Università degli Studi di Foggia

PhD Course

"MEDICINA TRASLAZIONALE E MANAGEMENT DEI SISTEMI SANITARI"

XXXIV Cicle

"Pediatric Obesity and GH/IGF-1 Axis: A New Insight"

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A mio Padre.

Custodirò per sempre nel cuore l'esemplare silenzio e la dignità con cui ha combattuto le sua battaglia. Vincitore e non vinto.

Tutto ha avuto inizio da qui.

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1 INTRODUCTION

1.1 Obesity in Paediatric age: an overview

Obesity in children has acquired increasing importance in recent years for the direct implications on child's health and because excess weight represents a risk factor for the onset of diseases in adulthood. Childhood obesity is also a predictor of obesity in adult age. It is estimated that 55% of obese children will be obese in adolescence, 80% of obese adolescents will still be obese in adulthood and 70% will be obese over age 30.[1]

According to the World Health Organization (WHO), over 340 million children and adolescents aged 5-19 were overweight or obese in 2016, and 39 million children under the age of 5 were overweight or obese in 2020.[1] Most of the world's population live in countries where overweight and obesity kills more people than underweight.[2] In fact, in the last 30-40 years there has been a sharp increase in the prevalence rates of overweight / obesity in children, which today are tripled in industrialized countries.

However, the 2019 data from OKkio alla Salute, the National Surveillance System promoted by the Italian Ministry of Health for primary school children, show a slight decrease in the levels of overweight and obesity in Italy.[3] Data highlights that, in 2019, overweight children were 20.4% and obese 9.4% (threshold values according to the International Obesity Task Force, IOTF), with significant prevalence reduction from 2008 to 2019.[3]

The highest prevalence was, once again, recorded in the Southern and Central Regions. However, these data do not have the update related to the SARS CoV2 pandemic, and its detrimental effect on nutritional state of our children. The COVID-19 pandemic has caused changes in everyday life, including dietary pattern. Moreover, the restrictions imposed by lockdown have included not only social distancing but also reducing movements out of the house. Thus, physical activity was limited to what was absolutely necessary. Taking into consideration the alarming increase of weight gain in children and adolescents during the pandemics, the American Academy of Pediatrics recommends the assessment of BMI in every pediatric evaluation and to provide counseling concerning healthy diet and physical activity.[4]

So, we can assert that childhood obesity is one of the most serious public health challenges of the 21st century. The problem is global and is steadily affecting many low- and middle-income countries, particularly in urban settings.[2] The prevalence has increased at an alarming rate and so this epidemic is rapidly increasing the number of subjects who will experience type 2 diabetes and other related complications (hypertension, dyslipidemia, fatty liver disease) in young adults but also in pediatric age.

1.2 Diagnosis of obesity: key points

The term Obesity originates from the Latin "obesus" derived from "obedere", whose meaning is "to eat". Nutrition represents an important pathogenic element in its determinism, but it would be reductive to focus on obesity as a simple pathology caused by poor nutrition. Genes, food, environment are the basis of a complex interaction on the genesis of obesity: genes play a permissive role, interacting with external factors.[5]

Overweight and obesity are defined as an excessive fat accumulation able to impair human health. They represent increasing degrees of excess body fat in relation to the subject's lean mass.[6] So, it is easy to understand that measurement of body fat is a useful tool for the definition of nutritional state.[7] However, the diagnosis and definition of obesity cannot be separated from anamnestic and clinical evaluation. Medical history has some important following points: a) familial history of type II diabetes, hypertension and excess weight; b) excessive weight gain during pregnancy; c) neonatal history (small for gestational age or large for gestational age infants are at greater risk of developing excess weight in later ages); d) precocity of adiposity rebound, that is, the age at which the Body Mass Index begins to increase (if it occurs before the age of 5, the risk of developing obesity is increased); e) Breastfeeding and weaning period (early weaning and overeating with excess of protein intake promote obesity); f) the time of onset of excess weight; g) food history with evaluation of eating habits ; h) both programmed and spontaneous motor activity (lifestyle).

These key points summarize the current evidence, suggesting that several early-life factors significantly contribute to the development of obesity.[8] Particularly, the prenatal and infancy stages can be considered as the key steps in the determination of the individual risk for developing such condition. The concept of the "first 1000 days" has thereby been described throughout the most recent literature.[9] The period from conception to 2 years of age is considered the most critical for the induction of those pathophysiological derangements eventually leading up to childhood and then later-life obesity.[10]

1.3 Clinical evaluation

The quantification of fat mass can be achieved through the use of direct and indirect measurements: the former, such as Dual x-Ray Absorptiometry (DXA), although accurate, are not of routine use in pediatric age both for costs and both for the difficult reproducibility. Indirect methods include the evaluation of the Body Mass Index (BMI, Quetelet Index or Body Mass Index) calculated using the known formula:

$$BMI = \frac{Weight\ (kg)^2}{Height\ (m)^2}$$

The simplicity of measuring weight and height, the reproducibility of the same inter and intra-operator measurement, make BMI a useful and simple tool for clinical evaluation of excess weight: in children, BMI correlates well with the amount of body fat estimated through other adiposity indices.[11]

The WHO classification and the American guidelines for obesity in adults define overweight as a BMI of 25-30 kg /m^2 and obesity as a BMI of 30 kg/m^2 or higher.[2]

In the developmental age, the physiological variations induced by the increase in body weight and grow up trend, make necessary different values of reference in the various age groups and in the two sexes.[6]

The growth charts, with percentile table, are curves obtained from epidemiological observations that describe BMI trend in the different ages of life, in the two sexes, giving the opportunity to define obesity in children. The extrapolation of BMI cut-off for the diagnosis of obesity in adults (BMI = 30 kg/m^2) to the developmental age, gives the possibility to diagnose overweight and obesity in children. After BMI is calculated, it is expressed as a percentile for age and sex. Therefore, a child is defined as obese if his or her BMI percentile is greater than or equal to a cut off value that, for that age and for that sex, coincides with the value of 30 Kg/m^2 at age 18.

For children in the first two years of life Weight/Length growth charts are, instead, the right tool for nutritional status interpretation.

According to guidelines of Italian Society of Pediatric Endocrinology and Diabetology (ISPED), children and adolescents are diagnosed with obesity according criteria defined below:[12]

• Child up to 24 months: the diagnosis is based on the weight / length growth charts (WHO reference tables 2006) with the following threshold values:

overweight risk> 85th percentile

Overweight> 97th percentile

Obesity> 99th percentile

- Child from 2 to 5 years: the diagnosis of is based on WHO 2006 reference curves with the following cut-off values:

  Overweight risk> 85th percentile

  Overweight> 97th percentile

  Obesity> 99th percentile

- Child / Adolescent (5-18 years): the diagnosis is based on reference curves: WHO 2007

  Overweight> 85th percentile

  Obesity> 97th percentile

  Severe obesity> 99th percentile

In a small percentage of cases (about 2-3%) obesity can be defined as secondary. Early recognition of a secondary cause is critical, since in most cases, especially in the case of endocrine pathologies, adequate treatment of the primary pathology can allow the improvement of the condition and early management of other associated pathologies. In this case, obesity may be ascribed to a specific cause (endocrine,hypothalamic, genetic, iatrogenic). Therefore, clinical history, peculiar signs and symptoms must be accurately assessed such as: 1) onset of obesity before 5 years and/or rapid progression, especially in association with clues suggesting secondary causes (i.e. genetic forms); 2) continuous and/or rapid weight gain associated with reduced height velocity or short stature; 3) delayed cognitive development; 4) dismorphic features; and 5) use of drugs inducing hyperphagia (i.e. corticosteroids, sodium valproate, risperidone, phenothiazines, ciproeptadine). The clinical suspicion arises after a careful anamnestic, anthropometric and clinical evaluation.[12]

Main causes of secondary obesity are, as expressed in table 1:

• Endocrinopathies: hypothyroidism, hypercorticadrenalism (Cushing's and Cushing's disease), GH deficiency, pseudohypoparathyroidism, nesidioblastosis, insulinomas).

• Monogenic obesity: mutations of the leptin genes, leptin receptor, proopiomelacortin, prohormone convertase 1, melanocortin receptor.

- Syndromic obesities: Prader-Willi, Bardet-Biedl, Alström, Cohen, Carpenter and Borjeson-Forssman.

• Chromosomal disorders associated with obesity: Down, Klinefelter and Turner.

• Organic pathologies of the central nervous system: neuroendocrine alterations arising after cranio-cerebral trauma, post-neurosurgical injuries, meningitis / encephalitis, hypothalamic-pituitary tumors, craniopharyngioma, Rohhadnet.

• Drugs: corticosteroids, sodium valproate, risperidone, phenothiazines, cyproheptadine.

Endocrine causes
• Hypothyroidism
• Cushing disease
• Polycystic ovaries
• Growth hormone deficiency
• Hypothalamic obesity
• Hypogonadism
• Insulinoma
• Pseudohypoparathyroidism
Genetic causes
Monogenic obesity:
• Leptin and leptin receptor deficiency
• POMC deficiency
• Melanocortin Receptor 4 deficiency
• Prohormone convertase deficiency
• BDNF and TrkB insufficiency
• SIM 1 insufficiency
Syndromic obesity:
• Prader–Willi syndrome
• Bardet–Biedl syndromes
• Beckwith–Wiedemann syndrome
• Alstrom–Hallgren syndrome
• Carpenter syndrome

Table 1. *Endocrine and genetic causes of obesity*

1.4 Obesity and Endocrinopathies, Endocrinopathies and Obesity

The relationship between Obesity and Endocrinopathies is bidirectional. As we discussed, some forms of obesity are defined secondary, often related to endocrine pathologies such as Cushing Syndrome or Hypothyroidism.

