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Detection of impaired glucidic metabolism and insulin resistance in a pilot child sample

Detección de la alteración del metabolismo glucídico y resistencia a la insulina en una muestra piloto infantil: Aproximación metabolómica

Ismael San Mauro-Martin[1]* orcid.org/0000-0002-7415-0293

Sara López-Oliva[1] orcid.org/0000-0003-2639-6800

Elena Garicano-Vilar[1] orcid.org/0000-0001-6327-6758

Belén García-de Angulo[1] orcid.org/0000-0003-2257-0621

Javier Andrés Blumenfeld-Olivares[2] orcid.org/0000-0002-7168-840X

1. Nutrition and Health Research Centers. Madrid, Spain
2. Pediatric Endocrinology Service, El Escorial Hospital. Madrid, Spain

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Resumen

Introducción: La metabolómica permite estudiar la resistencia a insulina (RI), un factor de riesgo de pre-diabetes y diabetes. *Quantose IR™* es el único test que mide la RI mediante la abrazadera hiperinsulinémica euglicémica. **Objetivo:** Se comprobó la eficacia de un test metabolómico en la detección de marcadores de RI en población infantil. **Materiales y métodos:** Once niños, de edad 8,54±3,53 años y con factores de riesgo de diabetes, fueron reclutados del Hospital El Escorial. Se estableció como criterio diagnóstico para la prediabetes el estándar de la Asociación Americana de Diabetes (ADA) (HbA1C 5,7-6,4% y glucosa basal 100-125mg/dl). Se compararon las analíticas de sangre con la prueba de *Quantose IR™*, estudiando el perfil del metaboloma relacionado con la RI (ácido alfa-hidroxibutírico, ácido oleico, linoleo-glicerofosfocolina e insulina). Su análisis generó una puntuación *Quantose*© (escala 0-100), siendo >63 RI. **Resultados:** Ningún sujeto cumplió el criterio de la ADA para prediabetes: HbA1C fue 5,3±0,18 % y glucosa 86,6±5,6 mg/dl. Por el contrario, 10 sujetos cumplieron criterios del test *Quantose IR™* para la RI (score: 78,09 ± 9,24 (>63)). **Conclusiones:** El test *Quantose IR™* mide el porcentaje de hemoglobina unida a glucosa dentro de los glóbulos rojos. Permite prever el riesgo de diabetes, y tomar medidas preventivas.

Palabras clave: Diabetes mellitus; resistencia a la insulina; metabolómica; metabolismo de los hidratos de carbono; niño. (Fuente: DeCS, Bireme).

Abstract

Introduction: Metabolomics enables the study of insulin resistance (IR), a risk factor for pre-diabetes and diabetes. Quantose IR™ is the only test that measures IR using the euglycemic hyperinsulinemic clamp. **Objective:** The effectiveness of a metabolomic test for the detection of RI markers in a child population was verified. **Materials and methods:** Eleven children aged 8.54 ± 3.53 years with diabetes risk factors were recruited from the El Escorial Hospital. The American Diabetes Association (ADA) Standards (5,7-6,4% HbA1C and 100-125 mg/dl basal glucose) were established as diagnostic criteria for prediabetes. Blood tests were compared to the Quantose IR™ assay studying the metabolomic profile related to IR (alpha-hydroxybutyric acid, oleic acid, linoleo-glycerophosphocoline and insulin). This analysis generated a Quantose© score of IR > 63. **Results:** None of the subjects met the ADA criteria for prediabetes: HbA1C=5,3±0,18 and glucose=86,6± 5,6 mg/dl. On the contrary, 10 subjects met the Quantose IR™ test criterion for IR (score: 78,09 ± 9,24 (>63)). **Conclusions:** The Quantose IR™ test measures the percentage of glucose bound hemoglobin within red blood cells. This assay makes it possible to predict diabetes risk and take preventive measures.

Key words: Diabetes mellitus; insulin resistance; metabolomics; carbohydrate metabolism; child. (Source: DeCS, Bireme).

