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SPISE INDEX
Single Point Insulin Sensitivity Estimator: indicator of insulin resistance in children and adolescents with overweight and obesity

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Ai miei fratelli e ai miei genitori,

pilastri della mia vita

Sommario

INTRODUCTION

1.1 DEFINITION

Obesity is a complex multifactorial and severe disease characterized by an excess of body fat due to an overtime unbalanced energy expenditure [1].

During the past decades, prevalence of childhood obesity has dramatically increased worldwide, especially in low and middle-income countries [2]. After a misperception about this condition, in 1998 the National Institute of Health defined obesity as a chronic disease [3].

1.2 EPIDEMIOLOGY

In US it has been reported that one on five children aged 2-19 years are affected by obesity, and this rate is supposed to increase 130% over the next 2 decades [4]. In 2020 the World Health Organization (WHO) reported that 12% of children aged 7-9 years living in 33 European countries can be defined as obese, while 39 million of children aged up to 5 years are obese worldwide. Moreover, it has been estimated that obesity affects nearly 107.7 million of children and adolescents worldwide [5].

In the majority of developed countries, mainly in U.S., overweight and obesity is recognized as one of the

most common chronic diseases in childhood and adolescence and represents a major public health problem [6]. In fact, overweight and obesity are the fifth cause of death, responsible of 3.4 million of deaths annually worldwide [7].

1.3 PATHOGENESIS

Pathogenesis of obesity is multifactorial and includes the interaction between genetic predisposition, physiologic, socioeconomic and environmental factors [8]. Several causative contributors for pediatric obesity and type 2 diabetes mellitus have been analysed: early nutritional and epigenetic mechanisms, the thrifty epigenotype, maternal malnutrition and microbiota assessment [9][10].

As regards the environment, the place can affect obesity risk by means of two different pathways: first, the shared environment which can favourite unhealthy weight, and, second, the social contagion, which results in the spread of obesity from person to person via the norms, behaviours, attitudes, beliefs [11].

The link between place and obesity risk has been studied in the Military Teenagers Environment, Exercise and Nutrition Study [12]. The association between space and adolescents' obesity risk was not

explained by shared environment, suggesting the main role of social contagion.

Despite its increasing frequency, more than 90% of pediatric obesity the specific causative factor is unknown, and in less than 10% of cases is secondary to other conditions, such as syndromes, monogenic forms, endocrine disorders, drugs, cancer, psychiatric illnesses [13]. In particular, monogenic and syndromic obesities belong to a wide spectrum of hypothalamic diseases affecting the satiety signal, and are characterized by early onset weight gain (before 6 years of age), impaired satiety, uncontrolled food ingestion with disordered eating behaviour and extreme phenotype variability (*Table 1*) [14]. Recently obesity and its comorbidities are recognized as early-onset complications of pharmacologic and radiotherapy treatment in young pediatric cancer survivors [15].

Despite their pathogenesis, overweight and obesity starting in infancy and childhood are risk factors for its persistence in adolescence and adulthood and more than 60% of overweight prepubertal children maintain the condition overtime, with early-onset comorbidities and reduced life expectancy [16].

	Affected gene	Specific clinical features
Syndromic obesity		
Prader Willi syndrome	Abnormal parental genomic imprinting of paternal 15q11-q13 region.	Neonatal hypotonia, suckling disorders in the first months, hyperphagia and food impulsivity around 4 years, moderate intellectual disability, social interaction and behavioral disorders, endocrine abnormalities (growth hormone deficiency, hypogonadism), dysmorphia, scoliosis.
16p11.2 microdeletion syndrome	Autosomal dominant transmission, small region of chromosome 16.	Developmental delay, intellectual disability.
Fragile X syndrome	X-linked dominant transmission, CGG trinucleotide expansion of FMR1 promotor leading to a lack of transcription.	Intellectual deficiency and dysmorphic features of varying degree, more severe and frequent in males. 40% of obesity with some PWS-like phenotypes.
Bardet-Biedl syndrome	Autosomal recessive transmission, 22 genes known.	Retinal dystrophy, polydactyly, renal abnormalities, hypogonadism, hepatic fibrosis, learning disabilities.
Alström syndrome	Autosomal recessive transmission, ALMS1 gene.	Retinal dystrophy, dysmorphic features, short stature, central deafness, endocrine abnormalities (central or peripheral hypogonadism and hypothyroidism, polycystic ovary syndrome) dilated cardiomyopathy, liver and renal fibrosis and no intellectual disability.
Pseudohypoparathyroidism	Autosomal dominant transmission, GNAS gene.	Dysmorphia, shot bones, short stature, subcutaneous ossifications, variable developmental delay, hypocalcemia, hypothyroidism, pubertal delay, epilepsy.
MYT1L	Autosomal dominant transmission, MYT1L gene.	Developmental and language delay, intellectual disability, behavioral disorders and dysmorphic features.
Monogenic obesity		
LEP	LEP, LEPR, POMC, PCSK1, MC4R genes: Autosomal recessive transmission: severe, early-onset obesity and eating disorders with related signs (see beside). Milder phenotype in heterozygous patients without related signs and more metabolic obesity complications.	Endocrine abnormalities (gonadotropic and thyrotropic insufficiency).
LEPR		Endocrine abnormalities (gonadotropic, somatotropic and thyrotropic insufficiency).
POMC		Endocrine abnormalities (corticotropic, gonadotropic, somatotropic and mild thyrotropic insufficiency), red hair.
PCSK1		Severe neonatal diarrhea, endocrine abnormalities (corticotropic, gonadotropic, somatotropic and thyrotropic insufficiency), hypoglycemia.
MC4R		Increased height growth in childhood.

ALMS1: Alström syndrome associated protein 1, FMR1: fragile x messenger ribonucleoprotein 1, LEP: leptin, LEPR: leptin receptor, POMC: proopiomelanocortin, PCSK1: prohormone subtilisin/kexin 1 convertase, MC4R: melanocortin receptor type 4, MYT1L: myelin transcription factor 1 like.

Table 1. Most prevalent syndromic and monogenic obesities including the specific clinical features, and genetic alterations [14].

1.4 OBESITY COMPLICATIONS

The adverse consequences of obesity include several conditions, like insulin resistance and Type 2 Diabetes Mellitus (T2DM) [17]. Both increase the risk for cardiovascular and cerebrovascular morbidity and mortality. Moreover, obesity predisposes to Non Alcoholic Fatty Liver Disease, (NAFLD) particularly dangerous in young adolescents since it can evolve to non-alcoholic steatohepatitis, cirrhosis and liver cancer, that shorten life expectancy [18]. Similarly, obesity is strongly associated with early-onset hypertension and increases the risk of chronic kidney disease up to end-stage renal failure [19].

1.5 INSULIN RESISTANCE

Since adolescence, overweight and obesity are firstly characterized by altered metabolic status, including Insulin Resistance (IR), different degrees of dysglycemia, (i.e. fasting hyperglycemia and impaired glucose tolerance), and abnormal lipid profile [20].

Among obese children the frequency of IR varies from 33.2 to 52.1%, due to different methods and their cut off values [21].

