

Type 2 diabetes mellitus in pediatrics: a new challenge

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Background: The increased prevalence of childhood obesity in the last few years has been accompanied by the increase in prevalence of type 2 diabetes in pediatrics. In this paper, we will review the risk factors and the pathogenic determinants leading to type 2 diabetes in youth.

Data sources: We searched on PubMed with the key words: obesity, type 2 diabetes, children, adolescents, youth, non-alcoholic fatty liver disease, genes and selected those publications written in English that we judged to be relevant to the topic of the review.

Results: Based on the data present in the literature, we reviewed the following three topics: 1) the role of ectopic fat deposition, in particular of fatty liver, in the pathogenesis of pediatric type 2 diabetes; 2) the progression to type 2 diabetes in pediatrics and how it differs from adults, and 3) current therapeutic options.

Conclusion: Type 2 diabetes in youth is a complex disease, creating new challenges in treatment and prevention.

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Key words: non-alcoholic fatty liver disease; obesity; type 2 diabetes mellitus

Introduction

The prevalence of childhood obesity has been increasing in the last four decades.^[1] In 2010, it was estimated that 43 million children were overweight worldwide, and this number is expected to increase up to 60 million by 2020.^[1]

The rise in pediatric obesity is accompanied by an increased prevalence of type 2 diabetes in children.^[2] The SEARCH for Diabetes in Youth study (SEARCH) showed that in the U.S. in 2010, over 20 000 individuals below 20 years of age had type 2 diabetes.^[2] Moreover, the study predicted that this number may increase up to 30 000 by 2020 and up to 84 000 by 2050.^[2]

The SEARCH study collects data regarding diabetes in children and adolescents, and is limited to the U.S. Very little is known about the progression of the disease in the so called emerging countries, where the prevalence of childhood obesity has been dramatically yet silently increasing in the last few years and where these types of studies are lacking.^[1] In fact, an increase in the prevalence of childhood obesity has been documented from 1990 to 2010 in Africa, South America and Asia, but information about its complications in these territories is quite sparse; this lack of knowledge is distressing given the frightening epidemic on the horizon.

Definition of type 2 diabetes and pre-diabetes

According to the American Diabetes Association (ADA) criteria, type 2 diabetes is defined as fasting plasma glucose levels of 126 mg/dL and above or plasma glucose levels of 200 mg/dL and above two hours after an oral glucose tolerance test (OGTT), while impaired glucose tolerance (IGT) is defined as plasma glucose levels of 140 mg/dL and above after an OGTT (Table).^[3] In addition to IGT, another prediabetic state has been described: impaired fasting glucose

Table. Criteria for the diagnosis of type 2 diabetes and prediabetes according to the American Diabetes Association^[3]

Criteria for diagnosis of increased risk for diabetes (Prediabetes)	Criteria for diagnosis of diabetes
HbA1c: 5.7-6.4%	HbA1c: $\geq 6.5\%$
IFG: fasting plasma glucose ≥ 100 -125 mg/dL	Fasting plasma glucose ≥ 126 mg/dL
IGT: 2h plasma glucose ≥ 140 -199 mg/dL	2h plasma glucose ≥ 200 mg/dL
	Random plasma glucose ≥ 200 mg/dL in patients with symptoms

HbA1c: hemoglobin A1c; IFG: impaired fasting glucose; IGT: impaired glucose tolerance.

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(IFG). IFG is defined as serum fasting glucose levels from 100 mg/dL to 125 mg/dL. Epidemiological studies indicate that IFG and IGT are two distinct categories of individuals and only a small number of subjects meet both criteria, showing that these categories overlap only to a very limited extent in children.^[4] Recently, the ADA has recommended testing the hemoglobin A1c (HbA1c) to diagnose diabetes.^[3] In particular, 6.5% is the lower limit used to diagnose type 2 diabetes. This value was chosen on the basis of cross sectional and longitudinal studies conducted in adult subjects showing that a lower limit of 6.5% identifies about one third of cases of undiagnosed diabetes and that subjects with HbA1c above that cut-off have a long term higher prevalence of microvascular complications.^[3,5-7] Subjects with a HbA1c between 5.7% and 6.4% have been defined as "at increased risk of diabetes".^[3] Hepatic steatosis is only the first step of a more complex disease.