Moreover, the same primary form of Obesity is cause of endocrine interferences, creating some disturbance on endocrine system. Obesity-related endocrine and metabolic manifestations represent the most common concurrent and/or long-term consequences of childhood obesity, and represent an important risk factor for atherosclerotic cardiovascular disease later in life. Obesity is associated with

increased secretion of adipokines, such as leptin, resistin, RBP-4, TNF-alpha, IL-6 and visfatin, and decreased secretion of adiponectin. These proteins are released into the systemic circulation, and they are involved in the pathophysiology of changes in insulin sensitivity and in Metabolic Syndrome pathway, as well as in food intake, energy consumption, neuroendocrine function, inflammation, immunity and altered vascular tone (Figure 1).[13]

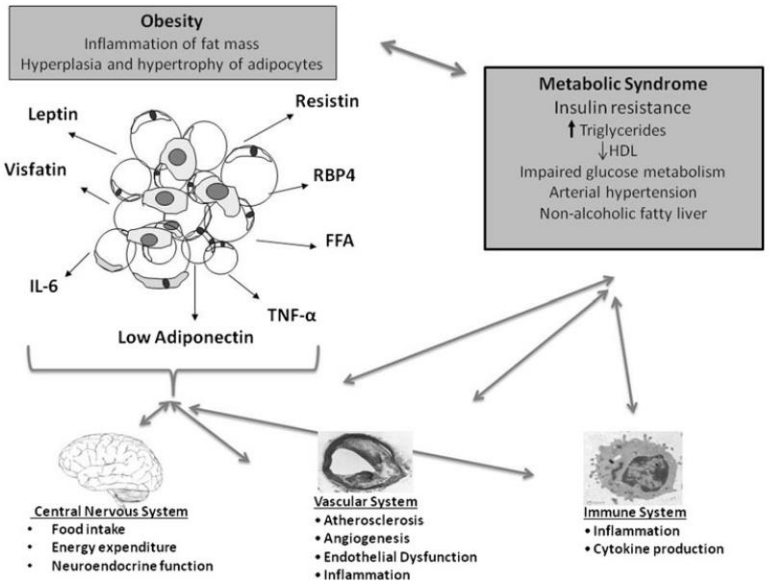


Figure 1 *Endocrine and metabolic role of adipose tissue*[13]

However, there are very common alterations in endocrine functions in obese children and adolescents which are a characteristic of the increased body fat mass. These alterations include disturbances of insulin secretion and insulin sensitivity, alterations in the adrenal gland function, alterations in the growth hormone somatomedin axis (GH, growth hormone, Insulin like Growth Factor, IGF) and the thyroid gland as well as gastrointestinal hormones. Secondary endocrine changes may facilitate further weight gain. The altered secretory profile of adipose tissue and also the single fat cell in the obese state is responsible for various metabolic and endocrine disturbances resulting in clinical end points such as type 2 diabetes, Non-Alcoholic Fatty Liver Disease (NAFLD), atherosclerosis and certain types of cancer. Most of these are created by the role of adipocyte, as an active cell responsible of the production of substances with autocrine, paracrine and endocrine effects. [13]

1.5 Fat mass: distribution and metabolic role

Various forms of adiposity are described in relation to the body area in which the adipose accumulation occurs: the subcutaneous adipose tissue, visceral adiposity and hepatic. The thickness and distribution of subcutaneous adipose tissue is influenced by various factors such as sex, puberty, nutritional status and hormonal status. In any case, a certain amount of fat in the subcutaneous area

is physiological. In fact, in case of deficiency (excessive thinness) important clinical pictures occur, including pubertal delay, amenorrhea in women and osteoporosis. The main factor secreted by the subcutaneous adipose tissue is leptin, a hormone capable of influencing appetite and gonadal function through its direct action at hypothalamic level.[14]

The role of leptin was first discovered in studies of severely obese ob/ob mice, which harbor mutations in the LEP gene resulting in a complete lack of circulating leptin; The leptin/melanocortin pathway plays a key role in the hypothalamic control of food intake. It is activated following the systemic release of the adipokine leptin (LEP) and its subsequent interaction with the leptin receptor (LEPR) located on the surface of neurons of the arcuate nucleus region in the hypothalamus.[15]

In pediatric age, conspicuous changes in the adipose tissue distribution are observed, and this correlates with the different phases of body growth and sexual differentiation.

In the first months after birth there is a rapid increase in body fat which reaches a maximum at 12 months of life. Subsequently, there is a slow and progressive reduction to a minimum reached at 6 years of age. In these phases, the dynamics observed in the two sexes are similar. After age of 6 years old, body fat growth is progressive in both sexes, but significantly greater in girls than in male peers. Adiposity rebound is, therefore, defined the age corresponding to the minimum BMI value before its increasing: if it occurs before the age of 5, the risk of developing obesity is augmented.[16]

All this fine mechanism is regulated by fine hormonal pathways. Adipose tissue can be divided into truncal region or peripheral region. Truncal adipose tissue includes subcutaneous fat in thoracic and abdominal region and also fat in intrathoracic and intraabdominal regions. Peripheral adipose tissue includes subcutaneous depots in upper and lower extremities. The association between regional fat distribution and cardio-metabolic complications was first suggested by Vague in 1947.[17] Vague described two patterns of adipose tissue distribution : *android* (upper body) and *gynoid* (lower body). He suggested that android obesity was associated with diabetes, coronary artery disease, gout and uric acid renal stones.[17,18]

Visceral fat is the part of adipose tissue concentrated within the abdominal cavity and distributed between organs. The accumulation of visceral fat is unequivocally associated with the risk of cardio-vascular complications and is one of the most important risk factors for type 2 diabetes. Intra-abdominal adipose tissue has increased metabolic (both lipogenesis and lipolysis) activity.

Free fatty acids (FFA), as a product of lipolysis, directly enter the liver via portal vein and lead to increased lipid synthesis, gluconeogenesis, and insulin resistance. This can result in hyperlipidemia, glucose intolerance, hypertension and atherosclerosis.[19]

Visceral fat adipocytes absorb glucose from the blood and release glycerol, free fatty acids and substances, such as adipokines, with local, central and peripheral effects. Through the release of these

substances, visceral fat "controls" appetite and consequently the energy balance, insulin sensitivity and lipid metabolism.

Adipose tissue should be considered an organ with endocrine functions responsible of secreting, in response to specific extra-cellular stimuli or changes in metabolic conditions, biologically active substances known, properly, as adipokines.[20] Among the adipokines secreted by visceral fat, a predominant role goes to adiponectin, whose levels are lower in obese subjects and which is able to improve insulin sensitivity and perform an anti-atherogenic and anti-inflammatory activity. [21]

Excess of visceral fat increases the release of adipokines with pro-inflammatory, pro-thrombotic activity and stimulation of triglyceride synthesis, all known as cardiovascular risk factors.[19] The adipokines released from visceral fat are directed into the portal venous system and from there transported to the liver.[19] Furthermore, free fatty acids act on the pancreas, compromising the functionality of beta cells (which produce insulin), and on muscles (inducing insulin resistance).

So, obesity and overweight are associated with a number of endocrine and metabolic changes, as described in table 2.[22]

The relationship between obesity and cardio-metabolic disorders, however, is more complex than it appears. The cluster of these metabolic and vascular complications is also called Metabolic Syndrome (MS) (hypertension, hyperlipidemia, fatty liver, atherosclerosis, type 2 diabetes).

Endocrine gland	Hormonal change
(1) Endocrine pancreas	Hyperinsulinemia
(2) Adipose tissue	Hyperleptinemia Adiponectin decrease
(3) Pituitary gland	Decrease in basal and stimulated GH Decreased response to prolactin stimuli
(4) Gonads	Women: decreased SHBG. Increased free estradiol and testosterone Men: decreased SHBG. Decreased total and free testosterone
(5) Adrenal glands	Increased free urinary cortisol and normal plasma cortisol
(6) Gastrointestinal hormones	Ghrelin decrease
(7) Thyroid gland	TSH and free T3 increase

Table 2. *Main endocrine changes in obesity*[22]

According to these observations, Visceral fat correlates directly with waist circumference which therefore assumes the role of primary cardio-vascular risk index becoming a fundamental measure in the assessment of body composition. Since waist circumference is a parameter strongly influenced by the patient's age, Waist to Height ratio (see methods) is considered a simple tool to identify

children at higher metabolic risk.[24] Central obesity and cardiometabolic risks have been closely associated with this ratio.[24]

1.6 Comorbidities associated with obesity

Obese child can develop short-term and long-term complications. Comorbidities associated with obesity, as previously reported, include metabolic (Insulin resistance and hyperinsulinism, type II diabetes, metabolic syndrome, hyperuricaemia), cardiovascular (hypercholesterolemia, hypertriglyceridemia, arterial hypertension, inflammatory and pro-thrombotic status) complications, endocrine (functional hypogonadotropic hypogonadism in males, early puberty in females, menstrual irregularities, polycystic ovary syndrome), acceleration of growth rate with untimely closure of the growth plates, gastrointestinal problems (Non Alcholic Fatty Liver Disease, cholelithiasis, gastro-oesophageal reflux, slowdown transit time), orthopedic and musculoskeletal (valgus knee, flat foot, scoliotic attitudes, Blount's disease, Osgod-Schlatter disease and femoral head epiphysiolysis), respiratory (sleep apnea) and psycho-social discomfort. [25,26]

1.7 Obesity and insulin resistance

Insulin is a peptide hormone composed of 51 amino acids coded on the short arm of chromosome 11 and synthesized in the pancreas within β-cells in the islets of Langerhans.[8] Insulin production in response to food intake is carried out in a rhythmic, two-phase fashion. The first phase (or quick secretion phase) begins within the first minute after food intake and reaches its maximum in 3-5 min. This phase lasts about 10 min and releases insulin that was already synthesized. The second phase (or slow secretion phase) starts 10 min after food intake. Secretion of insulin becomes apparent after 10 min of food ingestion. Duration of this phase is proportional to the time circulating glucose levels remain high. Under normal conditions this period extends up to 120-180 min. Insulin is an anabolic hormone that plays an essential role in carbohydrate metabolism by maintaining euglycemia.[27] Its main functions include glucose uptake of muscle and adipose tissue by favoring translocation of glucose transporter 4 (GLUT-4) to cell membrane, synthesizing hepatic and muscular glycogen, suppressing hepatic glucose synthesis, activating Na/K-ATPase pump in adipose and muscular tissue, synthesizing proteins, uptake of amino acids, and gene expression.[28,29,30]

Therefore, appropriate insulin sensitivity is based on the efficiency of this hormone to reduce glycemia by promoting glucose uptake by muscle and adipose tissue, increasing hepatic glycogen production and reducing hepatic glucose production.[30]

The term of insulin resistance (IR) describes a state of dysregulation of glucose-insulin homeostasis, characterized by a reduced ability of insulin to stimulate its actions in the liver, muscles and adipose

tissue. In particular, in condition of IR, a reduction in glucose consumption is observed at level of adipose and muscle tissue, while at the liver level there is a reduction in the suppression of glycogenolysis with increased gluconeogenesis, worsening the condition. Confirming that IR represents one of the most important factors in the pathogenesis of metabolic syndrome, Weiss et al. showed how the increase in IR is strictly related to the risk of MS in obese children and adolescents.[26] The most frequent metabolic alteration found in obese children is, in fact, IR.[31] This reduced response of the target tissues to insulin action leading to the need for higher plasma levels of the hormone (compensatory hyperinsulinemia) to obtain a normal biological effect.[32]

The activation of pancreatic beta cells allows an initial compensation to the insulin signal, but in the long time the compensation can be exhausted, due to the functional damage and stress condition of the beta cells, determining important changes in the glucose, lipid and protein metabolism.[33] A typical example of this occurrence is represented by type 2 diabetes mellitus.

