickness of the carotid artery intima-media, in adolescents and young adults.

Importantly, in pediatrics, the goal is not to successfully treat MetS, but to prevent it. In fact, to date, lifestyle intervention strategies for overweight and obese children and adolescents have shown only limited effects, being often insufficient to prevent long-term cardiometabolic complications in most world countries. Therefore, obesity prevention should be the main goal for pediatricians and should start from the first years of life through education to a balanced diet and a healthy lifestyle. It is essential to raise awareness and encourage screening campaigns aimed at identifying patients at higher risk of insulin resistance and long-term risk of chronic cardiometabolic disease from the earliest stages, thereby implementing careful follow-up and tailored preventive interventions for MetS and its components. Hence, pediatricians should keep in mind what the main cardiometabolic risk factors are from the first stages of life (i.e., family

history of T2DM or CVD; maternal gestational diabetes; small for gestational age at birth; the presence of signs or conditions associated with insulin resistance and MetS such as hypertension, dyslipidemia, polycystic ovary syndrome, acanthosis nigricans). Early diagnosis and effective treatment represent the first step to reduce adult MetS-related morbidity and mortality and limit the overall burden of T2DM and CVD, safeguarding the future health of children and adolescents.

Highlights/Take home messages

- The prevalence of MetS among children is increasing, concurrent with the obesity epidemic;
- Central obesity is the key driver of the imbalance of cellular metabolism leading to an overall increase in systemic inflammation and insulin resistance;
- Lifestyle interventions, i.e., diet and physical activity, are the current cornerstones of treatment for MetS in childhood, as well as for obesity;
- Early treatment of complications is recommended to reduce cardiovascular future risk;
- The prevention of childhood obesity and its complications is key to reduce MetS and the global burden of T2DM and CVD.

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SECTION 4 CHAPTER 3

OBESITY RELATED LIVER INVOLVEMENT, PCOS

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Type 2 diabetes (T2D) in childhood is closely related to obesity and insulin resistance (IR) (1). The liver represents one of the most important targets affected by the cardiometabolic burden of the aforementioned diseases (1, 2). In fact, Non Alcoholic Fatty Liver Disease (NAFLD) is largely recognized as the most common pediatric chronic hepatic disease mainly due to both the obesity and T2D epidemics (2). Recent data indicate an overall increased prevalence of NAFLD, with an estimated pediatric rate of up to 36.1% in obese children (1, 3). The relationship between T2D and NAFLD is complex and bidirectional, but it is still less defined in childhood (2, 4). Compelling evidence demonstrated not only that glucose metabolism derangements (such as T2D and prediabetes) are highly prevalent in pediatric NAFLD patients, in turn at higher risk of progressive liver diseases (e.g., non alcoholic steatohepatitis (NASH)), but also an increased prevalence of NAFLD among patients with T2D (2–4). NAFLD encompasses a wide histopathological spectrum of liver diseases, ranging from simple hepatic steatosis to NASH, advanced fibrosis, cirrhosis, and hepatocellular carcinoma. The pathophysiological basis of NAFLD might be summarized into the “multiple hit” hypothesis, in which a large number of triggers (such as genetic, epigenetic, and environmental factors) are closely interrelated (1, 5). According to the most recent European guidelines, screening for NAFLD (including liver function tests and hepatic ultrasound) (1, 5) should be performed in all obese children. NAFLD diagnosis is suggested in pediatric clinical practice by detected ultrasound hepatic steatosis and elevated serum alanine transaminase

levels (5). Despite the major feasibility of noninvasive imaging techniques (e.g., ultrasonography and magnetic resonance) to detect hepatic steatosis, the diagnostic gold standard is still represented by liver biopsy (5). Additionally due to the cost and the invasiveness of this procedure, its use also poses ethical issues in childhood and must be reserved for selected cases (5). Robust evidence showed a close association between NAFLD and Metabolic Syndrome, glucose metabolism derangements, cardiovascular disease and chronic kidney disease already in childhood, leading to an increased cardiometabolic risk (4). Given the plethora of extra-hepatic organ systems involved, NAFLD has been considered a multisystem disease with a remarkable impact on both morbidity and mortality (1, 5). To date, lifestyle modifications (including diet and physical activity) remain the cornerstone of the NAFLD treatment in childhood. Due to the intrinsic difficulty in achieving long-term weight loss, several pharmacological options have been studied but with limited results (5). In fact, there is currently a lack of licensed drug therapy of proven benefit for pediatric patients with NAFLD (1, 5). Promising alternative treatments targeting the main NAFLD pathogenic factors (such as oxidative stress, IR, gut-liver axis, dyslipidemia, and proinflammatory pathways) are being investigated, but results need to be validated on a larger scale (5). Liver involvement related to both obesity and T2D is mainly mediated by insulin resistance (IR) through a complex relationship that seems to be also bidirectional. In fact, it has been well assessed that the Western diet and IR favor NAFLD, which in turn contributes to hepatic IR. Moreover, IR increases hepatic de novo lipogenesis and impaired inhibition of adipose tissue lipolysis, resulting in an increased free fatty acids (FFAs) flux to the liver (1, 4). This not only decreases both insulin sensitivity and glucose uptake in intramyocellular lipids but also contributes to the promotion of insulin resistance through the activation of the serine/threonine kinases and reduced tyrosine phosphorylation of Insulin Receptor Substrate-1 (IRS-1). Taking into account this tangled link, it is clear the key role of insulin beyond the liver as a mediator of several different metabolic pathways. In fact, it has been largely studied the contribution of insulin in the phosphatidylinositol 3-kinase (PI-3K)/Akt and the mitogen-activated protein kinase (MAPK) pathways with metabolic and proliferating cells effects, respectively. These two molecular signaling pathways are involved in the pathogenesis of Polycystic Ovarian Syndrome (PCOS) by promoting steroid synthesis. In addition, a direct effect of insulin in increasing androstenedione secretion in thecal cells by enhancing CYP17A1 activity has been demonstrated (6). IR aggravates hyperandrogenism not only by stimulating both androstenedione and testosterone production via the

CYP17A1 pathway but also by reducing sex hormone-binding protein (SHBG), which in turn is involved in the PI3K/Akt pathway (including the IRS-1 phosphorylation) (6). More, central obesity not only increases the risk of IR but also impairs ovarian function through chronic inflammation and induced oxidative stress (including several mediators FFAs related to IL- 6, C-reactive protein, adiponectin, and TNF-α) (6). Thus, IR, central obesity and hyperandrogenism in PCOS are strictly interrelated via several pathways, resulting in a vicious cycle that exacerbates PCOS. PCOS represents a prevalent endocrine disorder in adolescent girls (almost 10%) (7). Although there is no global consensus on the definition, adolescent PCOS is currently defined by the coexistence of irregular menses (oligo-/amenorrhea) and clinical and/or biochemical evidence of hyperandrogenism >2 years beyond menarche, after exclusion of other causes of menstrual irregularity and androgen excess (e.g., 21-hydroxylase deficiency, prolactin excess, androgen secreting tumor). Due to intrinsic technical limitations of the transabdominal ultrasound, the use of ovarian morphology as a diagnostic criterion at this age is not recommended (7). Adolescent PCOS may have a high degree of phenotypical variability depending on different parameters such as age, body weight, ethnicity, genetic and environmental factors (6, 7). The main features of adolescent PCOS are hirsutism, acne and/or seborrhea, and oligo-amenorrhea (7, 8). Particularly, hirsutism (assessed by the modified Ferriman & Gallwey score) represents the first clinical marker of androgen excess, whose biochemical confirmation is documented by increased circulating testosterone levels and/or an estimate of free testosterone (7). The exact pathogenic mechanism is still unclear, but an imbalance between obesogenic environment and epigenetic background leading to a central (hepatovisceral) fat excess plays a prominent role. To date, studies focused on PCOS genetic susceptibility have demonstrated both epigenetic modulation (e.g., differential methylation, miRNAs) and genetic control exerted by at least 19 loci linked to genes associated with metabolic traits. Typically, central obesity, IR, LH hypersecretion, and low concentrations of circulating high-molecular-weight adiponectin act as drivers of PCOS in adolescence. In this scenario, central fat exerts a crucial role. In fact, it seems to drive PCOS patients to an adaptive endocrine-metabolic mode (Postpubertal central obesity syndrome) (9). Similar to NAFLD, these patients present a greater cardiometabolic risk (4). Of note, recent data showed a 4-fold increased prevalence of coexisting NAFLD in patients with PCOS (4). In view of the unclear PCOS pathogenesis, no FDA-approved treatments are currently available but this research area has gained remarkable interest in recent years. Given the major role of central obesity, weight loss represents

the mainstay therapeutic goal. It should be achieved through lifestyle modifications in order to reduce not only excess body fat but also the risk of related cardiometabolic outcomes (7, 8). Recommendations for combined oral contraceptives (COCs) as first-line medical treatment are available to improve both menstrual irregularity and clinical hyperandrogenism (8). In addition, metformin, an insulin sensitizer, might use as a second-line therapeutic modality in patients with PCOS and glucose metabolism impairments in case of failure of previous lifestyle interventions or contraindications to COCs. However, the use of both drugs is not supported by robust evidence in this age group, so further studies are required for adolescent PCOS management. Limited but promising adolescent data are provided by treatment with a low-dose combination of spironolactone, an aldosterone- and androgen-receptor antagonist, and pioglitazone/metformin, two insulin sensitizers (SPIOMET), with an overall metabolic improvement in these patients (8). Of note, a recent trial investigating the effects of one-year SPIOMET treatment confirms this finding and adds knowledge in the complex PCOS context by highlighting the role of C-X-C-motif chemokine ligand-14 (CXCL14), a chemokine released by active brown/beige adipose tissue with protective metabolic effects, as a novel potential biomarker (10). In conclusion, a better understanding of the pathogenic link among central obesity, liver, and IR might be helpful in carrying out target-specific strategies for this group of patients at higher cardiometabolic risk.

Summary

- Obesity and type 2 diabetes (T2D) are strictly interrelated and increase the overall cardiometabolic risk by affecting several organ systems already in childhood.
- Non Alcoholic Fatty Liver Disease (NAFLD) represents one of the most common comorbidities of both obesity and T2D. Patients with NAFLD are at greater risk of Metabolic Syndrome, glucose metabolism impairments and both cardiovascular and chronic kidney disease. Similarly, patients with glucose metabolism derangements have an increased prevalence of NAFLD.
- As metabolic consequence of obesity, Polycystic Ovarian Syndrome (PCOS) constitutes a prevalent heterogeneous endocrine disorder in adolescence. Central obesity, insulin resistance (IR), and hyperandrogenism are closely interrelated in a vicious cycle that aggravates PCOS. These patients have the same cardiometabolic risk of NAFLD subjects.

- IR plays a crucial role in the aforementioned comorbidities through several molecular signaling pathways (e.g., PI-3K/Akt, MAPK, IRS-1).
- Lifestyle modifications (including diet and physical activity) remain the mainstay of treatment of these comorbidities. Although promising drugs are being studied, there are currently no licensed pharmacological treatments for both NAFLD and PCOS pediatric patients.