***Corresponding author at:**
Ismael San Mauro Martín
e-mail: info@grupocinusa.es

Introduction

The insulin resistance syndrome (IR) is currently one of the most important etiological factors not only of morbidity but also mortality worldwide, due to its association with obesity, high blood pressure, dyslipidemia, arteriosclerosis and the development of type 2 diabetes mellitus (DM)[1]. The overall IR prevalence rates fluctuate between 3.1% and 44%, according to population-based studies of children and adolescents[2].

IR is defined as a reduction in the physiological response of tissues to insulin activity, particularly at muscular and adipose tissue level. Insulin is an anabolic hormone secreted by the pancreatic β cells in response to diverse stimuli, with glucose being the most relevant stimulus[3]. The main function of insulin is to maintain glucose homeostasis and other energy substrates. As a result, this hormone suppresses the release of free fatty acids while promoting the synthesis of triglycerides in adipose tissue after each meal. On the other hand, insulin inhibits the hepatic production of glucose and its uptake by skeletal muscle and adipose tissues[4]. In IR, the action of this hormone is reduced at the cellular level, which leads to its increased secretion, compensation for the defect in insulin's tissue activity and, ultimately, maintenance of the glycemic homeostasis[5]. This phenomenon is responsible for the hyperinsulinemic state, which is a characteristic of IR patients. It is estimated that approximately 55% of the variability in insulin sensitivity in children is determined by adipose tissue content and gender[6]. Age and pubertal stage have an important impact on the distribution of adipose tissue and sensitivity to insulin action[7].

The increase in visceral fat deposition is the main independent risk factor associated with the development of IR states in children and adolescents[8]. Also, nutrition can be a factor that favors the development of IR. In fact, a hypercaloric diet with a high content of fat and carbohydrates as well as a low fiber amount is related to IR[9].

IR has been recognized as playing an important role in the pathogenesis of type 2 diabetes in children[10]. In pediatric obese populations, the existence of IR may be the starting point for the subsequent development of type 2 diabetes and/or metabolic syndrome[11]. Indeed, type 2 prediabetes have been recently defined in obese adolescents[12].

The increase in the prevalence of obesity has been accompanied by an increase in the incidence of Type 2 DM in the pediatric population[13]. Likewise, an increase in BMI in children as well as the metabolic and cardiovascular complications associated with obesity have been consistently associated with a higher risk of hyperinsulinemia and IR[14,15]. Given the alarming increment in obesity rates among young populations, there is a great concern that the incidence of diabetes will overcome those rates. Thus, this pandemic is particularly threatening since it remains undetected worldwide (one in two diabetics patients is undiagnosed)[16]. For decades, levels of glucose, hemoglobin A1c, insulin and C-peptide have been the laboratory tests of choice to detect and monitor diabetes. Nevertheless, these tests do not identify prediabetic individuals or their sub-phenotypes that are at risk of developing type 2 DM, which would be a requirement for individualized prevention[17].

The available diagnostic methods to assess insulin sensitivity in the pediatric population are: (i) the hyperinsulinemic-euglycemic clamp, which represents the gold standard for measuring tissue sensitivity to insulin and insulin secretion but is considered a highly complex assay[18]; (ii) the HOMA (Homeostasis Model Assessment)[19] and QUICKI (Quantitative Insulin Check Index)[20] indices that are the simplest and most commonly used methods to evaluate IR in the field; and (iii) the Matsuda-DeFronzo index (also called ISI-Compound) calculated from an oral glucose tolerance curve (OGTC) that provides additional information about glucose metabolism in the post-stimulatory state[21].

The oral glucose tolerance test is the sole method for the early and reliable identification of people in the prediabetes phase with impaired glucose tolerance. However, this procedure is very lengthy and expensive and is not recommended as a screening method during a medical consultation. Therefore, there is a need for innovative laboratory assays in order to simplify the early detection of alterations in glucose metabolism[17]. New diagnostic technologies based on metabolomics, are more sensitive, specific and useful tools compared to other modern techniques such as genomics.

The *Quantose™ IR* test (*Metabolom INC, USA*) is a metabolomic-based assay that analyzes IR and is able to generate a value as an early risk indicator for the development of prediabetes and type 2 DM[22]. α-

hydroxybutyrate is the most important metabolite associated with insulin sensitivity and is considered an early marker of both IR and dysglycemia (impaired glucose regulation)[23].