Several factors have been recognized as pathogenetic for IR, and obesity is the most prevalent. Genetic predisposition [22], gestational diabetes [23], born small for gestational age [24] early postnatal weight

gain [25], premature birth [26] and smoking during pregnancy [27] are intensively studied risk factors.

IR is a metabolic impairment consequence of reduced ability of insulin to stimulate glucose uptake by adipose tissue and muscles, together with reduced insulin capability to suppress hepatic glucose synthesis and output [28].

The subsequent excessive supply of free fatty acids further affects glucose transportation in the skeletal muscles and inhibits insulin activity [29]. Moreover, IR exacerbates oxidative stress leading to vascular endothelial damage [30].

1.6 VASCULAR ENDOTHELIUM

Vascular Endothelium (VE) is located on the luminal surface of blood vessels and represents a selective, permeable and protective barrier between bloodstream and vascular wall. VE plays several important physiological, paracrine, endocrine and autocrine functions, mainly to assure normal blood fluidity and flow, and to hinder the entry of microbes and other harmful entities, in order to maintain cardiovascular homeostasis [31]. VE also regulates vessel permeability and smooth muscle cell migration, fibrinolysis and thrombosis, platelet and leukocyte adhesion, angiogenesis and vascular tone [30]. Healthy

endothelium has also anti-inflammatory properties due to its capability of reaction against hemodynamic changes by production of numerous vasoactive molecules, mainly nitric oxide and prostacyclin [32].

1.6.1 ENDOTHELIAL DYSFUNCTION

Endothelial Dysfunction (ED) is the consequence of either mechanical stimuli, like increased endoluminal pressure and shear stress, or metabolic factors like hormones and vasoactive agents [33]. ED is characterized by an imbalance between vasodilator and vasoconstriction agents and is followed by the release of substances aimed to regulate hemostasis, vasomotor activity and inflammation [33]. Moreover, damaged endothelium produces agents stimulating either thrombosis, like plasminogen activator inhibitors and von Willebrand factor, or inflammation, like several adhesion molecules, Interleukin-6 and Ultrasensitive C-Reactive Protein. ED is one of the most important predictive and pathogenetic mechanism of a broad spectrum of life-threatening conditions, in particular cardiovascular diseases, and represents the primary causative agent of atherosclerosis [34].

Prompt diagnosis of overweight and obesity is mandatory for pediatricians in order to clearly define the clinical and laboratory characteristics, to assess primary prevention strategies, personalized and

multidisciplinary care program, to screen and prevent development of vascular complications [35].

ED is the basal step of vascular toxicity, and is the first *movens* in the pathogenesis of atherosclerosis and thrombosis leading to cardiovascular diseases (coronary heart disease, hemorrhagic or ischemic stroke, peripheral arterial disease and venous thromboembolism) [36]. A normal endothelial function relies on the balance between anti-platelet, anti-coagulant and anti-inflammatory actions. IR impairs the complex endothelial cell system, which supports the balance between vasodilating and vasoconstricting substances produced by (and acting on) endothelial cells [37]. The reaction against hemodynamic stress on the damaged endothelium leads to thrombosis by producing plasminogen activator inhibitor, which reduces the production of plasmin by inhibiting the plasminogen activators tissue and urokinase. Furthermore, the desquamation of endothelial cells exposes them on Willebrand factor (vWF), a stimulus for platelet activation and aggregation, at the level of the subendothelial basement membrane. The inflammation exerts a procoagulant state through the increase of adhesion molecules [E-selectin, vascular cell adhesion molecule (VCAM-1), vascular cell adhesion molecule

(ICAM1)], vasoconstrictor agents (endothelin-1, tissue factor), chemokines and pro-inflammatory cytokines (interleukin-1, interleukin-6, interleukin-8, INFγ) [38]. A link between prothrombotic and inflammation state has been described and termed ‘immunothrombosis’. This relationship is bidirectional, with the release of inflammatory mediators activating the endothelium towards a pro-coagulant and platelet-activating phenotype. On the other hand, the production of procoagulant agents and tissue factors causes vasculitis and a pro-inflammatory state [39].

1.7 METABOLIC SYNDROME

Among several health impairments secondary to pediatric obesity, Metabolic Syndrome (MS) and its consequences deserve attention [40]. The term MS, extensively described by Reaven [41] includes a clustering of central obesity, insulin resistance, high blood pressure levels, high levels of triglycerides, low levels of HDL cholesterol and different degrees of dysglycemia. MS prevalence is increasing worldwide also in adolescents, mainly linked to epidemic obesity, and represents a cardiometabolic risk factor for the development of atherosclerosis, cardiovascular disease morbidity and mortality, and T2DM. MS seriously

impairs not only adolescents' global health but also quality of life [42].

At present, a valid, globally accepted definition of MS is lacking, and more than 46 different classifications have been proposed, mainly based on parameters from adulthood [43]. Therefore, a correct diagnosis of MS is sometimes difficult, since age- and gender-specific parameters (i.e. blood pressure levels percentiles, insulin resistance indexes, lipid profile, BMI-SDS) are different from those available in adulthood. In 2007 the International Diabetes Federation (IDF) established a new set of diagnostic criteria [42]. At present, four different classifications of MS are recognized, all including abdominal obesity, hypertension, dyslipidemia and impaired fasting glucose (*Table 2*) [44][45]. As a consequence, results from epidemiological studies are controversial, reporting a prevalence of MS ranging from 0.3 to 26.4%, especially due to different weight of components (*Table 3*) [44].

The pathogenesis of MS is still unclear, and different mechanisms have been studied [46]. Insulin resistance together with central and visceral obesity trigger various pathways resulting in a proinflammatory and prothrombotic state leading to endothelial damage. Acting as endocrine organ, visceral fat rather than

subcutaneous adipose tissue plays a pathogenetic role in MS. In fact, adipocytes secrete several inflammatory markers and adipokines involved in energy expenditure, endothelial metabolism and atherogenesis.

As the process persists, glucotoxicity can occur, resulting in chronic hyperglycaemia and clinical T2DM.

	Abdominal obesity	Hypertension	Dyslipidemia	Fasting glucose
IDF [47] Central obesity + 2 of 4 [a]	10-15 years of age WC > 90th percentile >15 years of age WC 294 cm (♂) [b] WC ≥ 80 cm (♀) [b]	Systolic BP≥ 130 mmHg or diastolic BP> 85 mmHg or specific treatment	TG ≥150 mg/dl or specific treatment HDL < 40 mg/dl (♂) <50 mg/dl (♀)	≥100 mg/dl or diagnosis of type 2 diabetes mellitus
Cook et al. [48] 3 out of 5°	WC ≥ 90th Percentile [c]	≥90th percentile [d]	TG ≥ 110 mg/dl [e] HDL ≤ 40 mg/dl [f]	≥110 mg/dl
Ford et al. [49] 3 out of 5°	WC ≥ 90th Percentile [g]	≥90th percentile [d]	TG ≥ 110 mg/dl [e] HDL ≤ 40 mg/dl [f]	≥110 mg/dl additional analysis with ≥100 mg/dl
de Ferranti et al. [50] 3 out of 5 [a]	WC ≥ 75th percentile	≥90th percentile	TG ≥ 100 mg/dl HDL ≤ 50 mg/dl	≥110 mg/dl

[a] Number of criteria that must be fulfilled for diagnosing MetS.