It should be noted that these two methods of diagnosing type 2 diabetes are not mutually exclusive since there is little agreement between them.^[8] Thus, to ensure an accurate diagnosis and avoid misdiagnosis in some patients, it is useful to measure them both.

The link between obesity and type 2 diabetes: the role of ectopic fat accumulation

Type 2 diabetes results from an imbalance between insulin sensitivity and secretion. Several conditions can influence these two factors (e.g., genetics, diet, physical activity, etc). The first step in the development of type 2 diabetes in youth is the onset of obesity and its consequences. In particular, obesity is accompanied by an abnormal distribution of lipid partitioning with the excess lipids being diverted from adipose tissue into other organs (such as skeletal muscle and liver).^[9,10] The ectopic fat distribution causes intracellular modifications, which in turn lead to an insulin resistance. A previous study has shown that obese youth with impaired glucose tolerance show a higher intramyocellular lipid (IMCL) content, visceral fat content, and hepatic fat content than their age, gender, and body mass index matched pairs.^[11] These obese young people with impaired glucose tolerance have a pronounced defect in the non-oxidative pathway of glucose metabolism.^[11] From a molecular point of view, the link between IMCL and insulin resistance seems to be represented mostly by diacylglycerol (DAG).^[12] Increased IMCL has been shown to increase intracellular DAG, a signaling intermediate that activates members of the protein kinase C (PKC) family, thus altering insulin signaling.^[12]

These events occur not only in the skeletal muscle, but also in the liver contributing to hepatic insulin resistance.^[12] Hepatic insulin resistance is one of the

primary disturbances responsible for the metabolic changes that occur in obese individuals and eventually leads to type 2 diabetes.

Non-alcoholic fatty liver disease (NAFLD) in the pathogenesis of type 2 diabetes

Recent studies in obese children and adolescents have elucidated the effect of hepatic steatosis on insulin sensitivity. In a multiethnic group of 118 obese adolescents, Cali et al^[13] observed that independent of obesity, the severity of fatty liver was associated with the presence of pre-diabetes (IGT with and without IFG). Paralleling the severity of hepatic steatosis, there was a significant decrease in insulin sensitivity and impairment in beta-cell function as indicated by the fall in the disposition index.^[13] Moreover, the authors observed with increasing severity of fatty liver disease, there was a significant rise in the prevalence of the metabolic syndrome, suggesting that hepatic steatosis may be a predictive factor of metabolic syndrome in children.^[13] More recently, a study from D'Adamo et al^[14] has clearly elucidated the role of hepatic fat content in modulating insulin sensitivity. The authors studied two groups of adolescents, one group with hepatic steatosis and the other group without. The two groups had similar visceral fat and IMCL.^[14] The obese subjects with hepatic steatosis showed increased muscular and hepatic insulin resistance; although not statistically significant, a trend towards increased adipose tissue insulin resistance was noted.^[14] More recently, in a longitudinal study we observed that baseline hepatic fat content correlates with 2-hour glucose, insulin sensitivity, and insulin secretion at follow-up.^[15] These data clearly indicate that the deleterious effect of intra-hepatic fat accumulation influences the insulin sensitivity at a multi-organ level, playing a bigger role than the other ectopic compartments.^[15] In general, obese children and adolescents with hepatic steatosis tend to show an adverse metabolic pattern characterized by dyslipidemia and adverse changes in glucose metabolism.