The mechanisms that induce IR in the subject with excess weight are not yet completely understood; also in this case the pathogenesis would involve genetic factors and environmental mechanisms, this data would explain why some obese subjects develop IR and some others do not.[34,35]

Modulation of insulin sensitivity appears to be, at least in part, related to changes in redox balance and oxidative stress as well as inflammation, with a relevant underlying role for mitochondrial dysfunction that may exacerbate these alterations. [19,23]

Obesity is therefore a fundamental risk factor for the onset and development of insulin resistance, and IR, in turn, is the key in metabolic disturbance obesity related. It is thought that many of the metabolic alterations of obesity are caused by the increase in abdominal visceral fat, which causes an increased concentration of free fatty acids in the portal circulation and a reduction in hepatic insulin clearance, resulting in IR and hyperinsulinemia.[23]

Among possible causes of obesity-induced metabolic derangements, adipose organ dysfunction and altered adipose metabolic processes clearly play a fundamental role.[36] Oxidative stress resulting from imbalanced reactive oxygen species (ROS) generation and antioxidant defenses as well as chronic inflammation in non-adipose tissues are also major regulators of insulin sensitivity; in particular, elevated pro-inflammatory stimuli may be enhanced by excess ROS and directly impair insulin signaling in insulin target tissues.[36,37]

Nutrients and substrates as well as systems involved in host-nutrient interactions, including gut microbiota, have been also identified as modulators of metabolic pathways controlling insulin action.[23] Complex systemic networks including responses to nutrients and substrates, the gut and its microbiota are also key players acting by regulating substrate utilization and balance, thereby modulating oxidizing and inflammatory stimuli.[38,39]

1.8 Adiposity dysfunction

It is largely demonstrated that adipocytes secrete several hormones (collectively defined as adipokines or adipocytokines) with a large array of biological effects on metabolism, inflammation and hemostasis, thereby modifying the classical and currently obsolete view of adipose tissue as an inert fat storage tissue.[23,37]

The visceral obesity phenotype predisposes to a condition of chronic inflammation, as reported above. The age range between birth and 6 years could be very important for determining the inflammatory state associated with obesity in adulthood.[40,41] Here, pre-adipocytes, stimulated by dietary fatty acids and intestinal-derived substances (for example the lipopolysaccharide derived from gram-negative intestinal bacteria), that activate the inflammatory cascade by binding the "Toll-Like Receptors" of the adaptive immune system, can express pro-inflammatory molecules, differentiate into adipocytes, and induce IR of contiguous adipocytes. In this process, TNF-alpha and interleukin 6 appear to be the cytokines that mainly mediate inflammation and IR.[42]

The condition of chronic low-grade inflammation and IR of visceral fat quickly becomes systemic, thus causing endothelial dysfunction and promoting the formation and consequent rupture of atherosclerotic plaques. Obese children have very early infiltration of macrophages in the adipose tissue in the perivascular site.[43]

The degree of loco-regional inflammation is directly proportional to the degree of generalized obesity, as macrophages, granulocytes and lymphocytes infiltrate the adipose tissue.[44] It has been found that persistent high insulin levels are associated with an increased cardiovascular risk in young adults and that there is a correlation between IR and endothelial dysfunction.[45]

Adipocyte oxidative stress has been demonstrated both in vivo and in vitro to lead to secretion of metabolically harmful adipocytokine patterns. Of note, in classic studies adiponectin expression was lower and IL-6 expression was increased in adipocytes after induction of oxidative stress; antioxidant treatment notably reversed these changes.[46] Dysregulated production of "offensive" adipocytokines, such TNF-α, IL-6, MCP-1, and angiotensinogen, and of "defensive" adipocytokines, such as adiponectin and leptin , is critically involved in the pathogenesis of metabolic syndrome.[46] Fat accumulation closely correlated with the markers of systemic oxidative stress.

Hyperinsulinemia causes an increase in the hepatic synthesis of triglycerides, inducing in turn hyperlipidemia, and through various mechanisms, including an increase in renal sodium reabsorption and an increased activity of the sympathetic system, contributes to the onset of hypertension. These metabolic alterations, which have also been demonstrated in obese children, lead to an increased metabolic risk. [40,46]

1.9 Visceral obesity and cardiovascular risk

Childhood obesity is a condition that carries an increased risk for developing cardiovascular diseases in adulthood, but lot of comorbidities obesity-related are described also in children, many involving cardiovascular system, such as arterial hypertension or initial signs of atherosclerosis.

It is known, in fact, that atherosclerotic disease has its origins in the pediatric age and that alterations in plasma lipid concentrations play a fundamental role in this condition. The alterations observed post-mortem in the coronary vessels and in the aorta of pediatric subjects are represented by lipid striae, consisting of foam cells (containing lipids) and extracellular lipids; these changes are also present in the neonatal period. In 5-10% of children between the ages of 2 and 15 years, fibrous plaques are yet present in the coronary arteries. [48]

The mechanisms that trigger vascular damage in obese children have not yet been fully disclosed. A mechanism that plays a key role in the atherosclerotic process is, once again, systemic inflammation. Indeed, serum C-reactive protein levels were found to be elevated in obese children.

The visceral obesity phenotype predisposes to a condition of chronic inflammation compared to an obesity phenotype with fat distribution in other locations.

Moreover, early clinical manifestations in obese pediatric subjects are dyslipidemia, hypertension, IR and diabetes, which represent risk factors for MS and, in a broader sense, for cardiovascular diseases.[49]

In obese subjects, even in pediatric age, the increase in adipose mass is complemented by alterations in glucose and lipid metabolism and this induces IR and consequent increase in circulating levels of insulin.[11] These metabolic alterations, which have also been demonstrated in obese children, lead to an increased risk of developing cardiovascular diseases, including coronary heart disease.[50]

It has been found that persistently high insulin levels are associated with an increased cardiovascular risk in young adults and that there is a correlation between IR and endothelial dysfunction; these observations suggest a key role of IR, once again, in the pathogenesis of atherosclerosis.[50,51]

Hyperinsulinemia and IR may already be present in obese prepubescent children, but their prevalence increases in adolescence. [51,52]

In obesity there are also alterations in endocrine functions that can contribute to the onset of cardiovascular risk. The increase in the production of some adipokines has an impact on multiple functions, such as immunity, insulin sensitivity, blood pressure and lipid metabolism, all altered in cardiovascular disease.[51,52] On the other hand, in obesity there is a reduction in the production of adiponectin, an adipokine with important anti-atherogenic and antidiabetic properties whose serum level is inversely proportional to the visceral fat mass and directly related to insulin sensitivity.[51]

A recent theory, developed that obese subjects have different gut microbiota compared to lean subjects, supports the concept that habits and the type of diet, rich or not in saturated fatty acids and refined carbohydrates, can predispose to obesity.[53]

The microbiota of the host develops during the first year of life and the type of breastfeeding or other type of feeding significantly modulates the relationship between intestinal bacterial species and the production of short-chain fatty acids that act as substrates for lipogenesis and gluconeogenesis. In the years, gut microbiota undergoes changes acquiring those characteristics that the subject will keep for his entire life.[53] Experimental evidence also suggests that bacterial flora can modulate host lipogenesis, favoring the accumulation, even in adequate or ectopic sites, of fat.[54,55] The gut microbiota collaborates with the host to accomplish many physiological activities including the improvement of enteric digestion and absorption of nutrients, the defense against colonization by pathogen microbes, the maintenance of the gut barrier, the modulation of inflammation and of the entero-hormones, the promotion of immune system maturation, and the influence on the formation of capillary networks in the small intestinal mucosa. Obesity is associated with a reduction in Firmicutes/Bacteroidetes ratio.

A number of gut hormones called incretin system, which include Glucagone like peptide 1 (GLP1), Glucose-dependent insulinotropic hormone (GIP), and Oxyntomodulin, are central to metabolism control. The incretin hormones stimulate glucose dependent insulin secretion and sensitizing pancreatic beta cells to glucose, which result in a modulation of post prandial glucose excursion

So, all of these alterations contribute to the condition of chronic low-grade inflammation and IR of visceral fat quickly becomes systemic, thus causing endothelial dysfunction and promoting the formation and consequent rupture of atherosclerotic plaques.[56] Obese children have very early infiltration of macrophages in the adipose tissue in the perivascular site.

1.10 Obesity and NAFLD

The term Non Alcholic Fatty Liver Disease (NAFLD or MAFLD, as in the very last period the condition is interpreted as metabolic dysfunction associated fatty liver disease) refers to a broad spectrum liver disease, whose histological characteristics range from the accumulation of fat> 5% in adipocytes (simple hepatic steatosis), to the evolutionary form of the disease (non-alcoholic steatohepatitis, NASH), characterized by the presence of necrosis plus inflammation, sometimes associated with hepatic fibrosis, which can progress, even in adolescence, towards a picture of cirrhosis. Although initially considered a two-stage disease, now it is agreed that NAFLD is a multi-etiopathogenetic disease, in which multiple factors (genetics, epigenetics, excessive calorie intake,

poor physical activity, consumption of junk food) contribute to promote the onset of the disease and its progression.[57]

It has been observed that an increase in circulating free fatty acids is associated with an altered metabolism of fatty acids in the hepatocytes, thus determining a net accumulation of triglycerides in the liver. In turn, the accumulation of fat in the liver acts on insulin resistance by interfering with the phosphorylation of the substrates of the insulin receptor. The factors responsible for the progression of steatosis towards the more severe lesions of steatohepatitis and fibrosis are still poorly defined.[58]

Fatty Liver associated with metabolic dysfunction is a common condition. Hepatic steatosis can in fact evolve into hepatic fibrosis at least through three different molecular pathways:[59,60]

- oxidative stress and the consequent lipid peroxidation;
- the production and release of pro-inflammatory and profibrotic adipokines and / or the reduction of adiponectin levels;
- an increased synthesis of leptin resulting in fibrogenesis stimulation through direct stimulation of hepatic stellate cells or by paracrine effects on sinusoidal endothelial cells.