Quantose™ IR is a fasting blood test that measures a set of IR biomarkers that includes an organic acid (alpha-hydroxybutyric acid), two lipids (oleic acid and linoleolyl-glycerophosphocholin) and insulin. The test score was developed to estimate the value obtained from the hyperinsulinemic-euglycemic clamp, the gold standard for determining insulin sensitivity, in a prospective observational study of 1,277 clinically healthy, non-diabetic people recruited in 13 European countries[22]. The cut-off point at 63 was defined by the top tertile of scores in the study. Insulin levels are measured by immunochemiluminescence and the three metabolites are detected by mass spectrometry/liquid chromatography (UHPLC LC-MS/MS). The concentrations of the four biomarkers are combined and analyzed in an algorithm that generates the Quantose IR score (1-120 scale).

Most cases of type 2 DM can be detected and treated in a timely manner and the global burden of diabetes can be reduced by investing in new technologies as more efficient methods of prevention and early detection, which would also save billions in productivity loss and healthcare costs[16]. Consequently, the aim of this study is to verify the effectiveness of a metabolomic test in the detection of IR markers in children.

Materials and methods

A descriptive, cross-sectional, ambispective, pilot study was conducted on a population of 11 children (8 boys and 3 girls) with risk factors of metabolic complications (family history, obesity, alteration and increase in plasma basal glucose levels) from the pediatric service of San Lorenzo del Escorial Hospital (Madrid, Spain). The sample was incidental, pretending to have a first approximation of the use (clinical-economic effectiveness) of a commercial test for its possible future implementation in clinical practice.

Biochemical parameters

Venous blood was taken in EDTA vacutainer tubes after a 12-hour fasting period. Glucose levels were determined via an automated glucose oxidase method (Roche Hitachi® 917, Roche Diagnostics, Mannheim, Germany), whereas insulin and glycated hemoglobin (HbA1c) were assessed through radioimmunoassay (RIA) and high precision liquid chromatography, respectively.

The American Diabetes Association standard was used as diagnostic criterion for prediabetes[24]: 5.7-6.4% HbA1C and 100-125 mg/dl basal glucose. Particularly, exceeding or not exceeding those figures in any of the traditional metabolites was classified as prediabetes.

The inclusion criteria were: 6 to 12 years old male and female children who had not reached sexual maturity, with and without obesity or overweight, with at least two risk factors associated with IR (high blood glucose levels, family inheritance, overweight of obesity).

The exclusion criteria were: sexually mature children at Tanner II stage or higher. Subjects with type I diabetes, pancreatitis, hepatitis, cerebral palsy, cancer, neuromuscular and psychological diseases or other serious diseases. Subjects with pharmacological treatment or dietary supplement for glycemic control.

All children were invited to participate for a period of three weeks and informed in writing about the purpose of the study. Parents of those subjects who decided to participate signed an informed consent form, which specify: the objectives of the research, all the conducted procedures, the expected length of the study, the uncertainty regarding the safety and efficacy of the treatment and information about those responsible for the research.

Four out of the 15 originally recruited subjects were excluded, because of not having completed the tests (two), the decision of the parents (one) and a failure in the biochemical assessment in the laboratory (one). An equal number of subjects regarding sex and age was not achieved due to the incidental nature of the sample that was taken from the hospital and voluntary consultation.

Data were gathered by trained dietitians-nutritionists homogenizing a data collection protocol and study monitoring. The weight (kg), height (m), waist (cm), body mass index (BMI), total body fat percentage, visceral fat percentage and fat-free body mass (kg) of each participant were measured.

Weight, BMI and human body composition were assessed through a Tanita BP601 tetrapolar, multi-frequency, electrical bio-impedance analyzer and a flexible, non-elastic, metallic measuring tape that had a width of 0.1mm-150 cm. The standard protocol of this equipment[25] and the manufacturer's recommendations[26] were followed to carry out the bio-impedance analysis. This protocol was previously shared with the participants in order guarantee a greater reliability of results.