Hepatic steatosis is only the first step of a more complex disease known as NAFLD, which has become the most common cause of liver disease in pediatrics.^[16,17] NAFLD is defined by the presence of macrovesicular steatosis in more than 5% of the hepatocytes in the absence of drug consumption, alcohol abuse and other determinants that may result in fatty liver.^[16-18] NAFLD encompasses a range of disease severity, from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis.^[18] Although, the natural history of NAFLD in the pediatric population is not entirely known, recent data have shown that in

addition to the increasing prevalence of NASH,^[19] it may also progress to cirrhosis in this age group.^[20]

Therefore, the screening for NAFLD should be recommended to overweight and obese children. Although liver histology is the gold standard for diagnosing NAFLD, performing biopsies to determine disease prevalence is not always feasible. Children with NAFLD typically have slightly elevated liver enzyme values [aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] in absence of other causes of steatosis. Therefore, elevated serum levels of liver enzymes, even though they often misrepresent the entity of intrahepatic damage, are used as a non-invasive test to screen for pediatric NAFLD along with liver ultrasound (US), that can detect the disease when steatosis involves >30% of hepatocytes. Although it does not represent the imaging gold standard, performing US has several advantages as a screening tool: 1) relative low cost; 2) large diffusion in medical community, and 3) feasibility in the pediatric population. Computed tomography (CT) scan is not recommended in pediatric setting because of the unjustified radiation exposure involved in the process. Magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) have been demonstrated to be the best methods to assess and quantify the amount of lipids present in the liver,^[21] but these techniques are too expensive to be used in clinical practice.

We have recently shown that liver damage correlates with insulin resistance in obese children.^[21] In particular, the levels of the caspase-cleaved CK18 fragment (CK-18), a robust biomarker of liver damage, are inversely correlated with insulin sensitivity, meaning that not only the amount of intra-hepatic fat, but also the degree of steatohepatitis may affect insulin sensitivity.^[21] Interestingly, this association is present in obese Caucasian and Hispanic children and adolescents, but not in African Americans. In this latter population there seems to be dissociation between the degree of liver injury and insulin sensitivity.^[21] These data are consistent with the data shown by Guerrero et al^[22] showing a clear dissociation between the amount of liver fat and the degree of insulin sensitivity in African Americans. The cause of this difference among ethnic groups is not known, but genetic background and the interaction between gene variants and nutrients may be a major determinant of such differences.^[22-27]

Beta cell impairment is the last step before development of type 2 diabetes

The relationship between insulin demand and secretion is a key factor regulating the maintenance of normal glucose tolerance. In fact, the beta cell responds to

insulin resistance occurring in obese children and adolescents by producing a state of hyperinsulinemia, which maintains normal glucose levels.^[28] In the long run, however, some individuals have deteriorating beta cell function, and insulin secretion may not be sufficient to maintain glucose levels within the normal range.^[28,29]

In fact, when insulin secretion is estimated in the context of the "resistant milieu", IGT subjects show a significantly lower degree of insulin secretion than the group with a normal glucose tolerance (NGT).^[30] In particular, using hyperglycemic clamp studies, Weiss et al^[30] investigated the role of insulin secretion in glucose regulation in a group of 62 obese adolescents with varying states of glucose tolerance (30 NGT, 22 IGT, and 10 type 2 diabetes). This study showed that in comparison to NGT obese adolescents with similar insulin resistance, those with IGT show a progressive loss of glucose sensitivity of beta first-phase secretion and that the beta second-phase secretion is compromised in type 2 diabetes.^[30] This does not mean that the defect in the first phase is less influential than the defect of the second phase in causing hyperglycemia, yet simply recognizes that the decline of the first phase of the insulin secretion is present before overt diabetes and it may be considered the fingerprint of prediabetes, whereas the defect in the second phase is required for the development of type 2 diabetes. In fact, differences in beta cell function have been described in various pre-diabetic conditions seen in obese adolescents, such as IFG or IGT, or the combined IFG/IGT states. Cali et al^[4] documented that in obese adolescents: 1) IFG is primarily linked to alterations in glucose sensitivity of first-phase insulin secretion; 2) IGT is characterized by a more severe degree of peripheral insulin resistance and reduction in first-phase secretion, and 3) the co-occurrence of IFG and IGT is the result of a defect in second-phase insulin secretion and a profound insulin resistance.