All these scientific evidences support the hypothesis of multiple insult in the pathogenesis of NAFLD / NASH and indicates that often some of the factors responsible above are linked together and together are involved in worsening of metabolic disarrangements of obesity.

The true prevalence of pediatric NAFLD remains unknown. Population studies conducted so far report a wide variability in the prevalence of the disease.[61]

As described for adults, children with NAFLD also tend to have notes of systemic metabolic impairment, such as increased abdominal circumference, arterial hypertension, IR, dyslipidemia, all of which increase the risk of developing type 2 diabetes and cardiovascular disease. The most recent evidence, in fact, now tends to consider NAFLD as the hepatic manifestation of metabolic syndrome.[62]

1.11 Obesity, Metabolic Syndrome and Cardiovascular Disease

Metabolic Syndrome (MS), in past time known as X Syndrome, summarizes in itself a series of metabolic abnormalities such as hyperglycemia, hypertriglyceridemia, hypertension, low plasma HDL cholesterol levels and abdominal obesity, such as to predict the risk of developing type 2 diabetes mellitus and cardiovascular disease.[63]

Longitudinal studies have shown that adults with MS already presented cardiovascular risk factors in childhood, this justifies the need to research the syndrome an to pay attention to it in developmental age.[64]

Obesity is a predisposing factor: the prevalence of MS in pediatric age increases with the degree of obesity.[65] In addition, each element of the cluster of the syndrome would worsen with increasing degree of obesity, independently by age and gender.[66,67]

It has been suggested that the dysregulation of circulating levels of adipokines could represent the link between obesity and MS.[68] In particular, leptin and adiponectin are indicated by the International Diabetes Federation (IDF) biomarker of MS: many evidences have pointed out as hyperleptinemia and hypoadiponectinemia are associated with the occurrence of MS and type 2 diabetes mellitus.[69,70]

At present, the diagnosis of pediatric MS does not meet unanimous consensus due to the difficulty of defining the cut-off values of the various components at that time.[71] Some considerations limit their definition in Pediatrics: the transient physiological IR of the pubertal period and the wide variations of the lipid structure in the various ages, in the two sexes.[72]

However, even though there is no international commonly used definition of the metabolic syndrome in children and adolescents, all definitions include obesity as precondition for the development of MS even in children.

The diagnostic criteria for pediatric MS generally represent an adaptation of those used for adults, it is known that an early diagnosis and successful treatment are the cornerstones for the reduction of morbidity and mortality related to MS.[73]

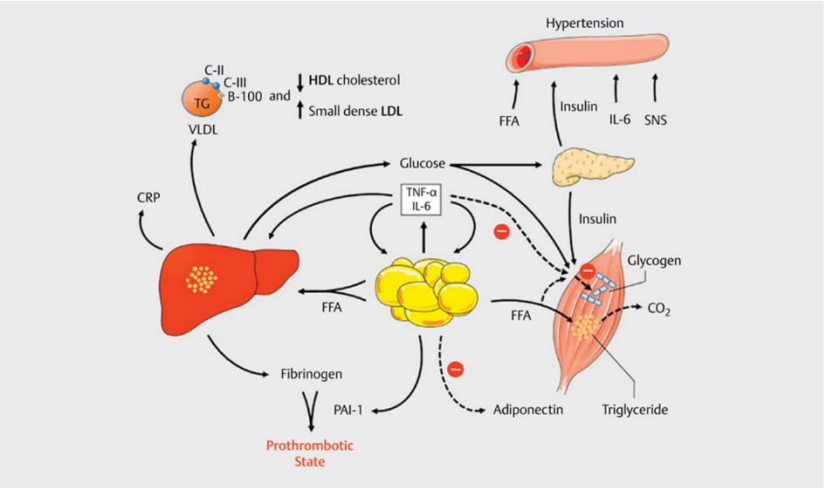
Actually, the pathogenesis of MS still remains poorly understood, due to multifactorial pathophysiology including causes of both genetic and environmental origin. For example, some ethnic groups (Hispanics and South Asians) appear to be more susceptible. On the contrary, the black population has a lower incidence of this syndrome than the white one.

The difficulty in studying the pathogenetic mechanisms of this condition is justified by the heterogeneity of the different phenotypes, determined by the different combination of the factors indicated as diagnostic and by the different definitions reported in the literature. The World Health Organization hypothesizes that IR is the key and driving factor in the development of MS. In states of IR, metabolic dysfunction across several organs occurs, creating the observed interplay of several concurrent metabolic abnormalities. [74]

So, the syndrome results from the complex interaction between genetic and environmental factors. Its pathophysiology is multifaceted, and the complex interaction between the many factors is still unclear.[75]

Although obesity and central fat accumulation are major risk factors, individuals with a normal or slightly overweight body mass index (BMI) may also present IR. On the other hand, central fat deposition has been recognized as highly damaging metabolically and continuously associated with IR and cardiovascular diseases.[76] The dysfunctional adipose tissue associated with VAT results in

increased levels and fluxes of plasma FFA, which in turn leads to ectopic lipid deposition and lipotoxicity. The chronic exposure to high circulating levels of FFA plays a crucial role in overall cellular dysfunction of the liver, pancreas, and skeletal muscle, with a cascade of events well described in figure 2, with a detrimental effect on health.[76]



Proposed Metabolic Syndrome Pathophysiology. CRP, C-reactive protein; HDL, high density lipoprotein; FFA, free fatty acids; IL-6, interleukin 6; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor 1; SNS, Sympathetic Nervous System; TG, triglyceride; TNF α, tumor necrosis factor α; VLDL, very low density lipoproteins.

Figure 2. *Pathophysiology of MS*[76]

So, the pathophysiological basis of MS can be enclosed in IR, which is often associated with the presence of visceral adiposity. In fact, the ability of visceral adipose tissue has been highlighted to interfere with the renin-angiotensin-aldosterone system, to produce adipokines (first of all, leptin and resistin), pro-thrombotic molecules (PAI-1) and pro-inflammatory cytokines (TNF-α, IL -1 and IL-6), which could have a fundamental function in the pathogenesis and perpetuating of IR. Visceral adiposity acts as a toxic / inflammatory area, altering insulin signaling at the receptor and post-receptor level, through the release of mediators, such as free fatty acids, TNF-α, IL-6, resistin, with the establishment of a vicious circle between adipose tissue and IR.[76,77] Furthermore, children with MS have increased levels of inflammatory markers, which counteract the peripheral effects of insulin.[78] In fact, a mechanism that plays a key role in the atherosclerotic process is the condition of systemic inflammation. Prospective studies conducted in adults have shown that the increase in blood levels of C-reactive protein has a predictive value of cardiovascular damage.[79]

Therefore, an increase in abdominal fat tissue can increase the risk of developing MS in people who are metabolically susceptible to this condition.

In addition, overweight and obesity, together with some morbidities associated with MS (lR, type 2 diabetes and dyslipidemia), represent risk factors for the development of NAFLD. Many authors

therefore suggest that NAFLD is the hepatic manifestation of MS, and that IR is the key element in the relationship between the two diseases.[62]

1.12 GH- IGF-1 and IGFBP-3

Growth Hormone (GH), Insulin-like growth factors (IGF-1) and their associated binding proteins (IGFBPs) play important roles in normal development and growth. The human GH gene cluster is located on chromosome 17 and it includes five GH variants, among which is the pituitary GH-N (or GH-1) variant and four placental GH-V (or GH-2) variants.[80]

GH is the product of GH-N gene which is primary expressed in the somatotropic cells of the pituitary gland, but also in other tissues. The major gene product is a peptide of 191 aminoacids, there is not only one natural GH, because due to alternative splicing, a shorter form is also produced which accounts for 10-20%. GH is primarily expressed, synthesized, stored within secretory granules in the somatotropic cells located in the anterior pituitary gland. Transcription of the GH gene is regulated by several transcription factors including Pit-1 (pituitary-specific transcription factor-1), Sp1 (specificity protein1), activator protein 2, nuclear factor-1, and upstream stimulating factor. Glucocorticoids have been shown to increase GH transcription as well as mRNA stabil ity; while thyroid hormone suppresses GH transcription.[80]

Its pulsatile secretion pattern reflects the interplay of multiple regulators, such as GH-relasing hormone and somatostatin. However, the name of Growth Hormone is limiting. Actually, GH has multiple actions on metabolism, in particular on anabolic and lipolytic pathways. The main target of GH action is the liver, where it stimulates Insulin Growth Factor 1 (IGF-1, somatomedin C) production. GH is an anabolic hormone that has important functions in regulating somatic growth either directly or indirectly via effectors such as insulin-like growth factor-1. How-ever, another facet of the biological effects exerted by GH includes its ability to modulate metabolism and energy homeostasis. The metabolic actions of GH are diverse and tissue-specific.

1.13 GH-IGF-1-IGFBPs: physiology and metabolic effects

Insulin-like growth factor I (IGF-1), also known as Somatomedin-C, is a 7.5 kDa peptide, structurally homologous to pro-insulin, so it' clear its name derivation.

It is mainly synthesized by the liver, under the stimulation of the Growth Hormone (GH), mediating most of its actions. It is physiologically similar to insulin, but it is a much more powerful mitogenic and anti-apoptotic agent. The action of IGF-1 is modulated by specific, high affinity binding proteins. In fact, most of IGF-1 circulates in the blood linked to IGF binding proteins (IGFBPs).[81] The main of these is IGF binding protein-3 (IGFBP-3), which therefore reduces the bioavailability of IGF-1.