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SECTION 5:

SECONDARY DIABETES/ NON DIABETIC HYPERGLYCEMIA

SECTION 5 CHAPTER 1

DYSGLYCEMIA IN CYSTIC FIBROSIS

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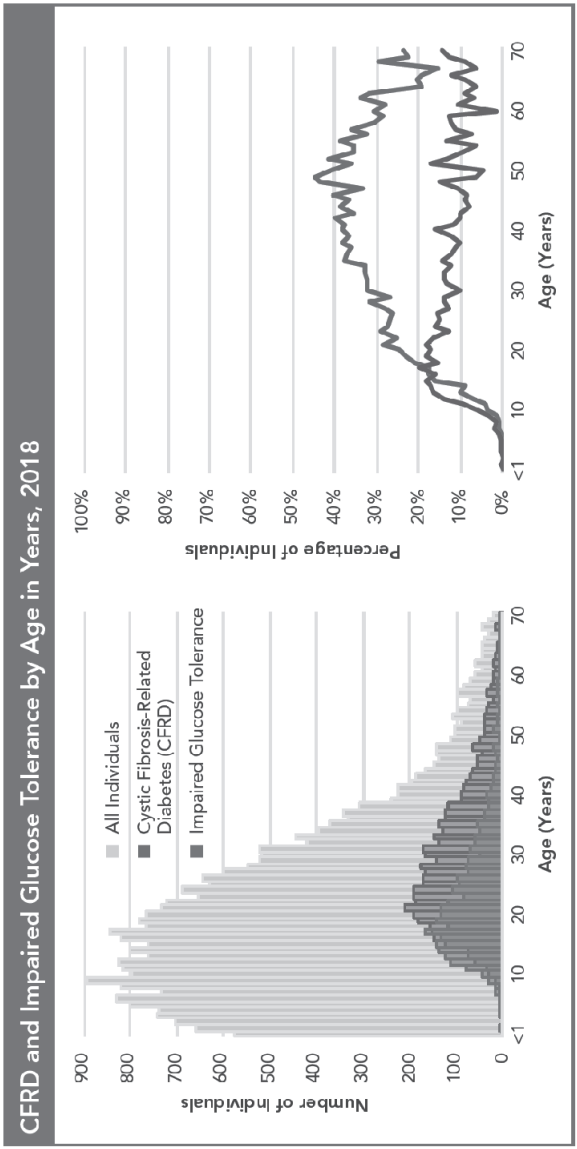
1. Introduction

Cystic fibrosis (CF) is caused by mutations leading to abnormalities in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a chloride channel involved in electrolyte and water movement across cell membranes and CFTR dysfunction leads to a chronic, multi organ disease. Glycemic derangements in CF include a spectrum of glucose tolerance abnormalities leading to progressive hyperglycemia and the diagnosis of cystic fibrosis related diabetes (CFRD). Spontaneous hypoglycemia including both fasting and reactive hypoglycemia in the absence of CFRD is also common in patients with CF. CFRD is the most common extra pulmonary complication of CF. An increased prevalence of CFRD has been observed due to improvements in CF patients' life expectancy and screening practices. The prevalence of CFRD increases with age. CFRD has been diagnosed in young children but diagnosis is uncommon before the age of ten. However, glucose tolerance abnormalities are common in young children with CF [1]. In a single CF center study,

Figure 1. Prevalence of CFRD and impaired glucose tolerance by age from Cystic Fibrosis Foundation Patient Registry, 2018 Annual Data Report (Reproduced with permission from ©2019 Cystic Fibrosis Foundation)



which followed > 500 patients, CFRD was present in around 2% of children 10 years and younger, 19% of adolescents, 40% of CF patients in their 20s and around 50% in those 30 and above in age [2]. Figure 1 shows the prevalence of impaired glucose tolerance and CFRD by age from Cystic Fibrosis Foundation Patient Registry, 2018 Annual Data Report [3]. The diagnosis of CFRD has significant implications on the overall health of patients with CF. CFRD is associated with increased morbidity and mortality, likely related to its negative impact on nutritional status and lung function. In this chapter, we will present a concise overview of the pathophysiology, screening and treatment strategies for CFRD.

2. Potential mechanism underlying the development of glucose intolerance and CFRD in CF

The pathophysiological mechanisms underlying the development of glucose impairment in subjects with CF is likely multifactorial and is not fully understood (Figure 2) [4, 5]. CFRD has features that overlap with both type 1 and type 2 diabetes (Table 1) but it is considered a distinct clinical entity. β cell dysfunction and reduction in β cell mass both likely contribute to the progressive insulin insufficiency seen in subjects with CF.

2.1 Structural islet abnormalities

Traditionally, CFRD was thought to be caused by the loss of β cells as a result of progressive destruction and fibrosis of the exocrine pancreas. This is the so-called “collateral damage hypothesis” or “bystander hypothesis” where continued inflammation, destruction and fibrosis of exocrine pancreatic tissue would then lead to dysfunction and ultimately destruction of β cells. This mechanism is likely a major contributor to the development of CFRD, as there is a strong association between exocrine pancreatic insufficiency and CFRD. However, this “bystander” effect does not explain the early changes in glucose tolerance and insulin secretion, such as impaired and abnormal glucose tolerance that can be seen in infants and prepubescent children with CF, well before the onset of pancreatic fibrosis. As a result, there are likely other etiological mechanisms to the development of CFRD (Figure 2).

Figure 2. Potential mechanisms underlying the development of CFRD.

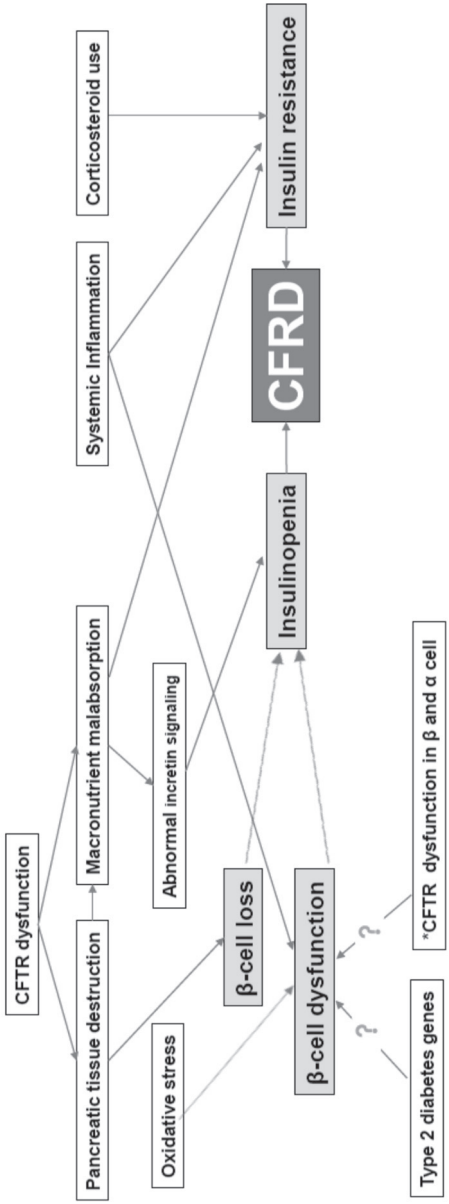


Table 1. Comparison of CFRD with type 1 and type 2 diabetes (adopted from ref [4])

	CFRD	Type 1 diabetes	Type 2 diabetes
Peak age of onset	Young adults	Primarily childhood (peaks at ages 4-7 and 10-14)	Adults
Onset	Insidious	Acute	Insidious
Antibodies (+)	Rare	Yes	No
Insulin secretion	Decreased	Decreased to absent	Decreased
Propensity to ketoacidosis	Rare	Yes	Rare
Diet recommendation	High calorie diet	Monitoring of carbohydrates. Normal balanced diet to allow normal growth	Monitoring of carbohydrates and calories to promote weight loss
Treatment	Insulin	Insulin	Diet, insulin, insulin secretagogues, sensitizers and others
Microvascular complications	Yes	Yes	Yes
Macrovascular complications	? No	Yes	Yes
Cause of death	Pulmonary disease	Cardiovascular disease	Cardiovascular disease

2.2 Functional islet abnormalities

Abnormal insulin secretion and glucose tolerance abnormalities can be seen, as early as infancy in subjects with CF. In one study, abnormal glucose metabolism was present in 39% of children between the age of three months and five years [1]. In a CF pig model, abnormal insulin secretion is seen in newborn pigs and these glucose tolerance abnormalities are not associated with reduced islet cell mass [6]. These findings suggest that functional defects in islet cell play a role in the pathogenesis of CFRD.

2.3 Role of CFTR mutation

There is conflicting evidence as to whether CFTR is expressed in the islet cells [4]. Some studies have demonstrated CFTR expression in the human and rodent islet cells and others have not. There is an ongoing debate about the potential direct effect of CFTR mutation in the pathogenesis of CFRD. CFTR defect could also indirectly influence insulin secretion through its impact on islet inflammation and exocrine pancreatic disease. There is also evidence to suggest that mutations in CFTR contribute to defective glucagon secretion from α cells. Small pilot studies in humans have shown improved insulin secretion after treatment with ivacaftor in CF subjects with G551D mutation. However, larger studies are needed to examine the impact of CFTR modulator therapies on glucose hemostasis.

2.4 Insulin resistance

Unlike type 2 diabetes, insulin resistance is not thought to be a typical feature of CFRD. During baseline stable health, patients with CF are thought to be insulin sensitive. However, insulin resistance can increase during times of acute illness, likely related to increased inflammation and concomitant use of glucocorticoid. Due to this, CF patients have fluctuating states of improved and worsening insulin sensitivity based on their overall wellness and steroid exposure.

2.5 Other factors contributing to CFRD

Genetic factors related to type 2 diabetes may modify the risk of CFRD. Several type 2 diabetes susceptibility genes such as TCF7L2 are also associated with increased risk for CFRD. A family history of type 2 diabetes is associated with an increased risk of CFRD. Autopsy studies have shown patients with CFRD had islet amyloid deposition while CF patients without diabetes did not. Islet amyloidosis is also seen in type 2 diabetes. Other factors including islet inflammation and changes in the incretion hormone axis have also been implicated in the etiology of dysglycemia in CF [4].

3. Clinical features and complications of CFRD

CFRD usually develops insidiously and the majority of patients with CFRD do not have classical symptoms of diabetes at diagnosis. Symptoms of CFRD could include an unexplained chronic decline in lung function, failure to gain or maintain weight, poor growth velocity and delayed

progression of puberty [7]. Classical symptoms of hyperglycemia like polyuria and polydipsia may be present but many patients are asymptomatic. Acute complications, such as diabetic ketoacidosis and hyperglycemic hyperosmolar state, as seen in type 1 and type 2 diabetes, are rare in CFRD.

3.1 Complications unique to CFRD

CFRD is associated with increased mortality in patients with CF. In one study, overall mortality for patients with CFRD was 1.8 per 100 person-years, compared to 0.5 per 100 person-years in patients with CF without CFRD [8]. The cause of increased mortality with diabetes in CF is not clear. Unlike type 1 and type 2 diabetes in which micro and macrovascular complications are the primary cause of morbidity and mortality; in patients with CF, pulmonary complications are the main cause of death. CFRD is implicated in the pathophysiology of lung function decline through its negative impact on nutritional status and lean body mass. Loss of lean body mass has been described as part of the natural history of dysglycemia in CF, reported as occurring as early as four years or as late as months before a formal diagnosis of CFRD is given. The mechanism for loss of lean body mass is unknown but is postulated to be related to catabolism induced by insulinopenia. A decline in pulmonary function, along with an increase in the frequency of pulmonary exacerbations, has been linked to abnormal glucose tolerance and the severity of insulinopenia. Glucose is normally not detected in airway secretions. In patients with CF, glucose in the respiratory secretions is shown to be elevated in the setting of systemic hyperglycemia and it is postulated that these glucose-containing secretions may contribute to bacterial growth and a pro- inflammatory environment.