Surely, genetic predisposition plays an important role in the development of type 2 diabetes. This idea is supported by clinical studies showing that youths developing IGT or type 2 diabetes have less insulin secretion even before the onset of IGT or type 2 diabetes. The role of a "pre-existing" dysfunction in obese adolescents with normal glucose tolerance has been shown by Cali et al in a longitudinal study.^[31] In a group of obese NGT adolescents who underwent repeated OGTT over a period of 3 years, those who progressed to IGT had less function at baseline compared to those who did not progress. This finding has been recently confirmed by Giannini et al,^[32] who have shown by using hyperglycemic hyperinsulinemic clamp studies an early impairment of beta cells in subjects developing IGT or type 2 diabetes.

It is worth mentioning that changes in insulin secretion and sensitivity in youth occur quicker than in adults. While in adults the transition to type 2 diabetes takes about 10 years,^[33,34] in obese youth it has been estimated that beta cell function reduction occurs at a rate of 15% per year,^[35] with a mean transition time from prediabetes to overt diabetes of around 2.5 years.^[32] This means that type 2 diabetes in youth might be a more severe disease than in adults.

Genetics of type 2 diabetes

So far several genome wide association studies (GWAS) have helped highlight the genetic basis of type 2 diabetes and several single nucleotide polymorphisms (SNPs) have been discovered to be associated with type 2 diabetes.^[36] The majority of these are in non coding regions or nearby a gene and few are missense mutations (such as the rs1801282 in the PPAR-gamma characterized by a C-to-G substitution encoding a proline to alanine substitution at codon 12).^[37] As pointed out by Billings and Florez recently,^[38] we have learned from GWAS studies that the majority of gene variants associated with type 2 diabetes are in genes expressed in the beta cells. Moreover, GWAS studies have unveiled the function of genes previously not known to be involved in the glucose metabolism pathways and whose action has been further studied through animal and cellular studies.^[39,40] While the majority of these studies have been conducted in large cohorts of adults, information about these associations in youth is sparse. Dabelea et al^[41] genotyped the rs12255372 and rs7903146 variants in or near the *TCF7L2* gene in a multiethnic cohort with 1239 (240 cases and 999 controls) youths enrolled in the SEARCH study; they observed that in African Americans the rs7903146 variant was associated with almost two folds increased odds of type 2 diabetes occurrence. More recently, Barker et al^[42] genotyped 16 SNPs, previously found to be associated with diabetes by GWAS studies, in a population of over 6000 children and adolescents, and investigated whether they may be additionally associated with fasting glucose levels. The authors observed that 9 loci were associated with the fasting glucose levels. In particular, they confirmed 5 previously discovered SNPs and discovered 4 more loci associated with fasting glucose. The strongest associations were with the *G6PC2* rs560887, *MTNR1B* rs10830963, and *GCK* rs4607517, and the effect size of the confirmed loci was similar to that observed in adults.^[42] The latter observation suggests that the effect of certain gene variants is constant over time and may not

be influenced by changes in insulin secretion and sensitivity occurring with age.

Some studies in adults have also tried to determine whether the quantity of gene variants may improve the ability to predict type 2 diabetes over time. These studies have shown that the co-occurrence of more risk alleles does not improve the ability to predict the development of type 2 diabetes when compared to the clinical risk factors, such as BMI or family history of diabetes.^[43-49] One exception is represented by a landmark article from the Malmo Preventive Study.^[43] The authors showed that the co-occurrence of 11 risk variants improved the prediction models for the diagnosis of diabetes over a median of 24.8 years of follow up.^[43] More recently, a significant association between the co-occurrence of risk alleles and type 2 diabetes has been observed by the investigators of the CARDIA study.^[50] They followed young adults into middle adulthood and observed that the co-occurrence of 38 gene variants predicted the incidence of diabetes over 24 years follow up.^[50]