IGFBP-3, and also IGFBP-5, after binding to IGF-1, join a third protein, the acid-labile subunit (ALS), forming a ternary complex with a high molecular weight (150 kDa). This complex prolongs the half-life of IGFs, from 10 minutes of the free form and from about 30-90 minutes of the binary complexes (IGF-IGFBP) to about 16 hours, also preventing their clearance and therefore modulating their action. Only a small fraction of IGF-1, about 2%, circulates as free, in an active form.

The relationship between IGF-1 and IGFBP-3 gives us a reliable estimate of the concentration of free and, therefore, bioavailable IGF-1. The dosage of IGFBP-3 can be used as a substitute for free IGF-1. Concentrations of IGF-1, ALS and, to a lesser extent, IGFBP-3 are mainly regulated by GH. However, it should not be forgotten that also genetic and nutritional factors, age and the concentrations of other hormones, such as thyroid and sex hormones and cortisol, as well as some cytokines (IL1, IL6, TNF), contribute to the regulation of circulating levels of IGF-1 (Table 3).[83]

IGF-1 has insulin-sensitizing effects. As discussed, It has a remarkable homology of structure with the insulin to which receptors is able to bind and, on the metabolic level, exerts insulin-like and hypoglycemic effects, opposite to those of GH. The action of IGF-1 is modulated by the degree of binding with the different transport proteins and is further regulated by the concentrations of some proteases capable of cleaving IGFBPs.

- Nutritional Status
- GH
- Immune System
- Genetic Factors
- IGFBPs
- Insulin
- Sexual Hormones
- T4
- Cortisol
- Age

Table 3. *Factors regulating serum IGF-1 concentration*[83]

The main action of GH, mediated by IGF-1, in children is linear growth, for its action on plates of the long bones. IGF-1 stimulates protein synthesis; this process is particularly evident in the muscles and in the growth plates, where it stimulates chondrocytes to cell division, causing bone growth and height-increase in children. GH, through IGF-1, stimulates protein synthesis but contemporary reduces proteolysis at the level of skeletal muscle.[85,86]

However, GH also has a lipolytic effect, favoring the release of FFA, an energy source for metabolism, that allows to save proteins, which are thus usable in the growth process.[83]

The hormone also influences carbohydrates metabolism, stimulating gluconeogenesis. On the other hand, IGF-1 can play itself a role in glucose homeostasis, promoting glucose uptake and improving insulin sensitivity. Therefore, IGF-1 and GH presents double and opposed effects on glucose metabolism according to physiological on not physiological levels. In fact, the chronic excess of GH (and also of IGF-1) has a diabetic effect, since it reduces the entry of glucose into the cells and its use, generating IR and insulin hypersecretion.

Several data support the thesis that IGF-1 deficiency increases IR, alters lipid metabolism, promotes oxidative damage and disrupts the neuro-hormonal axis.[83,84] According to these observations, it' clear the importance to observe this axis in obesity.

Experimental studies on mice have shown that the partial deficiency of IGF-1 alone is sufficient to reduce the hepatic expression of genes involved in lipid and glucose metabolism, leading to dyslipidemia and hyperglycemia.[85] Low levels of IGF-1 have been linked to the development of type II diabetes. As discussed, IGF-1 has opposite effects to GH on glucose metabolism, because it reduces the hepatic production of glucose (probably by binding to hepatic insulin receptors) and stimulates the uptake of glucose by the muscle.[86] Therefore, unlike GH and in physiological range concentrations, it improves insulin sensitivity and both insulin and IGF-1 have an antilipolytic effect on mature adipocytes (an effect probably mediated by insulin receptors for both ligands).

The insulin sensitizing effect of IGF-1 can be partially explained by its remarkable homology of structure with the insulin to which receptors it is able to bind. Therefore, on the metabolic level, it exerts insulin-like and hypoglycemic effects, opposite to those of the hyperglycemic GH. However, it is a much more powerful mitogen and anti-apoptotic agent than insulin. In particular, IGF-1 receptor (IGF-1R) binds the IGF with high affinity, just as the insulin receptor binds insulin with high affinity; however, since both receptors share a high degree of structural and functional homology, IGF-1R can bind insulin and the insulin receptor can bind to IGF-1 with less affinity. [87]These two receptors, which are expressed differently in different tissues, can also form heterodimers, which bind both ligands. The insulin receptor is highly expressed in the liver, muscle and white adipose tissue in adults, while IGF-1R is poorly present in the liver and white adipose tissue, while in muscles it is expressed in a manner comparable to the insulin receptor.[88] The action of IGF-1 is also modulated by the degree of binding with the different transport proteins and is further regulated by the concentrations of some proteases capable of cleaving IGFBPs.[89,90]

IGF-1 seems to have, indeed, a functional relationship with the adiponectin produced by adipose tissue, providing a link between obesity and the risk of metabolic syndrome.[90] It seems to have protective effects against inflammation and IR, thanks to its ability to improve the carbohydrate and lipid profile. [91]

Several studies have found a correlation between reduced levels of adiponectin and IGF-1 and IR, hyperinsulinemia, DM2, obesity and MS.[92]

Small adipocytes produce insulin-sensitive hormones, adiponectin, leptin and other hormones. The hypertrophy of these cells (large adipocytes), induced by obesity, causes a decrease in the production and secretion of the insulin-sensitive hormone and increases the insulin-resistant one, leading to an IR in obesity. Furthermore, TNF-α, which is increased in the visceral adipose tissue of obese subjects, appears to inhibit adiponectin production.[93] Adiponectin is well known for its anti-.inflammatory effects through the inhibition of IL6 production and the induction of the anti-inflammatory cytokines such as IL10 and the IL1 receptor antagonist. In addition, adiponectin reduces the induction of endothelial adhesion molecules ICAM-1 and VCAM-1, asserting a protective role in atherosclerotic processes.[94] So the relation between IGF-1 and adiponectin could partially explain some important effects of somatomedin C: one of this is, for example the role in aging processes. Likewise, lower serum IGF-1 levels were associated with protection from cognitive impairment.

Furthermore, the local production of IGFBPs can act in an autocrine or paracrine manner, regulating the action of IGF-1 in different tissues. In vitro and in vivo, studies suggest an independent action of the IGFBPs, as a modulator of GH. For example, IGFBP-3, like GH, has opposite effects to IGF-1 on glucose metabolism, increasing the circulating levels of glucose and insulin.[95]

1.14 GH and Obesity

The GH/IGF- 1 axis shows typical changes in obese growing children as showed in table 4.[96]

Impaired GH secretion in stimulation tests
Increased circulating levels of GH- binding protein
Increased circulation levels of IGF- 1, IGFBP- 1, IGFBP- 2, IGFBP- 3 in the pre- and intrapubertal period
Reduced levels of IGF- 1, IGFBP- 1, IGFBP- 2, IGFBP- 3 in the postpubertal stage
Reduced ghrelin secretion

Table 4. *Typical changes of the GH/IGF- 1 axis in overweight and obese children and adolescents*

The accelerated prepubertal longitudinal growth rate is followed by a slightly reduced pubertal growth spurt. Several cohort studies have demonstrated that growing up in an obese state will lead towards an impaired final height. In obese children there was a negative correlation between the increase in BMI and peak height velocity.[96]

Endogenous and post-stimulus GH production seems to be reduced in obesity. GH secretion in obese children may be as low as in poorly growing GH- deficient children. Despite normal growth obesity is characterized by a reduced GH secretion in provocative tests, GHRH tests and 24- hour secretion profile. It has been shown that the half- life of endogenous GH is shorter in obese individuals and that there are fewer GH secretory bursts than not obese children.[97] The impaired central GH secretion rate is mainly due to a reduction in pulse amplitude and less to a reduction of pulse frequency.

Factors that might be involved in GH- independent growth in obese children comprise leptin, adrenal androgens, GH- binding protein, IGF- 1 and IGFBP- 1, and insulin. Leptin may contribute to a stimulation of growth by its permissive role for puberty induction.[96,98]

Although the underlying mechanism is not fully known, clinical data indicate that the endogenous secretion of GH is markedly reduced in visceral obesity, compared to controls. In addition, the response to provocation tests for the dosage of GH, such as the gold standard insulin tolerance test, or moreover the maximal test GHRH + arginine, is reduced. The mechanisms underlying GH reduction include: alteration of GHRH pathway, somatostatin and ghrelin stimuli; but although the key role of GH impairment is principally due to metabolic alterations associated with obesity, such as hyperinsulinemia and excess FFA.[100] On the other hand, Ghrelin is a strong GH secretagogue and it stimulates GH secretion in a GHRH- independent way via the pituitary secretagogue receptors Ghrelin consists of 28 amino acids and is secreted from neuroendocrine cells of the gut found mainly in the fundus of the stomach as well as in the nucleus arcuatus of the hypothalamus. Ghrelin secretion in the gut is increased in the fasted state and is significantly reduced in the postprandial state.[103] Within the gut, ghrelin stimulates motility and the production of acid secretion. Ghrelin has multiple effects and exerts its orexigenic action in the hypothalamus by stimulating the Agouti related peptide/neuropeptide Y neurons and inhibiting the anorectic Proopiomelanocortin/Cocaine and amphetamine-regulated transcript neurons in the arcuate nucleus. Ghrelin plasma levels are elevated during fasting and in conditions related to malnutrition like cachexia and anorexia nervosa, while, plasmatic Ghrelin is reduced in obesity and in insulin resistance, T2DM, and hypertension. Ghrelin is a type of GH secretagogue that stimulates the release of GH. It is a first hormone linking gastrointestinal-pituitary axis. So, there is tan important link between the regulation of energy homeostasis and the activity of the GH/IGF- 1 axis in different, and not fully understood, ways.[104]

Obese subjects with low GH levels present changes in body composition (reduced muscle mass and increased visceral adipose tissue) and cardiovascular risk, with cardio-metabolic sequelae, significantly worse metabolic profile than obese subjects without alterations of the somatotropic axis.[99]

It is suggested that the reduced levels of circulating GH in obese result in a reduced lipolytic activity in adipose tissue and a reduced rate of protein synthesis in muscle tissue.[100] The same higher levels of circulating free fatty acids in obesity are able to reduce the GH response after application of GHRH stimuli, through a negative feedback mechanism.[101,102,105]

Hyperinsulinemia and IR of obesity are also reported as a contributing cause of GH reduction, presumably through the stimulation of the hypothalamic release of somatostatin, among the others. So, FFA levels, the chronic state of excess leptin and insulin, the chronic low grade inflammation are contributing factors of altered GH secretion, and this defective production seems to be .proportional to the degree of obesity.[100]

So, this condition creates a vicious circle.