3.2 Micro and macrovascular disease

Patients with CFRD are also at risk for microvascular complications like retinopathy and nephropathy. However, these complications occur at lower rates and are thought to be less severe compared to type 1 and type 2 diabetes [9]. This is likely due to persistent endogenous insulin secretion, which allows for better glycemic control and lack of metabolic abnormalities that are typically seen in type 2 diabetes. Current guidelines recommend starting annual screening for microvascular complications five years after diagnosis of CF [9]. Macrovascular disease is rare in CFRD and in the cases that are described, there are often other contributors to the development of macrovascular disease. Hyperlipidemia is also rare in CF and CFRD except for hypertriglyceridemia.

4. Impact of impaired glucose tolerance

In patients with CF, a decline in body mass index and pulmonary function can be seen in the early stages of glucose abnormalities, prior to diagnosis of CFRD. Impaired glucose tolerance (IGT) and indeterminate glycaemia (Table 2) as measured by the oral glucose tolerance test (OGTT) have been associated with increased risk of progression to CFRD. Despite data showing an association between these pre-diabetes stages and decline in clinical outcomes, there are insufficient data to show that early treatment of pre-diabetes stages in CF leads to better outcomes. Current guidelines do not recommend pharmacological treatment of IGT in CF. There are ongoing clinical studies evaluating the benefit of insulin therapy for pre-diabetes stages in CF.

Table 2. Classification of glucose tolerance categories in CF [9]

Glucose tolerance category	Fasting plasma glucose	1 h glucose	2 h glucose
Normal glucose tolerance	<100 mg/dL	<200 mg/dL	<140 mg/dL
Indeterminate glucose tolerance	<126 mg/dL	≥200 mg/dl	<140 mg/dL
Impaired glucose tolerance	<100 mg/dL	N/A	140–199 mg/dL
Impaired fasting glucose	100–125 mg/dL	N/A	<140 mg/dL
CFRD	≥126 mg/dL	N/A	≥200 mg/dl

5. Spontaneous hypoglycemia

Spontaneous hypoglycemia is common in patients with CF without CFRD, who are not on glucose lowering therapy [10]. Spontaneous hypoglycemia in CF can occur both in the fasting and postprandial states. Fasting hypoglycemia in CF is attributed to poor nutritional status and increased energy expenditure related to underlying inflammation or acute infection. Reactive or postprandial hypoglycemia is attributed to delayed and extended insulin release with blunted glucagon response. Dietary modifications with modest carbohydrate reduction at meals and adding a carbohydrate containing snack between meals can help prevent reactive hypoglycemia in most patients.

6. Screening and diagnosis of CFRD

6.1 Screening for CFRD

As CFRD is often clinically silent and is associated with morbidity and mortality, it is vital to screen for CFRD. Clinical practice guidelines for CFRD published by the American Diabetes Association [9] and the International Society for Pediatric and Adolescent Diabetes [7] recommend starting annual screening by age ten. Based on current guidelines, standard OGTT (patient fasted for 8 hours, 1.75 g/kg bodyweight oral glucose up to a maximum of 75 g, 2-hour test) is, at present, the only accepted screening test [7]. Other potential screening tests, like Hemoglobin A1c, fasting and random glucose are not recommended for screening due to their low sensitivity in patients with CF. Continuous glucose monitoring (CGM) has been proposed as an alternative screening tool but further research is needed to determine which CGM based outcomes and thresholds correlate with clinical outcomes in CF.

6.2 Diagnosis of CFRD

According to the current guidelines, during stable health, the diagnosis of CFRD can be made according to the standard ADA criteria used for the diagnosis of type 1 and type 2 diabetes (Table 3). In most patients with CF, a diagnosis of CFRD is made using the OGTT. Glucose tolerance is categorized into the following categories: 1) normal glucose tolerance, 2) indeterminate, 3) impaired glucose tolerance (IGT), and 4) CFRD (Table 2). Patients may fluctuate between glucose tolerance categories before the development of overt CFRD. A diagnosis of CFRD can also be made during acute illness or while on continuous enteral tube feeding (Table 3).

Table 3. Diagnostic criteria for the diagnosis of CFRD [9].

Clinical condition/setting	Diagnostic criteria
Stable baseline health	• FPG ≥126 mg/dl • 2-h OGTT glucose ≥200 mg/dl • HbA1C ≥6.5% (HbA1C <6.5% does not rule out CFRD) • Classical symptoms of diabetes (polyuria and polydipsia) in the presence of a casual glucose level ≥200 mg/dl
During acute illness	• If FPG levels ≥126 mg/dl or 2h postprandial plasma glucose levels ≥200 mg/dl persists for more than 48 h
Continuous enteral drip feeding	• Mid or immediate post feeding glucose ≥200 mg/dl, confirmed on two separate days

FPG: fasting plasma glucose; OGTT: oral glucose tolerance test.

7. Management of CFRD

The dietary recommendations for CFRD differ from those recommended for type 1 or type 2 diabetes. All patients with CF, including those with CFRD, require a high calorie, high salt, high fat diet. Patients with CF have a high caloric need to maintain nutritional status. Thus, calorie restriction is not appropriate for most cases of CFRD management. Current guidelines recommend insulin as the only medical treatment for CFRD [7]. Insulin therapy in CF patients improves glycemic control, nutritional status and lung function. Due to the association between nutritional status and survival in CF, the potential anabolic effects of insulin are thought to be one of the most important aspects of therapy [7]. CFRD patients without fasting hyperglycemia can be managed with premeal rapid-acting insulin, preferably based on an insulin to carbohydrate ratio. Instead, a basal-bolus insulin regimen or an insulin pump should be considered in CFRD patients with fasting hyperglycemia. The current guidelines do not recommend non-insulin diabetes agents for CFRD, although this is an area of active research. General guidelines for insulin dosing in CFRD are shown in Table 4. The current recommendations for glycemic targets in CFRD are the same as type 1 and type 2 diabetes which are based on the risk of microvascular disease

[9]. However, it is unclear as to what glycemic targets, if any, are necessary to reduce the risk of morbidity and mortality surrounding pulmonary outcomes. There may be a benefit to initiating low dose insulin even when a patient is achieving traditional glycemic targets strictly for the anabolic effects of insulin. The introduction of CFTR modulator therapy has made a dramatic impact on the management of CF. In small pilot studies, ivacaftor therapy in patients with G551D mutations, improved insulin secretion and glucose tolerance. Ongoing and future studies of CFTR modulators will provide more insights into the potential role of these drugs in the prevention or treatment of CFRD.

Table 4. General guidelines for insulin dosing in CFRD [7].

Typical total daily insulin requirement in CFRD	• 0.5 to 0.8 units insulin per kg body weight per day
Prandial insulin	• Common starting dose: 0.5–1 units rapid-acting insulin for 15 g carbohydrate. Consider using less insulin if younger • Pre-prandial dosing is preferred. However, post-prandial dosing may be needed in younger children or if there is gastroparesis
Correction insulin	• Typical pre-meal correction dose: 0.5–1 units rapid-acting for every 50 mg/dL above 150 mg/dL and titrate as needed
Basal insulin	• Generally, the goal is about 0.25 units/kg/day. Can start at half this dose and adjust based on fasting glucose
Overnight drip feedings	• A single dose of NPH or mixed insulin (regular with NPH) could cover an overnight drip feeding • Starting dose: calculate the total grams of carbohydrate in the feeding, determine a total insulin dose based on the insulin to carbohydrate ratio (typically 0.5–1 units per 15 g), and deliver half of this as regular and half as NPH insulin • Dose titration: based on mid-feed blood glucose (regular insulin adjustment) and post feed glucose (NPH adjustment)

Take home messages

1. The prevalence of CFRD increases with age.
2. Outpatient screening should occur annually by OGTT once the patient is ten years of age.
3. The mechanisms underlying CFRD development are not fully understood but progressive insulin insufficiency is considered the primary defect.
4. CFRD is associated with worsening nutritional status and pulmonary function in patients with CF.
5. Insulin is the only recommended medical treatment for CFRD.
6. Insulin therapy improves glycemic control, nutritional status and lung outcomes in patients with CFRD.

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SECTION 5 CHAPTER 2

DIABETES RELATED TO GENETIC SYNDROMES: PRADER WILLI SYNDROME

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Introduction

Prader-Willi syndrome (PWS) is a rare and complex disorder due to absent expression of paternally inherited genes at the locus q11-q13 of chromosome 15 *(1)*. It is considered the most common cause of syndromic obesity, occurring in 1:10,000–1:30,000 live births. The phenotype typically includes neonatal hypotonia, poor eating and loss of appetite in infancy, followed by hyperphagia with onset of obesity in early childhood (if uncontrolled), dysmorphic features, behavioral and psychiatric issues, cognitive impairment, genetic short stature and multiple endocrine abnormalities (GH deficiency, hypogonadism, hypothyroidism, central adrenal insufficiency, hypoinsulinemia, hyperghrelinemia and low oxytocin levels) *(2)* (Table 1).

In the absence of intervention, obesity associated with PWS can be massive even in early childhood, and its repercussions represent an important cause of morbidity and mortality, including cardiovascular disease, venous thromboembolism, respiratory failure, alteration of the digestive tract, metabolic syndrome and diabetes mellitus.

Table 1: Clinical Manifestations of Prader-Willi Syndrome

- **Dysmorphic features**
  characteristic facial appearance, small hands and feet, narrow hands with straight ulna border
- **Musculoskeletal issues**
  neonatal central hypotonia (causing decreased fetal movements, initial poor feeding and failure to thrive), scoliosis and/or kyphosis, osteoporosis, hip dysplasia
- **Obesity and its comorbidities**
  hyperphagia and early childhood-onset of morbid obesity *(if uncontrolled),* type 2 diabetes - hyperlipemia and hyperuricemia, hypertension, metabolic syndrome, gallstones, hepatic steatosis
- **Sleep disturbances**
  Obstructive sleep apnea, central sleep apnea, narcolepsy, hypoventilation syndrome
- **Endocrine abnormalities**
  short stature *(for genetic background)* and GH-IGF-1 axis dysfunction, hypogonadism, premature adrenarche, central hypothyroidism, alteration of oxytocin system, central adrenal insufficiency *(very rare)*
- **Cognitive, behavioral and psychiatric disturbances**
  developmental delay, typical behavioral problems, skin-picking, psychiatric disturbances, speech articulation defect
- **Miscellaneous**
  hypopigmentation, esotropia and/or myopia, viscous saliva, high pain threshold, reducing incidence of vomiting, thermoregulatory disorders, seizures, unusual skill with jigsaw puzzles, gastrointestinal problems

Alteration of carbohydrate metabolism in PWS

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes in PWS patients, whereas both type 1 and monogenic diabetes occur very rarely *(3).* Altered glucose metabolism (AGM) seems to be less common in the pediatric age than in adults with PWS, although its prevalence is higher (about 10–25% of cases) than observed in the general population. A study performed on 129 children and adolescents with PWS showed the presence of impaired glucose tolerance (IGT) in 7 patients (5.4%) and T2DM in 6 subjects (4.6%) *(4).* IGT has been found only in 4% of patients of a large French cohort with PWS aged 2–18 years, while none of them had T2DM *(5).* Otherwise, an earlier onset of T2DM has been found in a cohort of Japanese PWS patients with a mean age of 15 years *(6)*.