[b] For Europid males/females; ethnic-specific percentiles are recommended for other population groups [25].

[c] Age- and sex-specific, recommended by NHANES III (National Health and Nutrition Examination Survey).

[d] Age-, sex-, and height-specific, recommended by NHBPEP (National High Blood Pressure Education Program).

[e] Age-specific, recommended by NCEP (National Cholesterol Education Program).

[f] All ages/sexes, recommended by NCEP.

[g] Sex-specific, recommended by NHANES.

Table 2 [44]. Different definitions of pediatric metabolic syndrome, proposed by the IDF [45], Cook et al. [48], Ford et al. [49], and de Ferranti et al. [50].

Author (year)	Definition	Diagnostic criterion: elevated BP	Prevalence of elevated BP
Kim et al. (2016)[51]	IDF	Systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg	2.4%
	Ford	≥90th percentile; specific for age, sex, and high	20.4%
Agudelo et al. (2014) [52]	IDF	Systolic BP > 130 mmHg or diastolic BP ≥ 85 mmHg	2.2%
	Cook	≥90th percentile; specific for age, sex, and high	10.3%
Villalobos et al. (2014) [53]	IDF	Systolic BP> 130 mmHg or diastolic BP ≥ 85 mmHg	0.7%
	Cook	≥90th percentile; specific for age, sex, and high	8.7%
Author year	Definition	Diagnostic criterion: high fasting blood glucose (FBG)	Prevalence of high FBG
Agudelo et al. (2014) [52]	Cook de Ferranti	FBG ≥ 110 mg/dl	0.6%
	IDF, Ford	FBG > 100 mg/dl	2.8%

Table 3 [44]. Different weighting of components of the metabolic syndrome.

1.8 TYPE 2 DIABETES MELLITUS

Even if in pediatric patients clinical evidence of T2DM is rarely encountered, other conditions associated to IR, like MS are frequently observed [54].

As observed in adulthood, T2DM may have an indolent presentation and heralds several years of asymptomatic illness [17]. On the other hand, some youths show a severe clinical onset, similar to type 1, autoimmune diabetes, making correct diagnosis quite difficult [20]. T2DM in youth was almost undiagnosed until 2 decades ago, being described only in grossly obese sibling of patients with diabetes related to genetic syndromes or belonging to ethnic minorities, like Pima Indians. Obesity and insulin resistance are recognized as the most important causative factors for early development of the disease [55].

1.9 INSULIN RESISTANCE MEASUREMENTS

The role of IR as independent predictor of a range of disorders is certain; however, its quantitative assessment is not regularly performed in routine clinical practice, despite several methods have been proposed [56].

In particular, euglycemic/hyperinsulinemic clamp is the gold standard to measure insulin sensitivity [57]. On the other hand, this procedure is invasive and laborious, requires multiple blood sample withdrawals,

insulin and glucose infusion rates adjustments, and it is not applicable on routine medical practice, especially in pediatric age group.

Among the markers of insulin resistance, several indexes describing glucose-insulin homeostasis by means of simple, mathematically derived equations based on fasting plasma glucose and insulin concentrations have been proposed [58].

1.9.1 HOMA

In particular, Homeostatic Model Assessment (HOMA) of IR (HOMA-IR), of β-cell activity (HOMA-β) and of insulin sensitivity (QUICKI) have been developed, even on fasting samples of glucose and insulin, making the evaluation of these parameters easier and reproducible also for follow-up studies. These methods measure insulin resistance, β-cell production and insulin sensitivity, respectively, and are useful tool the assess metabolic status. On the other hand insulin secretion is pulsatile [59], has short half – life [60] and standardized assays are lacking [61].

Normal values of IR indexes should consider age, pubertal stage and gender, being reported different results for normal values according to these parameters [62].

1.9.2 OGTT

Glycometabolic assessment can be also evaluated by Oral Glucose Tolerance Test (OGTT), developed more than 100 years ago [63]. Baseline fasting plasma glucose and glycaemic levels are measured every 30' after glucose ingestion. Glucose levels at +120' define normal glucose tolerance, impaired glucose tolerance and diabetes mellitus. Recently, glycaemic level higher than 155 mg/dl at + 60' after glucose ingestion has been defined as a risk factor for T2DM in obese adolescents [64]. Moreover, total insulin obtained by sum during all the times of the test is useful to define insulin resistance [65]. More recently, it has been reported that in obese children and adolescents total insulin sum during OGTT > 535 microU/ml has the highest sensitivity for T2DM risk [66].

Moreover, OGTT is still labour intensive and expensive.

At present conflicting data are reported about the prevalence of impaired fasting glucose, insulin resistance and abnormal glucose metabolism in obese subjects. The lack of uniformity seems attributable to ethnic differences among the group considered.

1.9.3 OTHER

Several non-insulin-derived indexes have been proposed. In particular, several years ago the Triglycerides/HDL-Cholesterol ratio (TG/HDL-C) has been introduced [67]. The main advantage is the universal availability of serum lipid measurement, in preclinical setting. On the other hand, this method has several limitations, due to great ethnic variability in the cut off points of the ratio [68].

A new marker of insulin resistance based on a mathematical model including BMI, fasting triglycerides and HDL-cholesterol values, named Single Point Insulin Sensitivity Estimator (SPISE) has been proposed [69]. It has been demonstrated that SPISE has a better predictive value of IR as compared to HOMA-IR and Quicki indexes [69]. Similarly, SPISE index is a useful tool for detecting abnormal glucose metabolism in overweight and obese children [70].

AIMS

The primary aim of our study was to evaluate the values of SPISE index in a group of children and adolescents with overweight/obesity.

Secondary aims were to establish the relationship between SPISE index and glycometabolic profile and its predictive value as compared to other known insulin resistance indexes.

PATIENTS AND METHODS

2.1 PATIENTS

In our cross-sectional retrospective study, we evaluated SPISE index and other biochemical/glycometabolic parameters in 232 obese children and adolescents (105 m and 127 f) median age 13.2 years (range 10.8-15.4 years) and followed at the outpatient clinic, Endocrinology and Diabetes Unit, Department of Paediatrics, G. Gaslini Institute, Genoa, Italy, between 2016 and 2020. Inclusion criteria were: absence of acute illnesses or administration of drugs affecting glucose metabolism Exclusion criteria were syndromic/genetic obesity or African American origin.

2.2 METHODS

In all patients, height, weight, body mass index, and pubertal stage according to Tanner were recorded. Measurements were performed with the subject wearing only light indoor clothing and no shoes. Height was measured with a portable Harpender stadiometer by Tanner technique. Weight was measured with a standardized portable scale. BMI was calculated as follows: (weight in Kg)/ (height in meters)^2. According to the WHO criteria, overweight was defined as BMI > 2 SDS and obesity as BMI > 3

SDS [71]. Severe obesity was defined as BMI-for-age above + 3 Z-scores relative to 2007 WHO growth reference median [72].