For this reason, the low levels of IGF-1 could play a role in amplifying the cardio-metabolic risk of obese individuals.

1.15 IGF-1 and Obesity

Despite the unequivocal effects of obesity on GH, the effects on IGF-1 are controversial. While some studies have shown reduced serum levels of IGF-1 in the obese, other studies have not shown different concentrations of IGF-1 in obese subjects, compared to those of normal weight; however, they still found higher free IGF-1 concentrations in the former.

This discrepancy may be secondary to variations in: IGFBPs, bioactive IGF-1 and/or hyperinsulinemia associated with obesity. Although many tissues produce IGF-1, it is now recognized that the hepatocyte is the primary source of circulating IGF-1; this could be an important link between IGF-1 and liver. Although a metabolic role for IGF-1 is well accepted, there are several challenges in regards to understanding how IGF-1 mediates these effects. In the following sections, we tried to discuss about this intricate role.

1.16 GH/GF1 and Insulin Sensitivity

The link between nutrition and growth is likely due to a complex interaction between insulin, GH, IGF-1 and IGFBPs.[206] The structural homology between IGF-1, insulin and the hypoglycemic activity regulated by IGFBPs suggest that IGF-1 and its transport proteins may have an intrinsic role in glucose metabolism and its homeostasis.

The infusion of recombinant IGF-1 leads to a rapid reduction in blood sugar and insulinemia.[107] Transgenic animal experiments have shown that hepatic inactivation of the IGF-1 gene leads to a marked decrease of 75% in the concentration of IGF-1, hyperinsulinemia and IR, with impaired insulin signaling in the musculoskeletal tissue.[108] These transgenic animals also show hypersomatropinemia, suggesting that IR could be secondary to the increased concentration of GH.[108,109] Therefore, IGF-1 can also indirectly contribute to the regulation of insulin sensitivity, through the suppression of the secretion of counter-regulatory hormones, such as GH (negative feedback) and glucagon, with enhancement of insulin action.[110] The infusion of IGF-1 has the effect of oxidizing lipids and can act directly on skeletal muscle, through the IGF-1 receptors.[110]

Nam et al. have shown that free IGF-1 is increased in chronic IR conditions, such as obesity; however, visceral adipose tissue, rather than adiposity per se, was inversely related to the total circulating IGF-1.[111] However, the biological activity of IGF-1 measured as bioactive IGF-1, a method that considers the complex interaction between IGF-1, IGF-2, IGFBPs, protease and the IGF-1 receptor, may not be reduced in obese subjects. [112] This observation may be attributable to the compensatory changes in IGFBPs, mediated by insulin.

In mice, low IGF-1 concentrations have been shown to be accompanied by high insulin and serum lipid levels.[113] Sesti et al. have identified IGF-1 as an IR marker: the concentration of IGF-1 correlates positively with insulin sensitivity, assessed with the HOMA.[114] Studies conducted in patients with extreme IR and type 2 diabetes have shown how glucose tolerance and insulin sensitivity can improve more than three times after recombinant IGF-1 infusion.[115] Similarly, administration of recombinant IGF-1 in patients with type 1 diabetes resulted in a notable reduction in insulin requirements, with no changes in nocturnal pulsatile secretion of GH.[116]

The mechanism by which low IGF-1 concentrations can be associated with IR is unclear. IGF-1 has hypoglycemic effects and increases insulin sensitivity in both humans and experimental animals. The biological activity of IGF-1 could be mediated through type 1 receptors and / or hybrid insulin / IGF-1 receptors. Hybrid receptors are widely distributed in different tissues, including skeletal muscle and adipose tissue. They act as receptors for IGF-1, rather than for insulin. The increased proportion of hybrid insulin / IGF-1 receptors could reduce sensitivity insulin in the tissues on which insulin acts, contributing to cellular IR. In obese subjects, in whom there is an increase in insulin and a reduction in serum IGF-1, the expression of hybrid receptors correlates with the reduction of insulin receptors and the increase of those for IGF-1, which could contribute to reduced insulin action, due to the use of insulin receptors in these unresponsive forms.[117]

1.17 GH/IGF-1 and Metabolic Syndrome

There is several evidence that IGF-1 and IGFBP-3 play a role in many components of the metabolic syndrome (MS).[118] In a German study, analysis of the adult population on cross-sectional sampling revealed a higher prevalence of MS, associated with higher concentrations of IGFBP-3 and a low IGF-1 / IGFBP-3 ratio. [119] On the other hand, also high concentrations of IGF-1 constituted a risk factor for the incidence of MS. [119] These data suggest how IGF-1 could play a role in the development of glucose intolerance. The mechanism by which IGF-1 and IGF-1 / IGFBP-3 can cause an increased risk of MS is still unknown. There are several reasons that could explain the changes observed in the prospective association between IGF-1 and MS.[120]

In recent years, several studies have been carried out on adults i that correlate IGF-1, IGFBP-3 or IGF-1 / IGF-BP3 to MS. [123]

In particular, IGF-1 is significantly lower in adults with higher clinical-laboratory parameters such as: abdominal circumference, triglycerides, blood pressure, fasting blood sugar; low HDL-cholesterol or diabetes.[123] The reduction of IGF-1 is directly related to the number of features of MS presented. [123,124]

A close association between low levels of IGF-1 and the pathogenesis of type 2 diabetes, cardiovascular diseases and chronic inflammation has been described in literature.[125]. Low serum levels of IGF-1 have been found in subjects at increased cardiovascular risk and elevated levels of IGBP-3 in subjects predisposed to developing IR.[126] Therefore, the IGF-1 / IGFBP-3 ratio could represent a balance between cardiac and metabolic risk, especially for MS and in IR state. [122]

C-reactive protein (CRP), a marker of subclinical inflammation in MS and a risk factor for cardiovascular diseases, is also negatively correlated with the concentration of IGF-1.[127]

IGF-1 concentration changes with age: very low at birth (20-60 ng /ml) peaks at the completion of pubertal development (7 times the levels of birth) and subsequently a slight and steady decline (at age 60 , the level is ¼ of the pubertal peak). [128,129] On the other hand, within the population, the levels of IGF-1, genetically determined, are distributed log-normally, with differences of more than three times between the 5th and 95th percentiles, within the same age group.[130] Reduction of IGF-1 with age could be an additive association between IGF-1 and MS cluster.[131,132]

1.18 GH/IGF-1 and Non Alcoholic Fatty Liver Disease

A low IGF-1 / IGFBP-3 ratio and reduced IGF-1 concentrations were found in individuals with hepatic steatosis (Non Alcoholic Fatty Liver Disease, NAFLD), as a marker of a common pathway that links hepatic steatosis and low IGF-1 concentrations with MS and atherosclerosis.[133]

In fact, a significant negative correlation was recorded between IGF-1 and the severity of hepatic steatosis in overweight / obese subjects.[134,135] The strongest evidence is the link between excess visceral adiposity and the reduction of the somatotropic axis. Several factors are related to accumulation of ectopic fat (eg liver, visceral or epicardial fat), including pro-inflammatory mediators, which independently contribute to altering the secretion of GH and IGF-1. [101] Savastano et al. provided a hypothetical mechanism that could underly the relationship between obesity and the somatotropic axis (Figure 3). The concordance between low GH and low IGF-1 could have a pathophysiological relevance in accelerating the maturation of adipose tissue and contribute, in obese subjects, to long-term metabolic and cardiovascular complications.[101]

In pediatric age, Cianfarani et al. evaluated the levels of IGF-1 and IGFBP-3 in patients with hepatic steatosis.[136] Results confirmed that GH-IGF-1 axis is involved in the evolution of NAFLD. In particular, authors have shown that the serum concentration of IGF-1 correlates with the histological stage of NAFLD, and that liver disease is an index of cardiometabolic risk in obese children. The serum concentration of IGF-1 seems to be reduce in individuals with hepatic steatosis and the hepatic expression of IGF-1 mRNA is negatively correlated with fibrosis.[139]

The incidence of hepatic steatosis is higher in patients with GH deficiency than in those without deficiency, and hypopituitarism or Laron dwarfism can be associated with obesity, IR and NAFLD.[137] GH replacement therapy can also improve fatty liver disease.[137]

Therefore the association between fatty liver disease and low IGF-1 concentrations may also be bidirectional. Genetic determinants explain about 40% of the variability of the serum concentration of IGF-1 and 60% of IGFBP-3. This could suggests that also genetic component of IGF-1 and IGFBP-3 secretion modulates the susceptibility to NAFLD development in obese subjects.[138] This could be an important factor in explaining why some obese children do not have NAFLD.

The altered adipokine profile that occurs in MS can play an additional role in the development of NAFLD, as supported by several publications.