The increased frequency of impaired glucose metabolism observed in pediatric patients with PWS seems to be linked to the presence of obesity, as in non-PWS populations. In this context, the frequency of glycemic alterations in PWS children has been found to be lower in non-obese subjects than in obese patients. The correlation between weight excess and development of T2DM in PWS patients, however, is unclear and may be different from what was observed in patients with non-syndromic obesity. Indeed, it has been widely demonstrated that PWS patients present a higher insulin sensitivity when compared to patients with essential obesity. Moreover, insulin sensitivity (assessed by QUICKI) was moderately but significantly increased in non-obese PWS patients compared to lean controls. Unlike simple obesity, children and adolescents with PWS show low fasting insulin levels and reduced insulin resistance (measured by HOMA-IR) *(3)*. More specifically, HOMA-IR in PWS patients seems to be significantly lower than in the controls from the age of 11. Despite a similar Body Mass Index (BMI = kg/m^2) and glycemic response, stimulated insulin levels to both a mixed meal and an oral glucose tolerance test (OGTT) were significantly lower in PWS children than in obese controls *(7)*.

The mechanisms underlying the peculiar alterations of glucose homeostasis observed in PWS remain to be fully clarified. One possible explanation for the reduced insulin resistance could be the lower trunk-to-appendicular fat mass ratio and visceral adiposity in PWS patients, unlike in non-syndromic obesity.

In addition, it has been suggested that hyperghrelinemia may contribute through the modulation of insulin secretion and insulin receptor signaling. The impaired GH secretion can also act through a reduction of its trophic action on pancreatic islets. Furthermore, since adiponectin has a significant insulin-sensitizing effect, its increased concentrations in PWS might promote higher insulin sensitivity in these subjects. Further proposed reasons for pancreatic β cell dysfunction in PWS is an altereted vagal parasympathetic response to the pancreas, as well as impaired transformation of proinsulin to insulin, due to prohormone convertase PC1 deficiency *(8)*.

No clear data are available on differences in insulin secretion in PWS patients. Other studies have shown that PWS children and BMI-matched controls had similar insulin levels and a degree of insulin resistance. In this light, an evident association between weight excess and insulin values is still observable in PWS children, as obese patients had higher glucose

levels, insulin values and insulin resistance than non-obese individuals. Moreover, insulin resistance in PWS showed a positive correlation with age with a peak in adolescents, as in the general population *(3)*.

These differences may be due to the different clinical characteristics of the patients enrolled in the studies, including age, BMI, body composition parameters, concomitant GH and/or sex steroids therapy. However, further studies are needed to better understand these differences..

Clinical Characteristics of PWS with altered glucose metabolism (AGM)

The clinical aspect of AGM in PWS is rather variable. Most of these patients are asymptomatic, but occasionally the classic symptoms of T2DM may be present and unexplained weight loss, despite hyperphagia, was reported in rare cases *(9)*.

Accordingly, the indications for screening for AGM and its consequences in PWS subjects, especially if obese, are the same as those used for the general population *(3)*.

Starting from the pediatric age, these patients should have yearly evaluations of glucose, insulin and HbA1c levels. A standard OGTT should be performed starting at 10 years of age, particularly in overweight PWS subjects.

Diabetes-related complications seem surprisingly infrequent in PWS. At least once a year, PWS patients with T2DM should be screened for microvascular complications as well as hypertension and cardiovascular disease.

Influence of GH therapy on glucose metabolism in PWS

GH therapy (GHT) is an effective treatment for children with PWS, with beneficial effects not only on linear growth but also on muscle strength, lipid profile, body composition, cognition and quality of life. Long-term GHT has no adverse effects on glucose metabolism if weight gain does not occur, but might increase fasting insulin levels and reduce insulin sensitivity, mainly in those PWS patients who are obese and/or over 10 years old and/or with diabetes or obesity in the family *(4)*. Therefore, a risk assessment of glucose alterations is recommended before initiating and during GHT for all PWS subjects (10).

Management of PWS with AGM

To date, no strategy for the treatment of AGM in PWS has been established. Obesity prevention remains the primary goal in all PWS patients, as excess weight increases the risk of developing T2DM *(3)*.

Unfortunately, up to now, no effective pharmacological treatment options for hyperphagia and binge eating in PWS are seen. Dietary restriction, reduced food intake, continuous lifelong supervision, and physical exercise are still the only alternatives available.

The medications used to treat T2DM in PWS patients, are the same as those used in nonsyndromic obesity-related diabetes *(11)*. Metformin should be considered as a first-line medication, especially in those patients with obesity and increased insulin resistance through its positive effect on insulin sensitivity. Gastrointestinal problems and in some cases vitamin B12 deficiency can be observed. Thiazolidinediones *(i.e., pioglitazone)*, sulfonylureas *(i.e., gliclazide, glimepiride, etc.)* and meglitinides *(repaglinide)* may not always be appropriate in obese PWS with T2DM because they can increase body weight.

Alpha-glucosidase inhibitors *(acarbose)* seem to reduce body weight and postprandial glycemia, as well as improving nocturnal hypoglycemia and reducing the daily insulin dosage.

The use of Glucagon-like peptide-1 (GLP-1) receptor agonists/analogs *(exenatide*, *liraglutide, dulaglutide, etc.)* in PWS is promising but has not yet been validated as a long-term therapy. These drugs can induce improvement/stabilization of AGM and promote weight loss. However, attention should be paid to the potential side effects of delayed gastric emptying *(12)*.

Dipeptidyl peptidase-4 (DPP4) inhibitors *(alogliptin, saxagliptin, sitagliptin, etc.)* and glucose-lowering SGLT2 inhibitors *(canagliflozin, dapagliflozin, empagliflozin, etc.)* have been successfully used in PWS, with positive effects on weight and HbA1c levels.

Insulin therapy should be considered for patients who do not achieve glycemic goals with other types of medications, despite its negative effect on body weight.. Unfortunately, patients with PWS do not always comply with self-monitoring of blood glucose and insulin injections. In fact, PWS is often associated with obstinacy and obsessive-compulsive symptoms which make daily diabetes management difficult. In addition, the risk of

severe hypoglycemia associated with insulin therapy should always be carefully evaluated, especially when access to food must be reduced.

Bariatric surgery (*i.e., laparoscopic sleeve gastrectomy and mini-gastric by-pass*) can often induce weight loss and favorable metabolic changes, with the improvement or healing of AGM in absence of major perioperative complications or mortality. In these cases, however, close dietary monitoring is still mandatory.

Even if experiences with bariatric surgery in PWS have been encouraging, recent studies demonstrated that this surgical procedure cannot guarantee long-term sustainable weight loss or resolution of comorbidities *(13)*.

However, bariatric surgery should be borne in mind only in critical and selected cases where severe obesity-related morbidities are present (e.g., T2DM) and where rapid weight loss is potentially beneficial, particularly if equally effective alternative solutions are not available (8).

Conclusions

Although published data regarding AGM in PWS are scarce, T2DM can occur with a variable prevalence, particularly in adulthood and in obese individuals. Since obesity has a major role in the onset and development of AGM in PWS, the control of body weight remains the most important goal in these patients. Poor exercise and early obesity should be prevented by early interventions, although patients with PWS tend to avoid exercise and behavioral disorders make lifestyle modifications and dietary interventions difficult.

T2DM in PWS is clinically identical to obesity-related T2DM, but diabetes-related complications are less common in these patients. Screening for AGM should be routine for PWS subjects, especially in obese individuals and during GHT. Indeed, early identification and treatment of individuals with impaired glucose homeostasis could reduce morbidity and prevent mortality.

The pharmacological intervention in PWS is similar to that of non-syndromic T2DM, while the role of bariatric surgery has yet to be clearly defined (3).

Summary (take home messages)

- *PWS children show low fasting insulin levels and reduced insulin resistance compared to BMI-matched controls.*
- *T2DM occurs in about 20–25% of all patients with PWS, especially in late adolescence and adulthood, while it is not frequent during childhood.*
- *Severe obesity is an important risk factor for T2DM in these subjects.*
- *Prevention of obesity and regular glucose monitoring are recommended in all PWS patients.*
- *Growth hormone therapy does not negatively influence glucose metabolism if weight gain is kept under control.*

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SECTION 5 CHAPTER 3

DIABETES RELATED TO GENETIC SYNDROMES: BARDET BIEDL, WOLFRAM, ALSTRÖM

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Introduction

Classically, diabetes mellitus in children and adolescents is assumed to be type 1, autoimmune and ketosis prone, which still represent the majority of cases. Nevertheless, not all forms of pediatric diabetes are type 1. In particular, type 2 diabetes (T2DM) prevalence, until the recent past unknown for pediatricians, is increasing, especially among obese adolescents. The T2DM phenotype is extremely variable, ranging from transient hyperglycemia to non ketoacidotic hyperosmolar coma or diabetic ketoacidosis. T2DM clinical onset is frequently preceded by the so-called metabolic syndrome, characterized by a cluster of obesity, either general or abdominal, dyslipidemia, hypertension, insulin resistance and different degrees of glucose intolerance (1). Hyperandrogenism, polycystic ovary syndrome, non alcoholic fatty liver disease, obstructive sleep apnea, chronic low grade inflammation, and hyperuricemia are other clinical characteristics frequently associated.

The term monogenic diabetes defines an heterogeneous group resulting from mutations in a single gene and accounts for about 1–2% of cases of diabetes. Mitochondrial and neonatal diabetes, maturity onset diabetes of the young, and rare forms of syndromic diabetes are included in this group. These forms are characterized by impaired beta-cell activity with a broad clinical spectrum ranging from incidental hyperglycemia to severe metabolic decompensation (2). In monogenic diabetes beta cell autoimmune

damage is usually absent, otherwise insulin resistance and/or impaired secretion is frequently associated.

More than 30 genes encoding for proteins involved in the insulin pathway and, more generally, in pancreatic beta-cell physiology, are associated with different subtypes of monogenic diabetes (2-4).

We describe three uncommon genetic syndromes characterized by young-onset diabetes mellitus: Bardet Biedl, Wolfram and Alström syndrome.

Bardet Biedl Syndrome

Bardet Biedl syndrome (BBS; OMIM#209900) is a rare genetic disorder with severe multiorgan impairment and a prevalence ranging from 1/125,000 to 1/175,000 births, even if in isolated communities its frequency is higher (5). The main clinical characteristics include severe widespread diffuse obesity, rod-cone dystrophy, postaxial polydactyly, genital abnormalities, and kidney defects. Other clinical features are developmental delay, speech defects, brachydactyly or syndactyly, dental abnormalities, olfactory deficit, ataxia and poor coordination, congenital heart disease and diabetes mellitus. The clinical phenotype is extremely variable and usually worsens during childhood and adolescence (5). At present 20 different genes (named BBS1-BBS20) have been mapped on different chromosomes and autosomal recessive transmission is usually observed, even if oligogenic inheritance has also been described.