BMI was calculated and BMI SDS score (BMI-SDS) was computed for each subject by using the formula BMI-SDS = (actual BMI – mean BMI for age and sex)/BMI SD for age, race, and gender, based on established standards and norms.

Pubertal development stages were assessed using Tanner staging criteria by well-trained physicians in pediatric endocrinology. Patients were divided according to the pubertal development as follows: Group 1: Tanner stage 1; Group 2: Tanner stage 2-3; Group 3: Tanner stage 4-5.

2.2.1 BIOCHEMICAL ANALYSES

SPISE index vas calculated according to the formula: $600 \times HDL^{0.185}/Triglycerides^{0.2} \times BMI^{1.338}$, with fasting HDL cholesterol and Triglycerides expressed in mg/dL and BMI as kg/m^2 [69].

Lipid profile including triglycerides, total, HDL and LDL cholesterol was detected using standard methods.

After 12 hours of overnight fasting, all subjects underwent baseline diagnostic blood sample withdrawals including fasting Plasma Glucose (PG),

HbA1c, insulin, triglycerides, and total cholesterol levels. Glucose was detected by the glucose oxidase method on venous whole blood, and results were modified into plasma glucose values. Insulin was measured with a radioimmunoassay method. All parameters were measured at the same Laboratory.

As estimates of insulin sensitivity we measured HOMA-IR using the following formula: [fasting plasma insulin in microU/ml × Fasting Plasma Glucose (FPG) in mmol/l] / 22.5], and QUICKI as 1 / (log10 fasting plasma insulin in microU/ml + log10 glucose in mmol/l) [21]. As an index of pancreatic β-cell function, we measured HOMA-β as (20 × fasting plasma insulin in microU/ml)/ (FPG in mmol/l – 3.5) [21]. Hyperinsulinism was defined as the sum of insulin levels at 0^{th}, 30^{th}, 60^{th}, 90^{th},120^{th} min during OGTT > 300 microU/ml [25].

OGTT was performed using a standard dose of 1.75 g of glucose/kg of body weight (max 75 g) according to the ADA guidelines [73]. Before starting the test, an intravenous line was placed in the upper limb, and a fasting blood sample (after 10 to 12 hours of fasting) was taken and recorded as T0 (T for time). Blood samples were withdrawn at 0^{th}, 30^{th}, 60^{th} ,90^{th},120^{th} min, results were evaluated according to ADA criteria [73].

After the load, glucose tolerance was defined using standard parameters, i.e.:

- Normal Glucose Tolerance (NGT) = PG < 140 mg/dl at 2-h of OGTT,
- Impaired Glucose Tolerance (IGT) = PG 140-200 mg/dl,
- and Diabetes Mellitus (DM) = PG >= 200 mg/dl [73].

We also considered 1-h PG > 155 mg/dl as a biomarker to define high risk for progression to diabetes mellitus at a stage when β-cell function is substantially intact [74].

Biochemical parameters were evaluated, defining the so-called prediabetes as:

- Impaired Fasting Glucose (IFG), i.e. FPG 100-125 mg/dl,
- or IGT post-OGTT, i.e. PG 140-200 mg/dl, or HbA1c 5.7-6.4% (endorsed by ADA for prediabetes diagnosis) [73].

STATISTICAL METHODS

Descriptive statistics were performed; categorical variables were reported in terms of absolute frequencies and percentages; quantitative variables were reported in terms of median values and first and third quartiles (1^{st}–3^{rd} q).

Body Mass Index (BMI) was calculated as the ratio of body weight (kg) to squared height (meters). BMI was standardized by the LMS method [75], with gender and age adjustments, and was expressed as z-score, using the WHO tables as standard reference [76].

Comparison of frequencies was done utilizing the Chi-square test or Fisher's exact test (in case of expected frequencies < 5).

Comparison of quantitative variables (*example:* SPISE index) in 2 different categories of patients (*example:* patients with normal glucose tolerance *vs* patients with glucose intolerance) was made by the Mann-Whitney U test.

Comparison of quantitative variables (*example:* SPISE index) in more than 2 (three or four) different categories of patients (*example:* patients with overweight *vs* patients with obesity *vs* patients with severe obesity) was made by the non-parametric Analysis of Variance (Kruskal-Wallis W test); post-hoc comparisons were made using the Bonferroni's

correction in order to avoid multiple-comparisons error; whenever the Bonferroni's correction was applied the corresponding P-value was indicated as P_B.

Correlation between quantitative parameters (e.g., HOMA-IR vs Total Insulin after OGTT) has been evaluated by means of Spearman's Rank order correlation coefficient (r_S). The correlation coefficient was considered as follows: $r_S < |0.4|$ weak, $\geq |0.4|$ to $|0.59|$ moderate, $\geq |0.6|$ to $|0.79|$ strong, and $\geq |0.8|$ very strong, according to Swinscow et al [77].

ROC curve analysis [78] has been used to find the best cut-off values for the SPISE index that was postulated as a possible predictor of abnormal glucose metabolism.

All the statistical tests were two-sided and a *P* value < 0.05 was considered statistically significant. "Statistica" (release 9.1, StatSoft Corporation, Tulsa, OK, USA) and "Stata" (release 17.0, College Station, TX, USA) were used for all the univariate and bivariate analyses; the software MedCalc was used for the ROC curve analysis.

RESULTS

Clinical data of the enrolled patients ($n = 232$) is reported in *Table 4.*

Patients of both genders with a median age at evaluation of 13.2 years were included in the study. Only overweight, obese, and severely obese patients (as defined in the method section) were included in this cohort and the distribution of these categories of weight is reported in *Table 4*. Among these patients, only a minority was in a pre-pubertal Tanner stage (18.8 %).

As regards glucometabolic assessment, 66% of cases showed normal glucose tolerance, 33% impaired glucose tolerance and only 2 patients (0.9%) showed OGTT compatible with T2DM. For some analyses these 2 patients have been merged with the category of those with impaired glucose tolerance.

In our case series we compared SPISE values stratifying categories on the basis of BMI (overweight, obesity and severe obesity) (*Table 4*). As regards pubertal development, patients were divided in 3 groups: prepubertal, undergoing puberty and full pubertal development (*Table 4*).

The SPISE index significantly decreased from Tanner I to Tanner V in all weight categories increased (p value < 0.0001) (*Table 5* and *Figure 1*).

Maintaining the pubertal stage group as previously described, we compared the SPISE values between patients with normal glucose tolerance at OGTT, with those with impaired glucose tolerance or T2DM. In this case, we did not find a statistically significant difference between the 2 groups studied (Tanner stage I: p value 0.66; Tanner stage II-III-IV: p value 0.64; Tanner Stage V: p value 0.95) (*Table 6*).