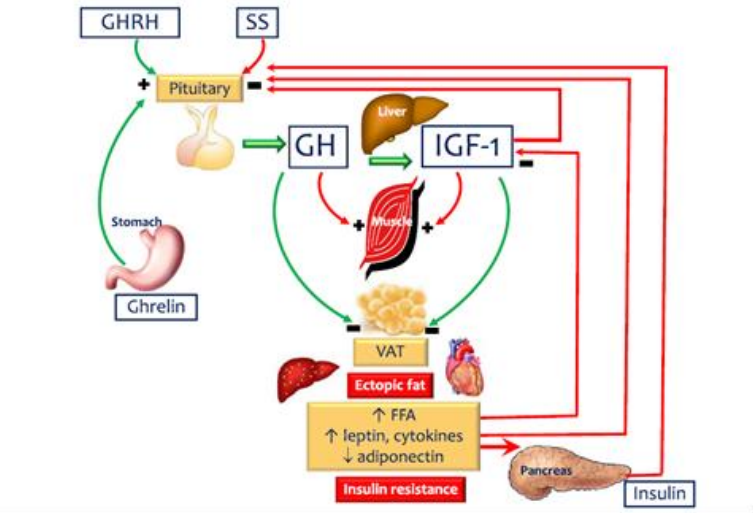


Figure 3. Hypothetical maladaptive mechanism involved in the etiopathogenesis of the alterations of the GH / IGF-1 axis, related to obesity. The increase in FFA, adipokines and cytokines, induced by the increase of visceral adipose tissue or by ectopic fat deposits (hepatic steatosis or epicardial fat), are responsible for insulin resistance and also alter the feed-back control system of the GH / IGF-1 axis. The functional reduction of the GH / IGF-1 axis, in turn, can act on body composition, causing unfavorable changes, similar to those found in GH deficiencies, contributing to worsening insulin resistance and its metabolic sequelae. Abbreviations: SS, somatostatin; GHRH, growth hormone-releasing hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1; FFA, free fatty acids, VAT, visceral adipose tissue[101]

The reduction of IGF-1 could be also consequence of liver inflammation. Since IGF-1 is mainly produced by the liver, a hypothetical serum reduction of IGF-1 in individuals with hepatic steatosis could represent the common enounced pathway that links hepatic steatosis to MS and atherosclerosis.[133]

1.19 IGF-1 receptor and its polymorphism

The insulin and insulin-like growth factor (IGF) system is critical for normal growth and development by regulating cell growth, differentiation, survival, and metabolism. The IGF system is composed of three ligands: IGF-1, IGF-2, and insulin; six high affinity ligand-binding proteins (IGFBPs) and several receptors, including the type I IGF receptor (IGF-1R), type II IGF receptor (IGF2R), insulin receptor (IR), and hybrid receptors composed of one chain of the IGF-1R and one chain of the IR.[140] Among the above three receptors in the IGF system, IGF-1R and IR belong to the receptor tyrosine kinases (RTKs) family, and IGF2R does not possess this biochemical activity. Many growth factors bind and activate RTKs by inducing the receptor dimer formation. However, cell surface IGF-1R or IR exists as homodimer or heterodimer without a requirement for ligand binding. Each dimer is composed of two disulfide linked polypeptide chains, also called a "half receptor". Each half-receptor

consists of an extracellular α-chain containing the ligand-binding domain and an intracellular β-chain with tyrosine kinase activities.[141]

Previous studies have revealed that IGF-1R and IR share a high similarity in both ligand-binding domains and kinase domains. Once the growth factor ligand binds to the extracellular domain of the receptors, the intracellular tyrosine kinase domain (TKD) of one β-subunit phosphorylates its apposing strand resulting in receptor auto-phosphorylation. Phosphorylation and activation of adaptor proteins induce the activation of downstream signaling such as PI3K/Akt and MAPK pathways, therefore regulating cell growth, survival, apoptosis, metabolism, and mediating normal physiological processes at both cellular level and systemic level.[142]

Recent literature has focused on IGF-1 Receptor and its role on human health, IGF-1R and IR are widely expressed in normal human tissue and play important roles in supporting the physiological function of the human body.[143] Epidemiological studies also suggest that the IGF system is extensively associated with the development and progression of several diseases such as cancer. Dysregulation of the IGF system contributes to the progression of multiple chronic liver diseases, such as nonalcoholic fatty liver disease (NAFLD), which may gradually promote hepatocarcinogenesis.[144] Recent experimental evidence suggests that the absence of one receptor cannot compensate for the lack of the other, suggesting the synergism of the two receptors. Insulin and IGF-I signaling are required for fat development.

Some polymorphic variants would affect blood levels of IGF-1, influencing the insulin / somatomedin C pathway.[145] Higher levels of IGF-1R mRNA have been recently reported in obese children and a single-nucleotide polymorphism (SNP), the G variant in codon 1013 (GAA1013¡GAG) of the IGF-1R may be associated with premature adrenarche in children, condition often associated with IR/hyperinsulinemia, obesity and policistic ovary syndrome. This way represents the potential future directions for targeting the IGF system in various disease, included obesity, and for new therapeutic approaches.

2 STUDY DESIGN

2.1 Background

Pediatric Obesity is an alarming problem due to direct influences on child health and direct-indirect effects on the onset of adult diseases, childhood obesity has become a major health problem all over the world.[1] Epidemic obesity should be considered an independent chronic metabolic disease, it is associated with the development of comorbidities previously considered "adult" diseases. Obesity is related with a complex imbalance of the endocrine regulation due to compensatory mechanisms. Although endocrine causes of pediatric obesity, such as Cushing syndrome, growth hormone deficiency or hypothyroidism, are rare, obesity-related endocrine and metabolic manifestations represent the most common concurrent and/or long-term consequences of childhood obesity, and consist a major risk for atherosclerotic cardiovascular disease later in life.[1,12]

So, even if there are various endocrine diseases including defined genetic syndromes which are the underlying cause for obesity in children and adolescents, these cases are very rare; instead, there are very common alterations in endocrine functions in obese children and adolescents which are a characteristic of the increased body fat mass. Hormonal implications of Pediatric Obesity are manifold: one of the most fascinating relationship, but less clear, concerns obesity - growth hormone-somatomedins' axis. Growth hormone, also called somatotropin (GH), is better known for its role on linear growth. Human GH is produced by somatotrope cells within the anterior pituitary, its pulsatile secretion pattern reflects the interplay of multiple regulators, such as GH-releasing hormone and somatostatin. Actually, GH has multiple actions on metabolism, in particular on anabolic and lipolytic pathways. [101] The main target of GH action is the liver, where it stimulates Insulin Growth Factor 1 (IGF-1, somatomedin C) production. Insulin-like growth factor 1 (IGF-1) is primarily produced in the liver upon stimulation by GH, and it plays key roles in regulating proliferation, differentiation, metabolism, and cell survival.

So, what is the interplay between GH axis and insulin in obesity, well known condition of hyperinsulinemia? Typical changes of the GH/IGF- 1 axis in overweight and obese children and adolescents are: Impaired GH secretion in stimulation tests, Increased circulating levels of GH-binding protein, Increased circulation levels of IGF- 1, IGFBP- 1, IGFBP- 2, IGFBP- 3 in the pre- and intrapubertal period, reduced levels of IGF- 1, IGFBP- 1, IGFBP- 2, IGFBP- 3 in the postpubertal stage, reduced ghrelin secretion.[8]

Researches on GH-IGF-1 system in obese have led to conflicting results. There are many evidences that obese children and experimental animals were often accompanied with low IGF-1 levels, especially in post-pubertal period. This condition could affect pubertal growth of obese children.

Increasing evidence suggests that IGF-1 plays an important role in glucose metabolism, low serum levels of IGF-1 have been linked to increased risk for development of type 2 diabetes. However, the physiological role of IGF-1 in glucose metabolism is not well characterized.

2.2 Aim of the Study

Aim of our Study was to analyze the relationship between GH-IGF-1 axis and obesity in Pediatrics, trying to identify the connection between the main co-morbidities of pediatric obesity and GH-IGF-1 axis, new markers of insulin-resistance and, in second phase the role of IGF-1R polymorphisms and possible association with obesity-mediated metabolic alterations. We also analyzed data according pubertal stage e linear height growth, to better understand the role of this axis in obese children.

2.3 Outcomes

Primary outcome

The role of GH/IGF-1 axis in pediatric obesity and its comorbidities

Secondary outcomes

Define the alterations of GH/IGF-1 axis in insulin resistance in basal condition and also during glycemic load.

The different metabolic profile accordine to IGF-1 state

The possible role of IGF-1R in insulin resistance

The role of GH/IGF-1 system in obese children growth

2.4 Patients and methods

Data of subjects referred to the Pediatrics Endocrine Service of our hospital for overweight and obesity, were collected from April 2019 to April 2021.

2.4.1 Inclusion and Exclusion Criteria.

Obese and overweight children and adolescents. Inclusion Criteria were: obesity/overweight status defined according to Italian Society of Pediatric Endocrinology and Diabetology Guidelines, Caucasian race, $6 \leq age < 18$ yrs.[12] Exclusion Criteria were: chronic diseases, precocious puberty, use of toxic/drugs, secondary obesity, GH deficiency, genetic diseases. Written parental informed consent was acquired before enrollment. We scheduled 80 children and adolescents meeting the inclusion criteria of a total 120 obese children and adolescents observed in our Service: 25 children were excluded for incomplete data, so a total of 48 children were enrolled.

The study was performed according to the World Medical Association Declaration of Helsinki for ethical principles in medical research involving human subjects. Written informed consent to the study was obtained by the parents/caegivers for the participation of their child in the study.

2.4.2 Clinical Evaluation

A detailed medical and family history and complete physical evaluation were performed. All clinical examinations were conducted by a trained medical staff according to standardized procedures: body weight was measured by the same physician scale to the nearest 0.1 kg in light underwear, before breakfast and after voiding; standing height was measured barefoot to the nearest 0.1 cm on a standardized wall-mounted height board. BMI was calculated as BMI = Weight/Height^2 (Kg/m^2) for each subject and BMI z-score was assessed according to WHO standards.[1,12] Waist circumference (WC) was measured midway between the lower rib margin and the iliac crest in standing position. The mean of three measurements made at the end of a normal expiration was used. For each patient, we calculated Waist to Height ratio (WHtR) according to: WC (cm)/Height (cm).[154]

Each patient underwent a pubertal evaluation according to Tanner's Criteria.[155]

Blood pressure was measured at the right arm, with appropriate sized cuff, after 10-minutes of rest in a comfortable supine position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed by standardized protocols. [156] The mean value of three measurements was registered and blood pressure z-scores were calculated. Systolic and diastolic blood pressure (mean of three measurements) were obtained on the right arm with the participant seated in a quite office and appropriately sized arm cuffs. Relative z-scores were calculated. [158,159]

According to AAP 2017 criteria: elevated blood pressure was defined as BP≥ 90°pc for age, gender and height in children aged <13 years, ≥120/80 in adolescents aged ≥13 years.[157]

The presence of acanthosis nigricans recorded. Pubertal development was evaluated according Tanner stage, no one had pubertal delay or precocious puberty; moreover, height velocity growth was not in pathological values.