A recent meta-analysis studied the genotype-phenotype association in BBS patients, demonstrating how different mutation types were predictors of clinical manifestation of the syndrome (6). All BBS genes encode for proteins involved in cilia development and function, being a part of BBSome. Cilia are microtubule-based organelles that protrude from the surface of a large number of cells. Cilia are divided in motile and immotile cilia. Defects in immotile cilia are responsible for retinitis pigmentosa, polydactyly, learning difficulties, situs inversus, cystic kidney, and liver and pancreas function impairment. BBS deficiencies are associated with increased appetite and reduced physical exercise, with peripheral leptin resistance and the risk of developing metabolic syndrome. Comorbidities characterizing BBS can be managed by symptomatic treatment and at present, it is not possible to prevent some complications, like loss of vision. Weight management is mandatory to delay the progression of metabolic syndrome as well as periodical endocrinological and glycometabolic evaluations for a prompt diagnosis of glucose intolerance

and diabetes mellitus. Diabetes mellitus is non autoimmune but secondary to insulin resistance, and the hyperglycemic hyperosmolar state has been reported as the first clinical presentation. Metabolic decompensation should be treated with adequate intravenous and subcutaneous insulin and, after recovery, adequate food plans and lifestyle modifications including increased exercise are the first choices. Pharmacological treatment with insulin sensitizing drugs like metformin is sometimes necessary but deserves attention due to its side effects, such as lactic acidosis and B12 vitamin deficiency, needing supplementation.

Wolfram Syndrome

Wolfram Syndrome (WS; OMIM#222300) is a rare autosomal-recessive disease (estimated prevalence of 1/550,000 children) characterized by diabetes mellitus, optic atrophy, diabetes insipidus, hearing loss and other neurological and endocrine dysfunctions (7). The acronym DIDMOADUD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness, Urinary Dysfunction) includes the main clinical characteristics of the syndrome. Glucose abnormalities, subclinical hearing loss, neuropsychiatric disturbance and other disorders have also been reported in heterozygous relatives of patients with WS (carrier frequency of 1/354). The prognosis is severe: nearly half of affected patients die before age 35 from central respiratory failure or renal failure secondary to infection.

The nuclear gene for WS is mapped to chromosome 4p16.1 and encodes a protein of 890 amino acids called Wolframin (WFS1). This gene is composed of eight exons: WFS1 mRNA is expressed in several organs such as pancreas, brain, heart, skeletal muscle, placenta, lung, liver, and kidney (8). In 2000, a second locus on chromosome 4q22-q24, called Wolframin 2 (WFS2), was identified by studying four Jordanian consanguineous families. The ZCD2-encoded protein, ERIS (Endoplasmic Reticulum Intermembrane Small protein), although also located in the endoplasmic reticulum (ER), does not interact directly with Wolframin (9). WFS2 patients show upper gastroenterological ulceration and bleeding, diabetes mellitus, visual impairment, and hearing loss, but do not develop diabetes insipidus.

Evidence suggests that WFS1 is a glycoprotein localized in the endoplasmic reticulum responsible for calcium homeostasis, acting as a calcium channel or a channel regulator. WFS gene mutations may activate several ER stress-mediated pro-apoptotic pathways leading to diabetes mellitus and multiorgan failure. Recently it has been demonstrated that WFS1 is a

calmomodulin (CaM)-binding protein responsible for mediation of a large number of signaling processes (8).

Diabetes mellitus is an invariable finding, usually the first clinical manifestation of the syndrome. It is an insulin-dependent non-autoimmune form of diabetes, caused by degeneration of β-cell. Autopsy studies have shown beta-cell loss or atrophy of the pancreatic islets without involvement of the exocrine portion of the gland or with focal areas of fibrosis. Thus, WS-associated diabetes is not caused by a functional defect in β-cells but by actual β-cells depletion. Diabetes mellitus clinical phenotype is usually mild, subcutaneous insulin therapy is the first choice of treatment and microangiopathic complications are almost absent, at least in adolescence. Regenerative medicine and glucagon-like peptide (GLP)-1 receptor agonists are promising treatment for allevieting ER-stress-mediated cell death in WS and improving diabetes clinical course. Recent studies indicate that loss of function of WFS1 causes mitochondrial dysfunction, accelerating neurodegeneration. From this point of view, mitochondrial modulating drugs might be able to delay neuronal dysfunction. WS is a devastating disease with severe morbidity, precocious mortality and quality of life impairment. At present no intervention program can alter disease progression and life expectancy.

Alström Syndrome

Alström syndrome (ALMS; OMIM#203800) is a rare multiorgan disorder with autosomal recessive inheritance and characterized by several metabolic deficits, including cone-rod dystrophy, hearing loss, childhood truncal obesity, hyperinsulinemia and insulin resistance followed by juvenile onset type 2 diabetes, hypertriglyceridemia, failure to thrive, cardiomyopathy, hepatic, pulmonary and renal dysfunctions. The prevalence of Alström syndrome is 1–9 cases per million, and about 950 cases have been reported (10). Symptoms usually appear in early infancy and progressive multiorgan involvement is responsible for reduced life expectancy. Different clinical phenotypes and ages at diagnosis within affected family members are likely to be due to genetic background. However, a genotype/phenotype correlation has not been demonstrated and clinical phenotype might be influenced by unknown genetic and environmental factors. Alström syndrome is caused by mutations in the ALMS1 gene. This gene, located on chromosome 2p13, consists of 23 exons and encodes for a protein of 4,169 amino acids (11). More than 200 variants have been described. The most common defects in this gene include insertions,

deletions, and nonsense mutations in exons 8, 10, and 16 resulting in protein truncations. ALMS1 protein has multiple functions and is located in centrosomes, basal bodies and cytosol. ALMS1 is a ciliary protein and the widespread clinical phenotype partially resembles that of other ciliopathies such as BBS. As regards diabetes, progressive insulin resistance with several features of metabolic syndrome have been described and the incidence of clinical diabetes ranges from 68 to 82% of patients over the age of 16. Treatment of type 2 diabetes includes lifestyle changes counseling, oral hypoglycemic agents like metformin (acarbose, repaglinide and glimepiride can be prescribed in experimental studies only). The early diagnosis of glycemic impairment is mandatory in order to prevent severe metabolic decompensation. Even if type 2 diabetes does not seem to be the major contributor to kidney dysfunction, it might have an additive effect on renal impairment progression, therefore strict metabolic control of glucose levels is mandatory (12).

Take Home Messages

1. Metabolic syndrome and T2DM develop in genetic forms of obesity
2. Clinical presentation is extremely variable
3. Lifestyle modification and adequate food plan are mandatory
4. Pharmacologic treatments include subcutaneous insulin and/or insulin sensitizer oral agents

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SECTION 5 CHAPTER 4

HYPERGLICEMIA RELATED TO STEROID AND OTHER DRUGS THERAPY

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Introduction

Several medications can induce acute or chronic hyperglycemia, including glucocorticoids, immunosuppressive drugs, chemotherapy agents, pentamidine, nicotinic acid, diazoxide, beta-adrenergic agonists, thiazides and phenytoin (Yaturu S 2008).

Drug-induced hyperglycemia in childhood has been often observed during acute lymphoblastic leukemia (ALL) treatment and related to the use of steroids and asparaginase (Hijiya N 2016).

Early diagnosis and careful management of hyperglycemia are essential in children with cancer in order to prevent severe complications such as diabetic ketoacidosis and hyperosmolar non-ketotic coma. In addition, hyperglycemia has been associated with high infection rates and poor prognosis, similarly to what is observed in adults (Shariff AI 2019).

Glucocorticoids

Adrenal glucocorticoid (GC) hormones contribute to energy homeostasis playing a role in glucose, lipid and protein metabolism. Short-term or chronic supra-physiological GC treatments are widely used in almost all medical fields, due to their well-known anti-inflammatory and immune-

suppressive activity. Despite their excellent efficacy and effectiveness profile, GCs cause insulin resistance and various metabolic side effects, as abdominal adiposity, skeletal muscle atrophy, hepatic steatosis and dyslipidemia with elevated triglycerides and non-esterified fatty acids (NEFA) (van Raalte DH 2009). Steroid-induced diabetes mellitus (SIDM) is defined according to the American Diabetes Association criteria (Diabetes Care 2012), as a form of diabetes associated with GC treatment, regardless of a previous history of diabetes.

The detrimental effects of GCs on glucose metabolism result from a complex pancreatic β cell dysfunction, characterized by impaired glucose sensitivity and insulin release, associated with hepatic and peripheral insulin resistance. The effects of GCs on β cell function depend on the dosage, treatment duration and individual susceptibility. *In vitro* and *in vivo* studies have shown that GCs acute exposure interferes with β cell glucose uptake and oxidation and inhibits insulin secretion. Although prolonged GC exposure induces hyperinsulinemia as compensation for insulin resistance, chronic GC treatment causes a progressive disruption of β cell function, due to a pro-apoptotic effect in susceptible individuals (van Raalte DH 2009). Skeletal muscle metabolism accounts for about 80% of postprandial glucose uptake from the circulation, through an insulin-mediated mechanism. Insulin resistance in muscle is caused by GCs interference with the insulin post-receptor signaling cascade (van Raalte DH 2009). Specifically, GCs decrease the activity of insulin receptor substrate (IRS)-1, phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (PKB)/Akt (van Raalte DH 2009). These molecules are responsible for the insulin-induced glucose uptake mediated by the translocation of glucose transporter 4 (GLUT4) from intracellular vesicles to the cell surface (Macut D 2017). In addition, GCs reduce insulin-induced glycogen synthesis by inactivating glycogen synthase (Figure 1) (Shariff AI 2019). GC-dependent skeletal muscle wasting results from a negative effect on insulin-mediated protein synthesis, associated with an augmented protein breakdown. Protein synthesis decreases through PKB/Akt inhibition and mammalian target of rapamycin (mTOR) phosphorylation. On the contrary, the augmented protein degradation is related to the upregulation of the genes encoding transcriptional factors FOXO 1 and 3, which cause the release of gluconeogenic substrates (such as alanine) from skeletal muscle (Figure 1). Finally, GC-induced lipolysis increases the accumulation of lipids (such as fatty acyl CoA, diacylglycerol and ceramide) inside skeletal muscle fibers, which interfere with insulin signaling and glucose uptake (van Raalte 2009).