As above mentioned, during 2022, we performed a retrospective clinical study on the same cohort of patients to evaluate insulin resistance parameters and compare them with each other [69]. In this retrospective study, the Total of Insulin Sum (TIS) during five points OGTT was correlated with other validated insulin resistance parameters. In the literature, a total sum of insulin > 300 microU/ml is considered a marker of insulin resistance [65], whereas in our study it was found that a TIS > 535 microU/ml, although with a lower sensitivity, had a higher specificity, with a greater area under the ROC curve [66].

We therefore compared the SPISE values, maintaining the division into pubertal stages, in patients classified as insulin resistant, i.e. a TIS at five points OGTT ≥ 535 microU/ml, compared to patients with a TIS < 535 microU/ml. In this case, the SPISE value was

significantly lower in the group of insulin-resistant patients compared to the others, in all 3 Tanner groups (Tanner stage I: p value 0.008; Tanner stage II-III-IV: p value 0.0008; Tanner Stage V: p value 0.002) (*Table 7*).

Similarly, we stratified insulin-resistant patients using as a cut-off a sum of insulin at OGTT ≥ 300 microU/ml. In this case, the SPISE index was significantly lower in prepubertal patients and in patients undergoing puberty, while did not show a statistically significant difference in the Tanner 5 patient group (Tanner stage I: p value 0.34; Tanner stage II-III-IV: p value 0.004; Tanner Stage V: p value 0.68) (*Table 8*).

Another widely used index of insulin resistance is the HOMA-IR [fasting plasma insulin in microU/ml x fasting plasma glucose (FPG) in mmol/l)/ 22.5]; analyzing clinical studies on HOMA-IR, a HOMA-IR > 75^{th} percentile or > 99.2^{th} percentile is considered high, and therefore a predictor of insulin resistance, depending on the different studies.

We compared the values of the SPISE index, using both the 75^{th} percentile of HOMA-IR (*Table 9*) and the 99.2^{th} percentile (*Table 10*) as insulin resistance cut off; in both cases we obtained a significantly lower SPISE value in patients categorized as insulin

resistant, in all Tanner groups (HOMA-IR > 75th percentile. Tanner stage I: p value 0.017; Tanner stage II-III-IV: p value 0.010; Tanner Stage V: p value < 0.0001) (HOMA-IR > 99.2th percentile. Tanner stage I: p value 0.08; Tanner stage II-III-IV: p value < 0.0001; Tanner Stage V: p value 0.008).

One of the main objectives of this study was to identify a reliable SPISE cut off, to identify patients at risk of insulin resistance, and consequently all the complications connected to this condition, using a simple blood sample that predicted HDL cholesterol, triglycerides together with the patient's BMI.

For this reason, we created ROC curves, trying to identify statistically significant areas under the curve. For this analysis we modified the division of patients based on Tanner, creating only 2 groups, one including Tanner stages I and II and the second including Tanner stages III-IV-V. We initially compared SPISE values with patients who showed a total insulin sum on OGTT ≥ 535 microU/ml; as shown in *Figure 2*, we obtained a significant area under the curve (AUC 0.75, 95% CI: 0.64 - 0.84) in the group of patients with Tanner I-II and the best cut off was ≤ 6.92 with a sensitivity of 87.2% and a specificity of 54.8%.

On the other hand, in the group of patients with Tanner III-IV-V, the area under the curve obtained a non-

significant value (AUC 0.64, 95% CI: 0.55-0.72), and the best cut off identified was ≤ 5.08 which showed a sensitivity of 59.3% and a specificity of 64.8% (Fig. 3).

In the same way we created a ROC curve, using a HOMA-IR ≥ 99.2th percentile as an insulin resistance parameter, maintaining the same division on the pubertal stage.

Also, in this case, for the Tanner I-II group we obtained an excellent area under the curve (AUC 0.84, 95% CI: 0.74 - 0.91), with good statistical validity, and the best cut off was ≤ 6.13, with an excellent sensitivity of 90% and good specificity of 67.7% (Fig. 4).

On the other hand, as already highlighted in the first ROC analysis, for the Tanner III-IV-V group, the area under the curve proved to be just satisfactory (AUC 0.69, 95% CI: 0.61 - 0.77) and the best cut off was ≤ 5, with a sensitivity of 66.7% and a specificity of 62.2% (*Figure 5*).

DISCUSSION AND CONCLUSIONS

In our cross sectional, retrospective study we detected the SPISE index in a group of children and adolescents with obesity.

Paulmichl modified the TG/HDL-C ratio, marker of insulin resistance, and defined the SPISE index including BMI, fasting triglyceride and HDL cholesterol [72]. The authors compared SPISE with other indexes of insulin resistance using area under the ROC curve (aROC) and $\chi 2$ test. They established a cut off value of 6.61, with a better aROC as compared to TG/HDL-C ratio.

Moreover, the stated there are large differences in the predictive power of TG/HDL-C ratio according to the ethnicities. In particular SPISE index should not be applied in African American; in this ethnicity higher insulin levels due to a compensatory reduction in hepatic insulin extraction with consequent reduced circulating TG levels have been reported [72].

Barchetta evaluated SPISE index in adolescents and adults with overweight/obesity as predictive of impaired glucose tolerance later in life [73]. The authors analysed 909 children and adolescents with overveight/obesity and 99 healthy controls. They reported SPISE index significantly lower in those with impaired glucose regulation, a positive correlation with

insulin sensitivity indexes and a negative relationship with age, blood pressure, HOMA-IR, basal and + 120th glucose levels during OGTT. The authors concluded that SPISE is associated with metabolic impairment and can be considered a predictor of future glucose abnormalities [73].

Correa-Burrows evaluates SPISE index in adolescents with obesity [79]. In prepubertal children a SPISE value of 6.3 showed the highest sensitivity and specificity, while in pubertal patients a SPISE value of 5.4 showed the highest sensitivity and specificity to screen patients for insulin resistance. Moreover, the predictive value was better in males than in females [79].

It is well known that insulin sensitivity decreases as children enter puberty, because of the physiological increased secretion and peripheral action of growth hormone/IGF1 axis and gonadal steroids.

Ha et al analysed SPISE and other risk factors for T2DM in 104 Korean adolescents with obesity and reported that fatty liver disease and family history positive for T2DM were significantly higher and SPISE index, whose cut off was 4.49, was significantly lower in those with T2DM as compared to normoglycemic adolescents [80].

Correa Burrows assessed the accuracy of SPISE to define cardiometabolic risk in 678 Chilean post pubertal adolescents with all weight status [81]. In males the SPISE cut off for MS was 5, and for IR the SPISE cut off was 5.9. In females the highest sensitivity and specificity of SPISE for MS was 6, and the SPISE cut off for IR was 6.4. The authors concluded that SPISE in a good diagnostic tool for MS and, especially in males, also for IR [81].

On the other hand, it is well established that NAFLD is associated with obesity.

To this purpose, Furthner et al evaluated SPISE as a marker of liver insulin resistance [82]. They found lower SPISE levels in pubertal patients with obesity-associated NAFLD.

Since insulin resistance is associated with obesity, it is mandatory the availability of easy, reproducible indicators of insulin resistance in order to assess prevention strategies aimed to avoid the clinical onset of T2DM and its severe complications even late adolescence [58].