In addition, and *ad hoc* questionnaire was conducted, which was based on different questionnaires that included personal information, clinical history, as well as lifestyle, exercise and dietary habits, together with the Food Consumption Frequency Questionnaire (resolution time between five to ten minutes).

Subsequently, blood concentration of the four metabolites related to IR (alpha-hydroxybutyrate, linoleolyl-glycerophosphocholine, oleic acid and insulin) was quantified via the *Quantose IR™* test, which relies on mass spectrometry/liquid chromatography (UHPLC LC-MS/MS). These quantitative absolute measurements are used to generate the *Quantose*™ score (0-100 scale), where values greater than 63 are indicative or IR and, thus, prediabetes. The *Quantose*™ score is also displayed on a color-coded scale. When the *Quantose*™ score increases, the color also changes from green to orange-red, which indicates a change in the patient from insulin sensitive to insulin resistant[27].

The data analysis was carried out with the SPSS 20 statistical analysis program. The results of the quantitative measurements of the four metabolites were combined in a logistic regression algorithm to generate a *Quantose*™ score that exceeds other estimates commonly used for IR.

Ethical considerations. This study was approved by the Bioethics Committee of the Puerta del Hierro Hospital, Madrid. The ethical standards of the committee in charge of supervising the human essays and the 1975 Helsinki declaration (as amended in 2004) were followed.

Results

The sample consisted of 11 children (eight boys and three girls) with a high risk of diabetes. The mean age was 8.54 ± 3.53 years old.

None of the children exceeded the criteria of the American Diabetes Association for prediabetes (5.7-6.4% HbA1C and 100-125 mg/dl basal glucose). Indeed, the averages of HbA1C and glucose were 5.3 ± 0.19% and 86.6 ± 5.6 mg/dl, respectively (Table 1).

On the contrary, the *Quantose IR©* metabolomic test identified 10 subjects with insulin resistance after the analysis of the algorithm of the four metabolites and the results of their scores (>63), *Quantose IR©*: 78.09 ± 9.25 (Figure 1).

Table 1. Descriptive analysis of the sample

	Total (n=11) Mean ± SD	Boys (n=8) Mean ± SD	Girls (n=3) Mean ± SD
Age (years)	8.5 ± 3.5	9.4 ± 3.9	6.3 ± 0.6
HbA1c (%)	5.3 ± 0.19	5.3 ± 0.2	5.6 ± 0
Glucose (mg/dl)	86.6 ± 5.6	87.8 ± 5.3	82 ± 5.7
α-hydroxybutyrate (µg/ml)	6.3 ± 2.4	6.4 ± 2.7	6.03 ± 1.5
linoleolyl-glycerophosphocholine (µg/ml)	11.7 ± 5.2	10.2 ± 2.7	15.9 ± 8.5
Oleic acid (µg/ml)	53.9 ± 29.0	59 ± 28.5	40.2 ± 31.2
Insulin (µU/ml)	13.6 ± 8.6	12.3 ± 6.3	17.2 ± 14.2
***Quantose IR©* score**	78.09 ± 9.25	79.9 ± 6.6	73.3 ± 15.2

SD: Standard Deviation

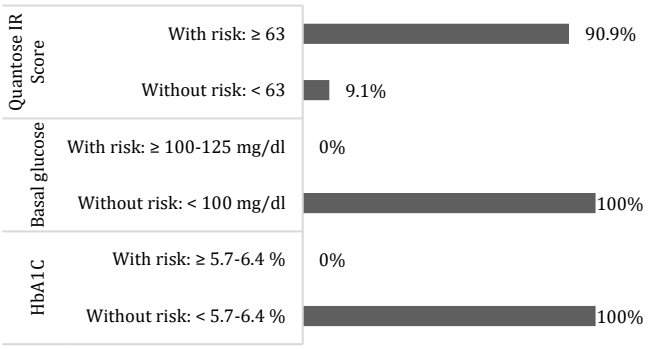

Figure 1. Percentage results obtained from the 3 main biomarkers. Each one shows the percentage of subjects estimated at risk or non-risk according to the *Quantonse IR*© metabolomics test, basal glucose and HbA1C

Discussion

Unfortunately, the general methods to detect biomarkers of metabolic disorders had had little success. As a result, metabolomics has become an important tool in the finding of new biomarkers for diagnosis and prognosis[28]. Scalbert, *et al.*[29], in their report on the identification of biomarkers by metabolomics, include supporting data on the validation of these biomarkers. However, the identification of new biomarkers to detect subjects at risk of IR and categorize the risk of progression to type 2 DM and/or cardiovascular disease remains poorly developed. New biomarkers would also facilitate to implement more efficient prevention strategies and monitor the response to treatment of these diseases[28].