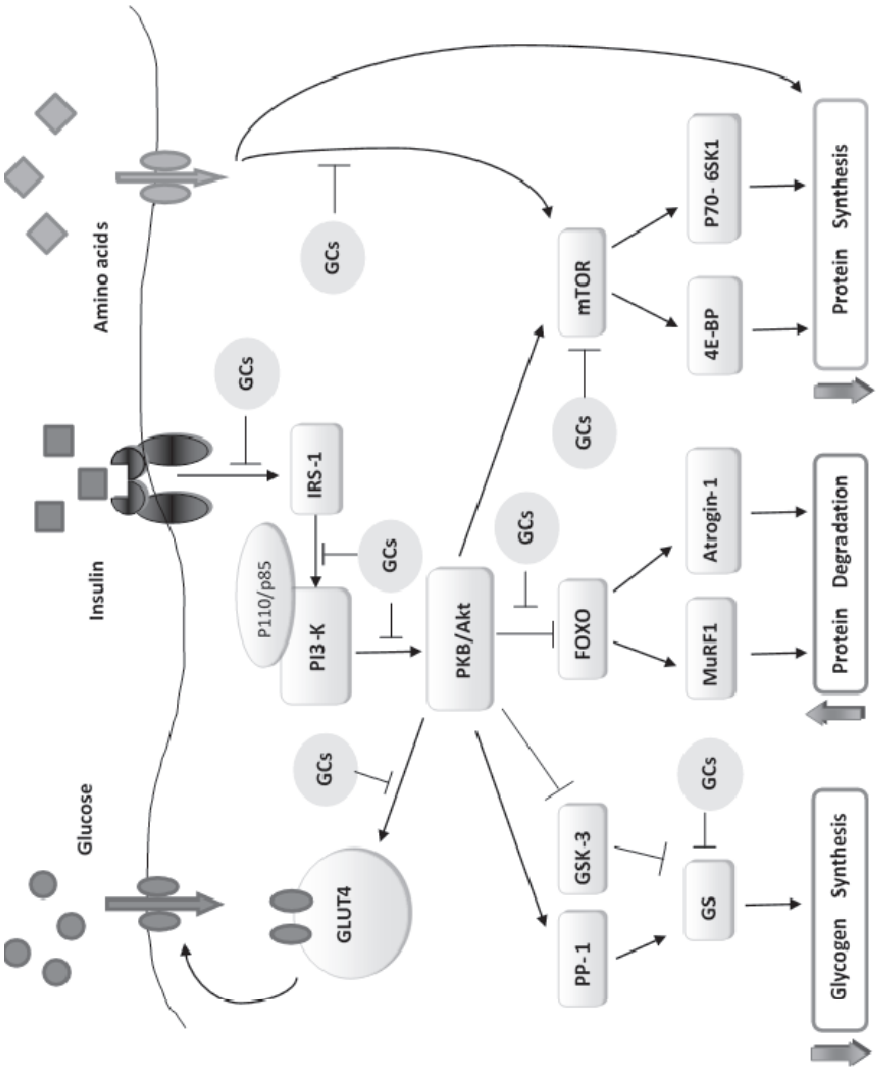

Figure 1. Metabolic effects of glucocorticoids on muscle tissue (adapted from van Raalte 2009).

Abbreviations: 4E-BP1, eIF4E-binding protein 1; GLUT4, glucose transporter 4; GS, glycogen synthase; GSK-3, glycogen synthase kinase-3; IRS-1, insulin receptor substrate-1; mTOR, mammalian target of rapamycin; MuRF-1, Muscle Ring Finger-1; PI3-K, phosphatidylinositol-3 kinase; PKB, protein kinase B; PP-1, protein phosphatase-1; S6K1, protein S6 kinase 1.

In addition, GCs stimulate endogenous glucose production, both by antagonizing the insulin-induced suppression of hepatic gluconeogenesis and by directly activating different genes encoding gluconeogenic enzymes such as phosphoenylpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase), and the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-alpha (van Raalte DH 2009). GCs inhibit the PEPCK enzyme gene expression involved in glyceroneogenesis, the metabolic pathway leading to glycerol 3-phosphate (G-3-P) from precursors other than glucose in adipose tissue. Concurrently, hepatic glycerol production, though the PEPCK enzyme is stimulated with increased NEFA release into the bloodstream (Hwang JL 2014) (Figure 2). Elevated circulating NEFA interfere with glucose utilization, as already described in skeletal muscle cells, and amplify insulin resistance.

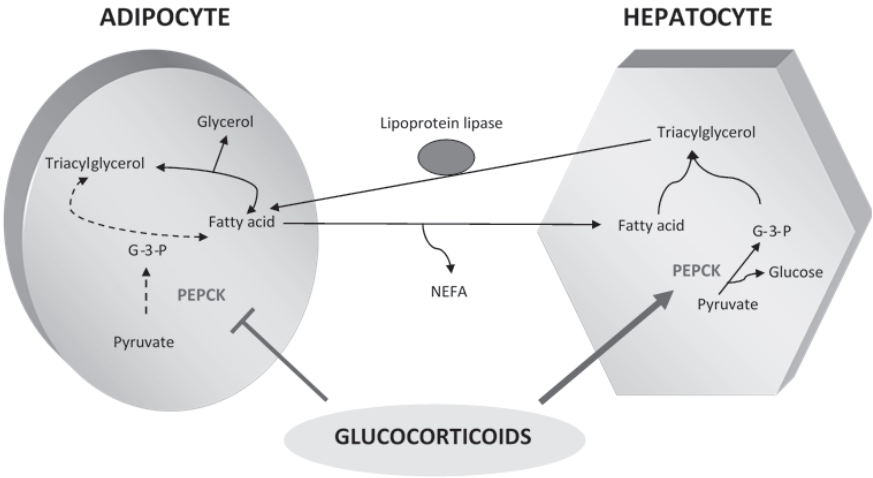

Figure 2. The effects of Glucocorticoids on glyceroneogenesis in adipose and hepatic cells (adapted from Hwang 2014).
Abbreviations: G-3-P, glyceraldehyde-3-phosphate; NEFA, non-esterified fatty acids; PEPCK, Phosphoenolpyruvate carboxykinase.

Asparaginase

Asparaginase is an essential drug used in multiagent chemotherapy regimens for childhood ALL. Asparaginase administration reduces plasma

concentrations of asparagine, catalyzing the deamination of asparagine into aspartic acid and ammonia (Hijiya 2016). Asparagine represents an essential amino acid for neoplastic cell survival, while healthy cells are able to synthesize it. Lack of asparagine causes leukemic cell death through a block of protein synthesis.

Asparaginase-related hyperglycemia has a reported incidence of between 4 and 27.5% among children with ALL and is more commonly observed when GC treatment is associated. Risk factors include age >10 years, overweight, family history of diabetes and Trisomy 21 (Fattorusso 2018).

Asparaginase damages insulin production without killing the β cells. This effect is related to insulin molecule destruction, interference with insulin synthesis and impaired insulin secretion. Decreased insulin receptor expression, insulin resistance and concurrent pancreatitis may potentiate hyperglycemia (Hijiya 2016).

Calcineurin inhibitors

Calcineurin inhibitors (tacrolimus, ciclosporin) are immunosuppressive drugs used in different autoimmune diseases and anti-rejection therapy. They inhibit calcineurin phosphatase activity, blocking the dephosphorylation and translocation of nuclear factor of activated T cells (NF-AT), a factor that starts the transcription pathway leading to the production of interleukin-2. Calcineurin is widely distributed in pancreatic islet cells, skeletal muscle, heart, neurons and adipocytes.

Calcineurin inhibitors act as a negative modulator of insulin secretion and β cell replication and survival. In addition, they inhibit glucose uptake in adipocytes and muscle cells, through the reduction of GLUT4 translocation to the cell surface (Chakkera HA 2017).

The mammalian target of rapamycin (mTOR) inhibitors

The mammalian target of rapamycin (mTOR) is an serine/threonine kinase encoded by the *MTOR* gene. The mTOR gene product is the central costituent of two distinct complexes, mTOR complex 1 (responsible for protein synthesis, cellular growth and inhibition of autophagy) and mTORC complex 2 (responsible for cell proliferation and survival) (Shariff AI 2019).

Inhibitors of the mTOR signaling pathway (rapamycin, everolimus and sirolimus) act as immune-modulators in anti-rejection and cancer therapy. The mechanisms underlying mTOR inhibitor-induced hyperglycemia are not completely clear, due to the limited number of human studies. Data from animal studies suggest that the mechanism underlying hyperglycemia induced by mTOR inhibitors may be a combination of impaired insulin secretion and insulin resistance. Moreover, mTOR inhibitors are able to reduce β cell proliferation and increase cellular apoptosis (Shariff AI 2019, Vergès B 2015).

Immune checkpoint inhibitors (ICPI)

Immune checkpoint inhibitors (ICPIs) represent a novel strategy to target and eradicate cancer cells through the immune system. At present, ICPIs include three different classes of monoclonal antibodies: anti-cytotoxic T-lymphocyte 4-antigen antibodies (anti-CTLA-4 Ab); programmed cell death-1 inhibitors (PD-1 inhibitors) and programmed cell death ligand-1 inhibitors (PDL-1 inhibitors) (Shariff AI 2019)..

CTLA-4 is a T-cell surface receptor that functions as an immune checkpoint molecule with inhibitory action on the immune response. Anti-CTLA-4 autoantibodies block the CTLA-4 receptor, leading to T-cell activation and cancer cell destruction.

PD-ligand is located on the cancer cell surface and binds to the PD-1 receptor, deactivating T cells. PD-1 and PDL-1 inhibitors enable the T cells to recognize and destroy cancer cells.

ICPIs block the immune modulation leading to cancer cell tolerance and may have “off-target” adverse effects known as immune-related events, related to the delatentization of an underlying autoimmune predisposition. The most commonly reported conditions are autoimmune colitis and dermatitis, while endocrinopathies as type 1 diabetes mellitus are rare. The clinical presentation is similar to classical autoimmune type 1 diabetes with hyperglycemia, ketoacidosis and positive pancreatic autoantibodies (Shariff AI 2019).

Insulin receptor signaling pathway inhibitors

The integrity of insulin-signaling pathway is essential for cancer cell survival and insulin receptor signaling pathway inhibitors represent emerging drugs in targeted cancer therapy. Hyperglycemia can be interpreted as a

indirect marker of the efficacy of these drugs. Tyrosine kinase inhibitors (TKIs) are antiangiogenic drugs blocking the first step of insulin signalling, mainly involved in the processes of cell proliferation and angiogenesis. TKIs may cause insulin resistance, although preclinical studies have shown a beneficial effect on hyperglycemia in patients with preexisting diabetes. This mechanism has not been fully clarified, but an increase in β cell mass has been reported in mice, probably representing a compensative mechanism for insulin resistance (Shariff AI 2019).

Phosphatidylinositol 3-kinase (PI3-K) inhibitors have been designed to block cancer progression since PI3-K plays a regulatory role on cell cycle checkpoints modulating apoptosis, proliferation, and growth. PI3-K blockage leads to insulin resistance interfering with insulin signaling.

Protein kinase B (Akt) inhibitors have been developed since PI3K/Akt pathway is crucial for cancer proliferation and progression. Akt is involved in the insulin-signalling pathway and Akt inhibitors are able to induce insulin resistance. In addition, they reduce glycogen synthesis and peripheral glucose uptake (Shariff AI 2019).

Management of drug-induced hyperglycemia

The correct management of drug-induced hyperglycemia in childhood must involve a multidisciplinary team including pediatric endocrinologists, pediatric oncologists, transplant physicians, nutritionists and psychosocial support services. The goal is to keep blood glucose values as close to normal as possible, avoiding the occurrence of hypoglycemic episodes.

In most cases, hyperglycemia during chemotherapy is transient and resolves once the GC or immunosuppressive therapies are reduced or discontinued. Specific treatment should be administered only if hyperglycemia persists. Insulin is the mainstay of available therapy, but there are no clear guidelines regarding timing and modalities of intervention, correct insulin dosing, and the potential role of new technologies as continuous subcutaneous insulin infusion (CSII) and continuous glucose monitoring (CGM).