In addition, it has been shown that the genetic predisposition to type 2 diabetes may be stronger in the pediatric population.^[51] In fact, it has been demonstrated that the co-occurrence of five common variants in or near genes modulating insulin secretion is associated with a higher risk of developing pre-diabetes and type 2 diabetes in youth.^[51] In particular, in that study the authors asked whether the co-occurrence of risk alleles in or near 5 genes discovered by GWAS (*TCF7L2* rs7903146, *IGF2BP2* rs4402960, *CDKAL1* rs7754840, the *HHEX* rs1111875, and *HNF1A* rs1169288) might be associated with a higher risk of IGT or type 2 diabetes in obese children and adolescents. The rationale for selecting these gene variants was based on studies recently performed in human islets indicating that these genes are involved in the release of insulin granules from the beta cell.^[51] For instance, variants near the *TCF7L2* were associated with reduced depolarization-evoked insulin exocytosis and susceptibility and variants near *HHEX* gene were associated with granule docking. The authors observed that the increase of risk alleles was associated with a progressive worsening of insulin secretion mainly due to an impairment of the dynamic phase of insulin secretion. With a higher number of risk alleles, there is a higher chance of progression from NGT to IGT or type 2 diabetes. For those who were IGT at baseline, a higher number of risk alleles was associated with lower odds to revert back to NGT.

Despite the strength of these associations, the portion of heritability explained by the identified loci is estimated to be less than 10%.^[52] Although the sample size of GWAS studies continues to increase revealing new associations, each newly associated variant has an

incrementally smaller effect size and contributes only marginally to the cumulative variation of the phenotype. GWAS may be reaching the limits of its ability to reveal genetic variations underlying complex traits. Additional genetic variations, such as rare variants with large individual effects, may contribute to the heritability of complex traits such as type 2 diabetes.

Therefore, very recently it has been proposed that rare variants may explain the so called "missing heritability" of type 2 diabetes. Although, several projects are ongoing to try to verify this hypothesis, so far there is no data available in the literature.

Therapy for youth with type 2 diabetes

In the pediatric population, there are few drugs approved to treat type 2 diabetes: insulin and metformin. The best approach to this disease in the pediatric population remains unclear.

Recently, the pediatric trial Treatment Options for type 2 diabetes in Adolescents and Youth (TODAY) has been completed.^[53] The TODAY study is a 15-center clinical trial sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, that examined the efficacy of three approaches to the treatment of type 2 diabetes in youth: metformin alone, metformin plus rosiglitazone, or metformin plus an intensive lifestyle intervention called the TODAY Lifestyle Program.^[53] The authors studied 699 subjects between 10 and 17 years of age, the patients were randomized to each arm and followed-up for about 3.86 years. Although the rate of failure in each arm was quite high (51.7% metformin alone, 38.6% metformin plus rosiglitazone, 46.6% metformin plus lifestyle) the treatment with metformin and rosiglitazone was more effective in maintaining glycemic control than the treatment with metformin alone ($P=0.006$). Additionally, metformin plus rosiglitazone seemed to be better than the metformin and lifestyle treatment however this difference was not statistically significant ($P=0.15$).^[53] Interestingly, when performing subgroup analyses the authors observed that metformin alone was less effective in non Hispanic black than in non Hispanic whites or Hispanics.^[53]

Overall, the high rate of failure in each arm of the TODAY study is quite striking. As it has been observed,^[54] this study was dealing with a very complex population: adolescents with type 2 diabetes, who grew up in a very sedentary environment.^[53] These data seem to suggest that any intervention in this population may be extremely challenging, thus more efforts should be aimed at the prevention of obesity and the progression to type 2 diabetes.

Conclusions

This overview shows that type 2 diabetes in youths is a quite complex disease, not only from a pathophysiological point view, but also from a public health perspective. The data present in the literature clearly show that this disease can be particularly aggressive in the youth. Moreover, since this is the first generation in which this phenomenon is so diffuse, longitudinal data showing the long-term natural history of the early onset type 2 diabetes are not yet available. It is possible that once available these data will show an unprecedented phenomenon: young people with a higher rate of mortality and morbidity than their parents. Thus childhood obesity represents a major problem of public health to be fought not only from a medical standpoint but also from a political perspective.

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