Multidisciplinary early intervention in children and adolescents with obesity improves insulin resistance and other inflammatory markers, therefore is important to have easily reproducible markers aimed to

precociously identify children at risk of even in adolescence or young adulthood of T2DM [83].

The availability of a reliable indicator of insulin resistance, measurable through an economical and easily performed blood test, could allow the correct stratification of patients.

The strength of this work is that we were able to verify that the SPISE index tends to reduce significantly both with increasing weight and with increasing Tanner stages. Moreover, we found significant results when compared SPISE with other indicators of insulin resistance.

Furthermore, regarding the identification of a valid cut off, we obtained encouraging results otherwise only in Tanner stages I-II.

On the other hand, in the Tanner III-IV-V patient group it was not possible to identify a significant area under the curve, and consequently a cut off with high sensitivity and specificity. This result can also be linked to a limited sample of patients, therefore one of the future objectives will be to analyse this parameter again by increasing the number of patients and define the SPISE level in healthy age- and gender-matched peers.

Another limitation of the study is linked to the absence of controls with normal BMI, to be able to compare SPISE values with the healthy population.

In conclusion, the SPISE index as a new indicator of insulin resistance in pediatric and adolescent obesity is very encouraging.

***Table 4**. Description of the study patients [N = 232].*

	N. (%)
Sex[##]:	
Male	105 (45.3 %)
Females	127 (54.7 %)
Age at visit (years); *median (1^{st} – 3^{rd} q)*	*13.2 (10.8 - 15.4)*
BMI SDS, *median (1^{st} – 3^{rd} q)*	*2.7 (2.2 - 3.1)*
Overweight	48 (20.7 %)
Obesity	110 (47.4 %)
Severe obesity	74 (31.9 %)
Pubertal stage [N=224]:	
Tanner: I	42 (18.8 %)
Tanner: II-III-IV	100 (44.6 %)
Tanner: V	82 (36.6 %)
Insulin resistance [N=229]:	
yes (TIS OGTT≥ 300 microU/ml)	197 (86 %)
No	32 (14 %)
Insulin resistance [N=229]:	
yes (TIS OGTT≥ 535 microU/ml)	129 (56.3 %)
No	100 (43.7 %)
Glucose tolerance [N=230]:	
Normal	152 (66.1 %)
Glucose intolerance	76 (33.0 %)
Type 2 Diabetes Mellitus	2 (0.9 %)

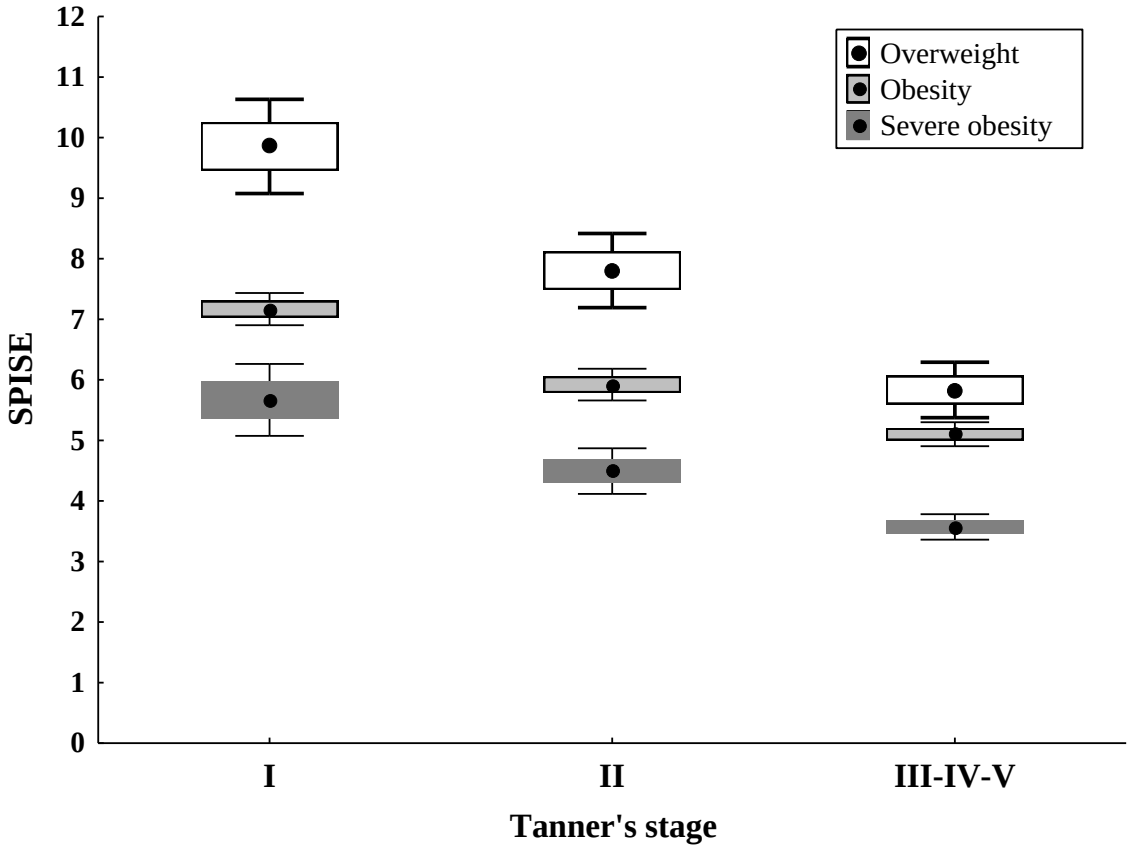
TIS: Total Insulin Sum during OGTT

Table 5. *SPISE index values in different categories of patients, by pubertal stage and category of weight.*

	Tanner stage: I		**Tanner stage: II-III-IV**		**Tanner stage: V**		**P**
	No.	**Median (1^{st}– 3^{rd} q)**	**No.**	**Median (1^{st}– 3^{rd} q)**	**No.**	**Median (1^{st}– 3^{rd} q)**	
Overweight (>1 SDS)	7	9.8 (8.9 - 10.4)	17	7.5 (7.1 - 8.6)	18	5.8 (5 - 6.7)	< 0.0001
Obesity (> 2 SDS)	14	7.2 (6.9 - 7.5)	53	5.7 (5.3 - 6.4)	38	5.1 (4.7 - 5.5)	< 0.0001
Severe obesity (> 3 SDS)	21	5.3 (4.7 - 6.4)	28	4.4 (3.9 - 5)	23	3.6 (3.2 - 3.9)	< 0.0001
	P	< 0.0001		< 0.0001		< 0.0001	

P: Kruskal-Wallis W test (non-parametric Analysis of Variance).

Figure 1. *SPISE index in different categories of patients, by pubertal stage and weight categories.*



As shown in ***Figure 1***, the SPISE index shows a statistically significant decrease from Tanner stage I to II and to III-IV-V, in all the weight categories. Median values and quartiles as well as P values are presented in ***Table 5***.