Gall *et al.*[30] were the first ones to identify α-hydroxybutyrate and linoleolyl-glycerophosphocholine as the first and second main biomarkers of IR, respectively. Given that these biomarkers had not been previously evaluated in clinical studies, the authors assessed their capacity to predict type 2 DM in a long-term observational cohort of subjects at risk. They concluded that abnormal levels of α-hydroxybutyrate and linoleolyl-glycerophosphocholine were predictive of dysglycemia.

In this context, *Quantose™* is a validated, simple and innovative test for IR, which requires a single fasting blood sample and may be used as an early risk indicator of the development of prediabetes and type 2 DM[22]. The three metabolites that are used in the test (α-hydroxybutyric acid, oleic acid and linoleolyl-glycerophosphocholine) have a number of potential functions in the metabolic pathways associated with insulin action, insulin secretion and pancreatic β cell function[22].

In the study by Cobb, *et al.*[22], the three biomarkers of the test showed a similar pattern that was characterized by significant differences in insulin-resistant subjects. Specifically, the levels of α-hydroxybutyric acid oleate increased in these patients. In addition, the *Quantose*™ score was significantly better for the detection of IR than insulin alone (areas under the curve of 0.79 vs. 0.74). Also, the *Quantose*™ score can be used as an early indicator to predict glucose intolerance. In fact, the test identified thin subjects with normal fasting glucose levels who were at risk of developing IR. The *Quantose*™ scores of glucose intolerance progressors decreased by 8% over the course of three years, indicating an increase in IR.

Ferranini, *et al*.[31] reported progressively higher levels of α-hydroxybutyric acid and lower levels of linoleolyl-glycerophosphocholine through the quartiles of IR and in individuals with glucose intolerance or type 2 DM. Furthermore, they demonstrated that such levels at the beginning of the study were more pronounced in individuals with a declined health compared to those in individuals displaying a stable glucose tolerance after three years of follow-up and in individuals who progressed to type 2 DM after 9.5 years of follow-up. When these variables were added to a model to predict either dysglycemia or type 2 DM, which included family history of diabetes, sex, age, BMI, fasting glucose and fasting levels of these two metabolites, the predictability improved. However, when the model included fasting glucose and its level after two hours, the two metabolites had a minimal impact on the predictability. Based on these results, Ferrannini *et al*.[31] confirmed that α-hydroxybutyric acid and linoleolyl-glycerophosphocholine fasting levels may represent new biomarkers to predict dysglycemia and type 2 DM.

Conclusions

Metabolomics-based tests could be used as better early predictors of prediabetes or type 2 DM compared to traditional blood glucose markers (fasting plasma glucose, hemoglobin A1c and oral glucose intolerance test), which increase relatively late during the disease progression. In the light of the

current global pandemic of type 2 diabetes, such preventive actions are extremely important.

Additional clinical data from various longitudinal populations are currently being analyzed in order to corroborate the full clinical potential of this new IR and prediabetes test.

Metabolomics has the potential to provide information abtout the important pathways involved in glucose metabolism and type 2 DM pathogenesis. It can also reveal biomarkers capable of improving the prediction of the risk of glucose tolerance impairment and type 2 DM. The *Quantose*™ test score could be a useful tool in clinical practice for personalized monitoring of the patients' progression to prediabetes or type 2 DM and, thus, potentially improving their glycemic status.

Limitations of the study

This is a pilot study with a very small sample size. The authors suggest that research should continue with a larger population and the incorporation of other biomarkers.

Conflict of interest: none

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