The first step to managing hyperglycemia is to promptly recognize it. Inappropriate surveillance approaches can cause a delayed diagnosis. Depending on the different drug-specific effects, fasting blood glucose may be misleadingly normal, while postprandial hyperglycemia is present due to severe insulin resistance. Serial urine samples may be used to detect glycosuria, but the diagnostic gold standard is represented by multiple daily

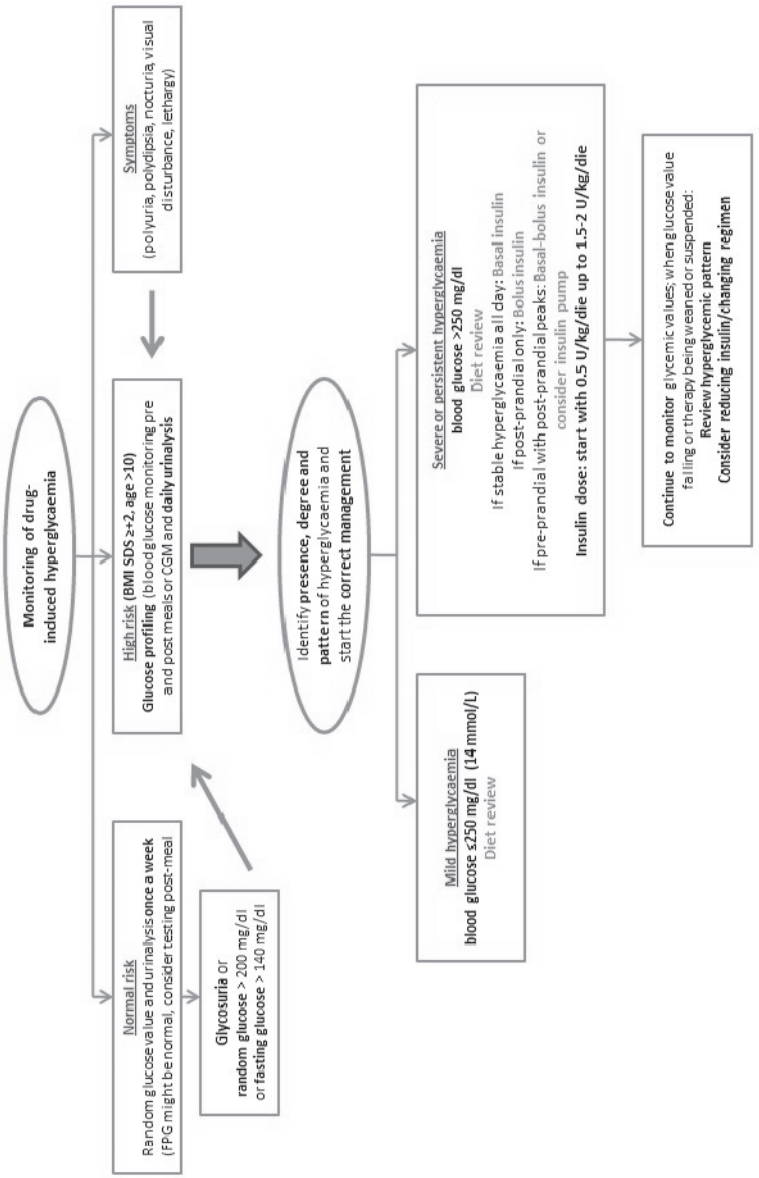

Figure 3. Monitoring and management of corticosteroid-induced hyperglycemia in pediatric ALL (adapted from Gregoriu 2020)
Abbreviations: *CGM, continuous glucose monitoring

capillary blood glucose testing, with laboratory confirmation of plasma glucose levels in patients with abnormal capillary values.

Glycated hemoglobin (HbA1c) assessment is not helpful, due to the acute onset of hyperglycemia and the commonly concurrent anemia, often requiring blood transfusions.

The oral glucose tolerance test (OGTT) is proposed during the follow-up of cancer survivors and transplanted patients as a screening tool (more reliable than fasting plasma glucose) to detect abnormal glucose metabolism complications as impaired glucose tolerance or type 2 diabetes.

CGM can facilitate glucose monitoring and management in frail subjects with long-lasting hyperglycemia (Gregoriou K 2020) (Figure 3).

Non-pharmacological treatment

In case of mild hyperglycemia (blood glucose ≤14 mmol/L or 250 mg/dL), the correct initial treatment is the regulation of the diet. Daily food intakes should be assessed and the carbohydrate intake should be set at 45–50% of the total daily calories, as recommended by the International Society for Pediatric and Adolescent Diabetes (ISPAD) (Smart CE 2018). Patient mobility and regular physical activity should be encouraged.

Pharmacological treatment

Insulin therapy should be considered in cases of persistent and severe hyperglycemia (blood glucose >14 mmol/L or 250 mg/dL) and requires close glucose monitoring. Insulin analogs are usually used, while the use of regular soluble insulin (human insulin) and intermediate-acting human insulin suspensions (isophane insulin) is now rare and restricted to specific indications (M.O. Regelmann 2015, K. Gregoriou 2020).

Insulin therapy with basal-bolus regimen should be modulated according to glycemic pattern. Basal insulin therapy (with glargine, degludec or determir) is the first step if hyperglycemia is evident after an overnight fast and persists as mild and stable throughout the day. For instance, subcutaneous basal insulin can be used alone in patients receiving continuous enteral or parenteral nutrition. In these patients, continuous intravenous insulin infusion represents an alternative, when multiple subcutaneous injections are contraindicated (e.g., thrombocytopenia). Bolus insulin therapy (with lispro, aspart or glulisine) at mealtimes should be preferred when

hyperglycemia is mostly postprandial, but fasting glucose levels are normal or nearly normal. Basal-bolus therapy is the preferred treatment when fasting hyperglycemia is associated with postprandial glucose peaks.

Glycemia should be monitored in any patient receiving GC treatment. GC-induced hyperglycemia is mostly postprandial, so the first-choice treatment is usually a bolus insulin regimen. Basal insulin is added when fasting hyperglycemia is associated. CSII may help in patients undergoing daily changes in GC dosing. CSII therapy is more flexible, but requires closer glucose monitoring and does not represent a routine clinical practice in these patients.

The recommended starting dose of insulin is 0.7–1 units/kg/day for pre-pubertal children and up to 1.2–2 units/kg/day during puberty (K. Gregoriou 2020). In our opinion, the starting dose may be even lower (0.4–0.5 units/kg/day). Initial doses are gradually increased according to glucose levels, aiming at nearly normal glucose levels without hypoglycemic episodes. Some patients are extremely insulin resistant and require five times the recommended dose. Likewise, tapering of insulin replacement is required to avoid rebound hypoglycemia after GC reduction or discontinuation (UKALL2011).

Metformin therapy improves insulin sensitivity and reduces gluconeogenesis. It has been proposed, as an alternative to insulin, in patients showing blood glucose levels ≤16.6 mmol/L (300 mg/dl) and no comorbidities. Metformin therapy has a common side effect represented by gastrointestinal upset and potential risks such as hepatic toxicity and lactic acidosis. Due to the specific action profile of metformin, a full effect can be obtained only after a few weeks and therapy must be discontinued before imaging studies with contrast medium, due to an increased risk of lactic acidosis.

Insulin secretagogues have been proposed to manage the complex metabolic pathways leading to GC-induced hyperglycemia, even if these medications can cause hypoglycemia and are associated with a potential risk of pancreatic β cell exhaustion. Some authors have proposed sulfonylureas and thiazides to treat new onset diabetes after transplant (NODAT), but safety and efficacy in children have not yet been established. Similarly, there is little experience with the use of DDP-4 inhibitors and GLP-1 agonists in the pediatric population (Regelmann MO 2015).

Long-term follow-up of patients with NODAT involves an annual screening of long-term complications as hypertension, dyslipidemia, nephropathy and retinopathy.

Despite recently published indications (Gregoriou 2020), evidence-based guidelines are still lacking and the decision to treat drug-induced hyperglycemia still relies on personal experience and clinical judgment.

Take-Home Messages

Drug-induced hyperglycemia in childhood has been mostly observed during the treatment of acute lymphoblastic leukemia and associated with the use of glucocorticoid and asparaginase, but new classes of drugs are emerging.

Early recognition and management of hyperglycemia are essential to prevent ketoacidosis and hyperosmolar nonketotic coma. In addition, hyperglycemia has been associated with increased infection rate and poor survival.

Insulin therapy should be considered in case of persistent and severe hyperglycemia (blood glucose >14 mmol/L) and it requires strict monitoring of glucose levels once initiated.

Basal and/or bolus insulin therapy should be modulated according to the pattern of hyperglycemia.

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SECTION 5 CHAPTER 5

STRESS HYPERGLYCEMIA IN SEVERE CRITICAL CONDITIONS

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Introduction

Stress hyperglycemia (SHG) refers to a state of self-limited transient hyperglycemia that occurs during acute critical illness. Critically ill children frequently suffer from hyperglycemia at some point during their illness.

Many studies have shown a worse prognosis for critically ill children with SHG,[1] and in specific processes such as traumatic brain injury,[2] severe burns,[3] septic shock,[4] and postoperative congenital heart disease.[5] Ranges

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for threshold levels of SHG in various studies range from >126 to > 250 mg/dL.

Pathophysiology

During periods of stress, the routine hormonal balance that keeps blood glucose levels within normal ranges becomes overwhelmed. SHG results from a combination of increased production of glucose, critical illness induced insulin resistance, and to a lesser extent insufficient levels of insulin relative to the degree of hyperglycemia. Increased production of glucose begins with rising levels of counter-regulatory hormones (epinephrine and norepinephrine, glucagon, cortisol, and growth hormone) and various pro-inflammatory cytokines. This leads to increased hepatic and renal production of glucose to supply increased metabolic demands during stress.

Insulin resistance is characterized by organ-specific changes in glucose utilization and impaired insulin-mediated uptake. While elevated insulin levels are common in adults, pro-inflammatory cytokines in critically ill children may lead to β cell dysfunction and impaired insulin production, resulting in relative insulin deficiency.[6] As with diabetes, insulin resistance promotes catabolism leading to lipolysis. Together, lipotoxicity, glucotoxicity, and inflammation form a triad of self-perpetuating bodily insults that characterize SHG.

Mechanisms of adverse outcomes from stress hyperglycemia

We do not have a definitive understanding of hyperglycemia-induced injury, but postulated mechanisms of SHG damage include direct cellular insults and alterations of essential organ function. Hyperglycemia results in excessive glycolysis and oxidative phosphorylation with consequent production of toxic reactive oxygen species. Such species cause mitochondrial dysfunction with subsequent cellular apoptosis and organ system failure.

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outcome in the postoperative pediatric cardiac patient. Pediatr Crit Care Med. 2006;7:351-355.

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Hyperglycemia is also a risk factor for infection in critically ill patients. Acute hyperglycemia can impair macrophage activity, reduce polymorphonuclear leukocyte chemotaxis and bactericidal capacity, and alter complement fixation.[7] Additionally, SHG impairs all major aspects of innate immunity and is associated with poor intestinal motility, which may contribute to bacterial overgrowth and translocation.[7]

Hyperglycemia is harmful also in its contribution to the already hypercoagulable state of critical illness. This effect is mediated partly through the increased expression of tissue factor, known to be both pro-coagulant and pro-inflammatory.[8]

Treatment considerations

Vasoactive-inotropic medications such as epinephrine, norepinephrine, and dopamine are frequently associated with SHG. Epinephrine stimulates β_2 receptors thus promoting glycogenolysis and gluconeogenesis and increasing insulin resistance by the release of glucagon and cortisol. Its stimulation of α_2 receptors also reduces insulin secretion. The effects of dopamine and norepinephrine are less potent due to lesser activity at the β_2 receptors.