In ***Figure 1***, differently from the table, means and 95% Confidence Intervals are presented, but the statistical test as well as the corresponding interpretation should refer to the previous table.

Table 6. *SPISE index values in different categories of patients, by pubertal stage and category of glucose tolerance.*

	Tanner stage: I		**Tanner stage: II-III-IV**		**Tanner stage: V**		
	No.	**Median (1^{st}– 3^{rd} q)**	**No.**	**Median (1^{st}– 3^{rd} q)**	**No.**	**Median (1^{st}– 3^{rd} q)**	**P[#]**
Normal glucose tolerance	28	6.7 (5.9 - 7.9)	63	5.6 (4.9 - 6.9)	54	4.7 (4.1 - 5.4)	< 0.0001
Glucose intolerance/T2DM[§]	14	6.7 (5.2 - 7.4)	35	5.3 (4.9 - 6.6)	25	4.8 (3.9 - 5.7)	0.005
	P[##]	0.66		0.64		0.95	

[#]P: Kruskal-Wallis W test (non-parametric Analysis of Variance); [##] P: Mann-Whitney U test; [§]T2DM: Type 2 Diabetes Mellitus; only 2 patients suffered from T2DM and therefore were included in the group of patients with glucose intolerance.

Table 7. *SPISE index values in different categories of patients, by pubertal stage and category of insulin resistance*.

	Tanner stage: I		**Tanner stage: II-III-IV**		**Tanner stage: V**		**P[#]**
	No.	**Median (1st– 3rd q)**	**No.**	**Median (1st– 3rd q)**	**No.**	**Median (1st– 3rd q)**	
Total insulin < 535 microU/ml	24	7.4 (6.2 - 8.9)	37	6.4 (5.4 - 7.3)	34	5.2 (4.7 - 5.7)	< 0.0001
Total insulin ≥ 535 microU/ml	17	6.1 (4.7 - 6.9)	61	5.3 (4.6 - 6.2)	45	4.5 (3.6 - 5.2)	0.0001
	P[##]	0.008		0.0008		0.002	

[#]P: Kruskal-Wallis W test (non-parametric Analysis of Variance); [##] P: Mann-Whitney U test.

Table 8. *SPISE index values in different categories of patients, by pubertal stage and category of insulin resistance (categorised according to a different cut-off value).*

	Tanner stage: I		**Tanner stage: II-III-IV**		**Tanner stage: V**		**P[#]**
	No.	**Median (1st– 3rd q)**	**No.**	**Median (1st– 3rd q)**	**No.**	**Median (1st– 3rd q)**	
TIS < 300 microU/ml	8	9.5 (6.4 - 10.1)	11	7.3 (6.4 - 8)	12	5 (4.2 - 5.8)	0.0025
TIS ≥ 300 microU/ml	33	6.5 (5.2 - 7.3)	87	5.4 (4.8 - 6.5)	67	4.8 (3.9 - 5.5)	< 0.0001
	P[##]	0.034		0.004		0.68	

[#]P: Kruskal-Wallis W test (non-parametric Analysis of Variance); [##] P: Mann-Whitney U test.

Table 9. *SPISE index values in different categories of patients, by pubertal stage and the HOMA-IR index (categorised as: >75th percentile vs≤75th percentile).*

	Tanner stage: I		**Tanner stage: II-III-IV**		**Tanner stage: V**		**P$^{\#}$**
	N	**Median (1° - 3° q)**	**N**	**Median (1° - 3° q)**	**N**	**Median (1° - 3° q)**	
HOMA-IR ≤ 75th percentile	17	7.53 (6.32 - 9.21)	29	6.57 (5.3 - 7.5)	29	5.48 (4.84 - 6.05)	0.0004
HOMA-IR > 75th percentile	25	6.43 (5.03 - 7.18)	69	5.38 (4.84 - 6.25)	50	4.52 (3.66 - 5.11)	0.0001
	P$^{\#\#}$	0.017		0.010		< 0.0001	

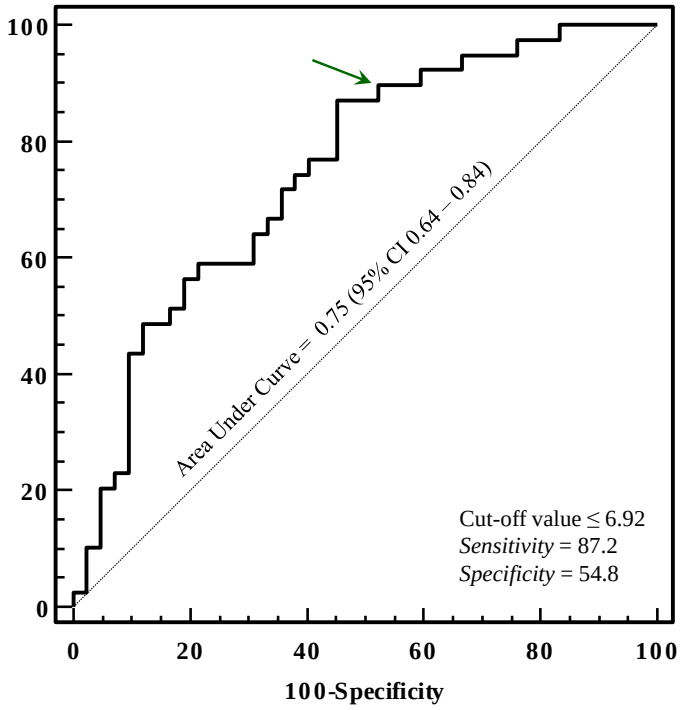
$^{\#}$P: Kruskal-Wallis W test (non-parametric Analysis of Variance); $^{\#\#}$ P: Mann-Whitney U test.

Table 10. *SPISE index values in different categories of patients, by pubertal stage and the HOMA-IR index (categorised according to a different cut-off value: > 99.2nd percentile vs≤ 99.2nd percentile).*

	Tanner stage I		**Tanner stage: II-III-IV**		**Tanner stage: V**		**P$^{\#}$**
	N	**Median (1° - 3° q)**	**N**	**Median (1° - 3° q)**	**N**	**Median (1° - 3° q)**	
HOMA-IR ≤ 99.2nd percentile	35	6.92 (5.74 - 8.47)	71	6.1 (5.1 - 7.07)	53	5.03 (4.49 - 5.69)	0.0001
HOMA-IR > 99.2nd percentile	7	5.65 (4.33 - 7.18)	27	4.86 (4.14 - 5.46)	26	4.5 (3.53 - 5.17)	0.028
	P$^{\#\#}$	0.08		< 0.0001		0.008	

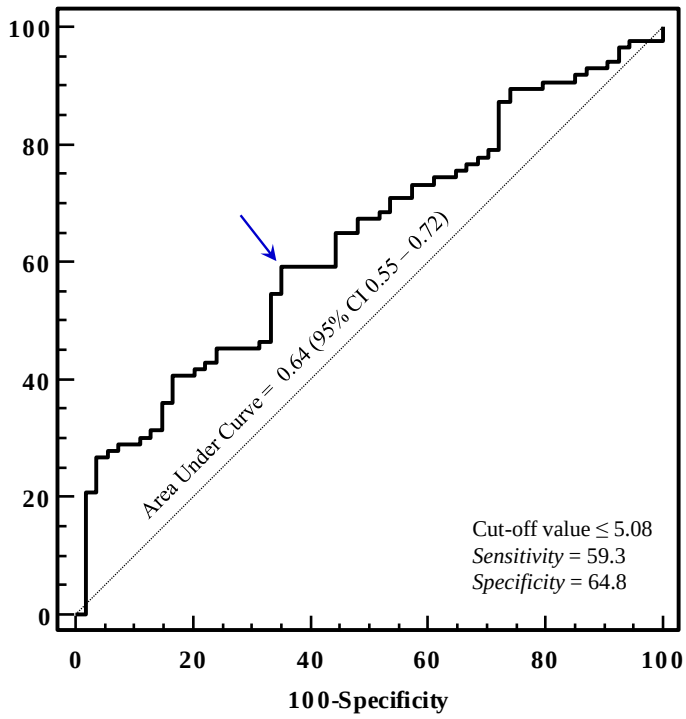
$^{\#}$P: Kruskal-Wallis test (non parametric Analysis of Variance); $^{\#\#}$P: Mann-Whitney U test.

Figure 2. *ROC curve of the SPISE Index against the categorized variable "Total Insulin Sum" (TIS) ≥ 535 microU/ml, in Tanner I-II patients [n = 39/81; 48.1 %].*



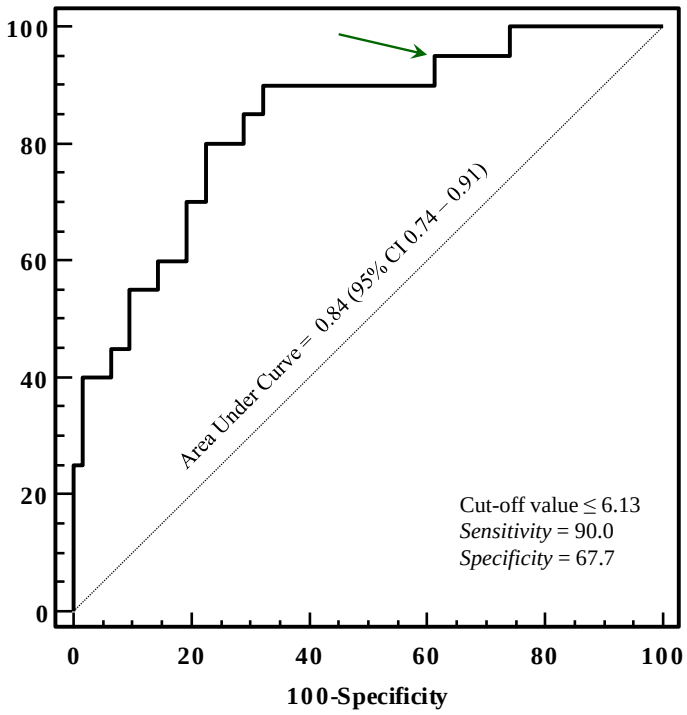
As shown in ***Figure 2***, the ROC curve of SPISE against the categorised variable "Total Insulin Sum" (TIS) after OGTT > 535 microU/mL, has a good value of Area Under Curve (AUC), being equal to 0.75 (95% CI: 0.64 – 0.84). The best cut-off value for SPISE in Tanner's stage I and II patients, was ≤ 6.92 corresponding to a sensitivity of 87.2% and a specificity of 54.8%.

Figure 3. *ROC curve of the SPISE Index against the categorised variable "Total Insulin Sum" (TIS) ≥ 535 microU/ml, in Tanner III-IV-V patients [n = 86/140; 61.4 %].*



As shown in ***Figure 3***, the ROC curve of SPISE against the categorised variable "Total Insulin Sum" (TIS) after OGTT > 535 microU/mL, has an unsatisfactory value of Area Under Curve (AUC), being equal to 0.64 (95% CI: 0.55 – 0.72). The best cut-off value for SPISE in Tanner's stage III-IV and V patients, was ≤ 5.08 corresponding to a sensitivity of 59.3% and a slightly better specificity of 64.8%.

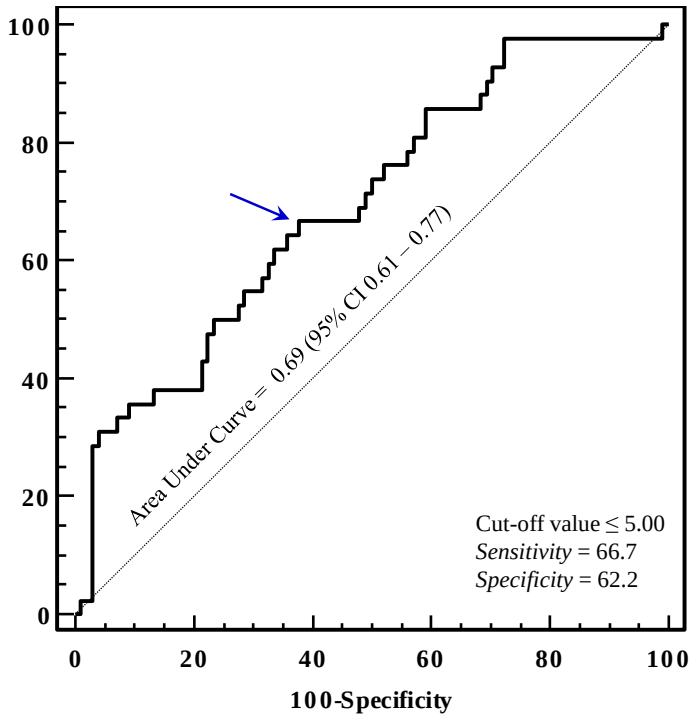
Figure 4. *ROC curve of the SPISE Index against the categorised variable "HOMA-IR > 99th percentile", in Tanner I-II patients [N = 82].*



As shown in ***Figure 4***, the ROC curve of SPISE against the categorised variable "HOMA-IR $>99^{th}$ percentile", has very good value of Area Under Curve (AUC), being equal to 0.84 (95% CI: 0.74 – 0.91). The best cut-off value for SPISE in Tanner's stage I-II patients, was ≤ 6.13 corresponding to a very good sensitivity of 90% and a specificity of 67.7%.

Patients with HOMA-IR > 99^{th} percentile, in Tanner's stage I-II, were 20 over 82, representing a percentage of 24.4 %].

Figure 5. *ROC curve of the SPISE Index against the categorised variable "HOMA-IR > 99th percentile", in Tanner III-IV-V patients [N = 140].*



As shown in ***Figure 5***, the ROC curve of SPISE against the categorised variable "HOMA-IR > 99th percentile", has only sufficient value of Area Under Curve (AUC), being equal to 0.69 (95% CI: 0.61 – 0.77). The best cut-off value for SPISE in Tanner's stage III-IV and V patients, was ≤ 5 corresponding to a sensitivity of 66.7% and a slightly better specificity of 62.2%.

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