Several medications commonly used in the intensive care unit (ICU) setting may compound SHG. One of the more insidious means of worsening hyperglycemia is the large volumes of dextrose-containing fluids used as carriers for intravenous antifungal and antibiotic medications. Corticosteroids, thiazide diuretics, and calcineurin inhibitors (tacrolimus and cyclosporine) can all cause or worsen SHG.

Nutrition practices also strongly affect SHG. Excessive carbohydrate loads, particularly in the form of parenteral nutrition, can worsen SHG. If the caloric needs of a critically ill child are in question, indirect calorimetry is the optimal method to assess their needs in order to modulate caloric intake.

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Glycemic control through insulin therapy is the mainstay of SHG treatment. *Tight* glycemic control (TGC) to a low target of 80–110 mg/dL (4.4–6.1 mmol/L) has been the subject of many studies and much discussion. In 2001, a single center adult study reported a 30% reduction in hospital mortality through the use of a TGC protocol.[9] Subsequent studies were unable to confirm these results though, and many found harm with TGC secondary to hypoglycemia. A large international multicenter randomized control trial involving more than 6,000 adult patients reported increased mortality and risk for severe hypoglycemia among adults in the ICU being treated with TGC protocols.[10] Guidelines for adults currently recommend a higher glucose threshold for initiation of insulin therapy at 150 mg/dL with a blood glucose target <180 mg/dL.[11]

Several prospective randomized clinical trials are available to guide practice in critically ill children. Published in 2009, a single center in Belgium enrolled a mixed medical/surgical patient population (75% were postoperative from cardiac surgery).[12] The study targeted age-adjusted glycemic range for infants and children: 50–80 mg/dL in children aged < 1 year and 70–100 mg/dL for all others. While the results showed improved mortality in the TGC group, the TGC group also had severe hypoglycemia (<40 mg/dL) at unacceptable rates (25% overall, and 44% in neonates) making it difficult to conclude that TGC at those ranges was a safe and beneficial therapy.

In 2012, a randomized trial reported results for 980 children below age three years who underwent cardiac surgery. They found that TGC in a target range of 80–110 mg/dL could be obtained with a low percentage of time spent in hypoglycemia. But the study found no clinical benefit for

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TGC with respect to risk of infection, length of hospitalization, mortality, or risk of organ failure compared with standard care.[13]

However, a post hoc analysis showed that TGC can lower the rate of infection in patients older than two months at the time of cardiac surgery compared when with standard care.[14]

Both studies added concern for the effect of hypoglycemia on neurocognitive outcomes.

The Belgian study ascertained that hypoglycemia associated with intensive insulin therapy and TGC was not a cause of worse neurocognitive otucome at a four-year follow-up.[14] Both treatment groups' outcomes, however, were similar to the few patients who developed moderate or severe hypoglycemia in the two-center, cardiac only trial. Notably, the group that had no hypoglycemia, as reported by continuous glucose monitoring, had a markedly better neurocognitive outcome than the other three groups. It is possible then that the "no hypoglycemia detected" group of the first trial may have had undetected hypoglycemia leading to the moderately impaired outcomes. Subsequent studies have confirmed the dangerous effects of hypoglycemia in critically ill children.[15] Overall, these data suggest that hypoglycemia should be avoided in order to ensure optimal short and long-term outcomes.

In 2014, a British multicenter randomized trial reported that TGC had no significant effect on the number of days alive and without mechanical ventilation at 30 days after enrollment in critically ill children (60% were postoperative from cardiac surgery). However, in patients in the TGC arm, there was a lower need for dialysis, as well as a slight reduction in the health care cost at 1 year. These effects were most pronounced in the non-cardiac population.[16]

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In 2017, results from a 25-center randomized clinical trial conducted in the US and Canada of 730 critically ill children with hyperglycemia compared target ranges 80–110 mg/dL or 150–180 mg/dL and showed no benefit of the lower target in ICU-free days (mortality-adjusted ICU length of stay), nor any other secondary outcome. Even with continuous glucose monitoring and a computer algorithm driving insulin dosing, severe hypoglycemia was not eliminated, and long-term follow-up once again suggested compromised neurocognitive outcomes in those with moderate or severe hypoglycemia.[17]

Pediatric burn patients are physiologically different from other critically ill pediatric patients, so a 2010 single center study focused on severely burned pediatric patients treated with TGC. The authors showed that intensive insulin therapy significantly reduced the rate of infection/sepsis and improved organ function by decreasing inflammation, supporting targeting a low range of 80–110 mg/dL in this specific population.[18]

In summary, the cumulative take-away from these randomized studies is that low target ranges produce little to no benefit, yet increase the risk of hypoglycemia and its associated harms. As in the adult world, consensus for general, non-burn, pediatric patients agrees that an insulin infusion should begin when glucose levels reach 150 mg/dL with the aim of maintaining blood glucose <180 mg/dL. In all cases, patients younger than one year old are at higher risk for spontaneous hypoglycemia and require special attention when treating hyperglycemia with insulin.[19]

Take Home Messages

1. Stress hyperglycemia (SHG) refers to states of self-limited transient hyperglycemia that occur during acute critical illnesses such as trauma, sepsis, burns, and after major surgeries.
2. SHG harm results from the three-fold sequelae of hyperglycemia: lipotoxicity, glucotoxicity, and inflammation.

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3. Tight glycemic control (TGC) as a means of treating pediatric SHG is important, but studies have shown that targeting higher, rather than lower, ranges of blood glucose levels, achieves the same positive effects while reducing the risks associated with hypoglycemia.

SECTION 5 CHAPTER 6

STRESS HYPERGLYCEMIA IN MINOR CRITICAL CONDITIONS

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Introduction

Nondiabetic hypoglycemia (NDHY) is usually a transient condition, not due to chronic insulinopenia or insulin resistance.

NDHY in the pediatric population can be induced by stress, drugs, surgery or traumatic injury.

Stress hyperglycemia (SHY) is defined as blood glucose levels of >8.33 mmol/L (150 mg/dL) occurring as a response to stress and reverts to normal independently after the solving of the illness in non-diabetic patients. [1]

There are two types of SHY: 1) SHY type 1 occurs during severe illness, surgery, emergency state and resuscitation areas; 2) SHY type 2 occurs during minor critical conditions like acute illness in non-life-threatening situations (febrile convulsion, traumatic injury, acute asthma). [2]

Pathophysiology

The target of SHY in minor critical conditions is to increase glycemia to satisfy the needs of vital organs at a time of increased metabolic demand. Though this situation promotes survival in acute illness, its persistence can

be harmful due to increased oxidative damage. SHY is the result of both increased production of glucose and peripheral insulin resistance.

Causes of stress hyperglycemia include an increased in secretion of counterregulatory hormones (glucagon, growth hormone, catecholamine and glucocorticoid) and pro-inflammatory cytokines (in particular tumor necrosis factors-α or TNFα, interleukin-1 and interleukin-6). These cytokines stimulate gluconeogenesis in the liver as well as in the kidney. In addition, counterregulatory hormones and pro-inflammatory cytokines promote both central (in the liver) and peripheral (in muscle and adipose tissue) insulin resistance by reducing the translocation of the insulin-dependent glucose transporter protein GLUT4 from internal membrane stores. This mechanism reduces cellular utilization of glucose and promotes hyperglycemia.[3]

Hyperglycemia, even if it is transitory, causes excessive oxidative phosphorylation with subsequent increased production of reactive oxygen species (ROS), causing mitochondrial dysfunction, increased cellular apoptosis and, consequently, organ failure. [3]

Materials

Some studies about SHY2 in the pediatric population are collected below:

Valerio et al. describe SHY2 in children with febrile seizures or traumatic injuries. In this work, two groups of children were studied: stress-exposed group (833 children with acute illness such as febrile seizures or traumatic injury) and non-stress-exposed group (366 healthy children) used as control group. SHY2, defined as blood glucose levels of ≥8.3 mmol/L (≥150 mg/dL) during acute illness, was found in 41 (4.9%) stress-exposed patients and in none of the controls. Noteworthy is that after a follow-up period of 3.5±0.6 years, none of the patients with stress hyperglycemia subsequently developed DM. [4]

Mobaireek et al., in a retrospective study regarding 166 children affected by acute asthma, found hyperglycemia of ≥11.11 mmol/L (200 mg/dL) in 38.6% of children. [5]

Weiss et al. studied a cohort of 55,120 patients hospitalized in the emergency room due to respiratory illness, trauma or seizure and found that SHY2 (blood glucose level ≥16.7 mmol/L or ≥300 mg/dL) occurred in 0.13% of all patients. Moreover, the authors reported a spontaneous

reduction of glycemia at 8.3 mmol/L (150 mg/dL) in two days. For this reason, they suggest the possibility of treating SHY2 with hydration only. [6]

Ovenusi et al. studied a group of 1045 patients with a mean age of 2.5±2.7 years. SHY2 was recorded in 135 patients (at a rate of 12.9%) with acute respiratory tract infections (17.4%), malaria (11%), septicemia (15.3%), gastroenteritis (14.9%), and burns (18.2%). [7]

Table: Studies on Stress hyperglycemia in minor critical condition

Authors	Disease	Number of children	Age	Hyperglycemia	Rate of children
Valerio et al. (2001)	Febrile convulsions and traumatic injury	833	5.2±4.5 years	≥ 8.3 mmol/L (≥150 mg/dL)	4.9%
Mobaireek et al. (2018)	Acute asthma	166	5.4±2.6 years	≥11.11 mmol/L (200 mg/dL)	38.6%
Weiss et al. (2010)	Patients in emergency room	55.120	2.5±2.7 years	≥ 16.7 mmol/L or ≥300 mg/dL	0.13%
Ovenusi et al. (2016)	Acute respiratory infections, septicemia, malaria, gastroenteritis and burn	1045	2.5±2.7 years	≥ 8.3 mmol/L (≥150 mg/dL)	12.9%

Management and Treatment

Since SHY is transient, its treatment is still under discussion. The hard issue is whether to treat hyperglycemia in non-life-threatening conditions, SHY2, which has a limited impact in term of severity and duration.

Currently there are no evidence-based recommendations concerning hyperglycemia treatment; however, after reviewing the literature, it is possible to suggest SHY2 to treat if blood glucose level is ≥11.11 mmol/L (200 mg/dL) and longer than 2 hours, using a rapid or ultrarapid-acting insulin analog at 0.1 IU/Kg in bolus via intravenous (IV) or subcutaneous (SC) administration. [2]

Take home messages

- Stress hyperglycemia type 2 (SHY2) occurs during minor critical conditions such as acute illness in non-life-threatening situations (febrile convulsion, traumatic injury, acute asthma).
- SHY2 occurs as a response to stress and reverts to normal independently after the solving of the illness in non-diabetic patients.
- SHY2, even if it is transitory, can be harmful because of increased oxidative damage and, consequently, organ failure.
- Since SHY is transient, its treatment is still under discussion. Many studies suggest the possibility of treating it with rapid-acting insulin analog in bolus (IV or SC).

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