2.4.3 Laboratory and instrumental investigations.

Blood Samples were collected, after an overnight fast, for determination of metabolic parameters. Plasma glucose was measured by using Beckam glucose analyser (Beckman, Fullerton, CA; USA). Serum levels of insulin were measured by Enzyme-Linked Immunosorbent Assay (Elecsys, Roches diagnostics, Mannheim, Germany). The Homeostasis Model Assessment Insulin resistance (HOMA)

was calculated as follows: fasting glucose (mmol/l) x fasting insulin (mUI/l) / 22.5, and relative percentile calculated.[147] Relative percentile of HOMA index were calculated.[149]

Serum Triglycerides (TG), Total cholesterol (TC), Low density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were determined by using an automated analyzer (Unicle DxC800 Synchron™ System, Beckman Coolter, Fullerton, CA; USA). The relative percentile for TG, TC, LDL and HDL was calculated according to data by Daniels et al.[160]

We recorded some clinical chemistry parameters: liver function (glutamic oxaloacetic transaminase-GOT, glutamic pyruvic transaminase- GPT, gamma glutamyl transferase – gamma GT). The blood count detection allowed us to exclude any anemic states. We studied the inflammatory state, with inflammation indices: Erythrocyte sedimentation rate - ESR and protein c reactive- PCR.

Glucose metabolism was analyzed with HbA1c and Oral Glucose Tolerance Test (OGTT). OGTT was performed with 1.75 g / kg (max 75 g) of glucose: glucose and insulin determinations were assessed at 0-30-60-90-120 minutes after load. Indices of insulin-resistance and beta cells function were calculated: Matsuda index (WBISI), Insulinogenic Index (IGI), Oral Disposition Index, HOMA (Homeostasis Model Assessment) index, Glucose Effectiveness, Hepatic Insulin resistance and insulin sensitivity index. [150,151,152,153]

Area Under the Curve (AUC) was calculated for glucose and insulin by trapezoidal method from 0 to 120 and from 0 to 30.

IGF-1 and IGFBP-3 were assayed with immunoenzymatic method on Siemens-Immulite 2000. IGF-1 and IGFBP-3 were considered as absolute values (ng / ml and mg / l, respectively) and as Standard Deviatin (SD), specific for age and sex. The IGF-1 / IGFBP-3 ratio was also calculated, as an estimate of the concentration of free and, therefore, bioavailable IGF-1. GH, IGF-1 and IGFBP3 were evaluated in basal condition and at 60-120 minutes after glucose load during OGTT. Standard deviations for IGF-1 were obtained according to cut off laboratory values for sex and age.[158]

2.4.4 Hepatic ultrasound

Liver ultrasonography was performed in all subjects, after at least 10 hours of fasting, by the same experienced radiologist using a high-resolution ultrasound system (LOGIQ® 7, GE Medical Systems, Milwaukee, Wisconsin, USA). Liver echogenicity was graded and patients were classified as "with or without hepatic steatosis" according to ultrasonographic findings.[161]

The diagnosis of NAFLD was made in the presence of hepatic hyperechogenicity assessed by ultrasound, in the absence of a positive history of alcohol, drug and / or toxic intake, excluding the main metabolic, infectious diseases and forms of secondary obesity.

2.4.5 Metabolic risk definitions

To avoid problems related to the lack of consensus on MS definition in Pediatric age, we used to assess the cardiovascular risk of our sample size a cardio metabolic score.

Metabolic risk parameters were considered: BMI z-score ≥ 2SD, HDL cholesterol ≤10°pc, cholesterol ≥90°pc, Triglycerides ≥ 90°, high fasting glucose ≥100 mg/dl, systolic blood pressure and/or diastolic blood pressure≥ 90°pc.[162]

2.4.6 Molecular Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes, the analysis were conducted by the Laboratory of Medical Genetics, Department of Clinical and Experimental Medicine University of Foggia. A 279-bp fragment of the IGF-1R gene (accession number: NM_000875) was amplified by using the following primers: forward, 5=-TGCTTTAATTACGGTTTCTTC-3= and reverse, 5=-GCTTTTCAGGAACTTTCTCTT-3=, as described elsewhere. [145]

Polymerase chain reaction amplifications were performed using APTaq polymerase (Applied Biosystems, Foster City, CA). After an initial denaturation step at 94°C for 3 minutes, the PCR reaction conditions were 30 cycles of denaturation at 94°C for 30 seconds, 30 seconds of annealing at 60°C, and 1 minute of extension at 72°C. With the last cycle, extension at 72°C for 7 minutes was performed. Restriction fragment length polymorphism analysis using the amplified genomic DNA was then performed. The endonuclease MnlI (New England Biolabs, Beverly, MA) cuts in positions 50, 182, and 202 in the presence of the G allele. In the presence of the A allele, the MnlI site in position 202 is lost. After digestion with MnlI, the G allele yields four fragments (132, 77, 50, and 20 bp), whereas the A allele yields three fragments (132, 97, and 50 bp). [145]

2.5 Statistical Analysis

Results are expressed as mean ± Standard Deviation (SD) with 95% confidence interval for continuous variables, as percentages for categorical and discrete variables. For the non-Gaussian distribution parameters, instead, the median and the interquartile range were used. The Kolmogorov-Smirnov goodness of fit test was used for assessing the hypothesis of normality of the data. Data were analyzed by t-Student test, Mann-Whitney U test, $\chi 2$ test. Bonferroni test and pairwise comparison according to Dunn-Bonferroni post hoc method were applied for multiple simultaneous comparisons The calculation of Spearman's rho and Pearson's coefficients, as appropriated, were used to evaluate the degree of association between variables. Trend analysis was carried out with Jonckheere-Terpstra test. Values of $P \leq 0.05$ were considered statistically significant. Statistical analysis was performed with SPSS Version 22 statistic software package.

3 Results

3.1 Population's Description

Forty eight children and adolescents, mean age 11.8 ±2.8 yrs (CI 95% 10.1-12.6, range 12-6-5.55 years): 23 males (mean age 12.2 ±2.1 yrs) and 24 females (mean age 11.5±3.4 yrs) (p=0.417).
33.3% of children were in prepubertal stage (37.5% of males and 30.4% of females), while 70% in pubertal state (62.5% of males and 69.6% of females, p=0.642).
Acanthosis nigricans was observed in 70% of overall sample.

3.1.1 Anamnestic Data

34.7% of children were born by cesarean delivery. Mean neonatal weight standard deviation was -0.288±1.5 (range -2.48 - 2.79)

3.1.2 Anthropometric Data

Mean BMI z-score was 2.9±0.6 (CI95% 2.7-3.1) according to WHO growth standards. No differences according to BMI z-score were recorded between males (2.9±0.5) and females (2.9±0.7) (p=0.097).
Mean WtHr was 0.6±0.08: 0.63±0.1 in males and 0.6±0.08 in females (p=0.678)
Mean Standard Deviation of Height was 0.47±1.17.

3.1.3 Blood Pressure

Mean z score of systolic (SBP) and diastolic (DBP) blood pressure are described in table 5.

				95% CI			
		Mean	SD	IL	SL	Min	Max
z-score SBP	boys	1,4304	1,95821	-,2067	3,0675	-1,22	4,99
	girls	1,6196	1,47533	,8860	2,3533	-,12	4,99
	Total	1,5614	1,60054	,9149	2,2079	-1,22	4,99
z-score DBP	boys	,3018	,53526	-,1456	,7493	-,25	1,38
	girls	,4002	,71180	,0462	,7542	-,43	2,13
	Total	,3699	,65337	,1060	,6338	-,43	2,13

Table 5. *z-score of SBP and DBP in boys and girls*

In particular, 46.2% of overall population had high systolic blood pressure and 16% of overall population had high diastolic one.

3.2 Laboratory Findings

3.2.1 Lipid metabolism

18.6% of overall population had total cholesterol levels > 90°pc for age and sex, 32.6% presented HDL cholesterol < 10°pc, while 40% had triglycerides levels > 90°pc. No differences were recorded between girls and boys.

3.2.2 Hepatic function

There was no statistical difference in sGOT, sGPT and gammaGT in males and girls. Mean values and p significance are expressed in table 6.

	Males	Females	Total	P values
sGOT	23.57±7.8	25.75±10.8	24.7±9.5	0.436
sGPT	35.22±13.8	33.75±12.1	34.5±12.8	0.700
gammaGT	21.55±13.7	18.88±5.5	20.1±10	0.385

Table 6. *sGOT, sGPT and gammaGT in our sample*

3.2.3 Phlogosis indexes

Protein C Reactive proteins levels were not statistically analyzed, cause of almost all children recruited had levels under the lower limits of laboratory range (<0.29 mg/dl). Data of erythrocyte sedimentation rate (ESR) were collected: mean values were 15.67±11 (range 2-45).

ESR levels were directly correlated with hepatic function indexes (table 7, Figure 4).

	sGOT	sGPT	gammaGT
ESR mm	r:0.624	r:0.616	r:0.625
	p=0.013	p=0.014	p=0.013

Table 7. *ESR and hepatic indexes correlation*

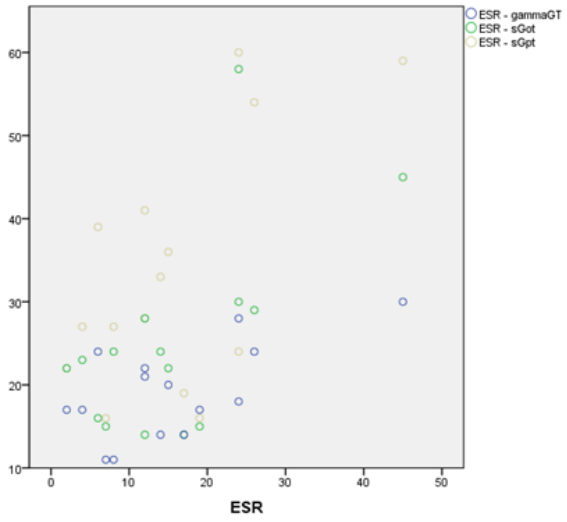


Figure 4. *Scatter plot hepatic function indexes and ESR*

3.3 Hepatic steatosis

Hepatic Steatosis (HS) was observed in 55.3% of overall population (62.5% of males and 50% of females, p=0.444).

All children with HS had acanthosis nigricans on clinical examination (p=0.015, likelihood ratio 7.5, p=0.006).

Children with HS presented higher levels of gammaGT (37.29±14.2 vs 30.8±11 U/l, p=0.046), but no differences in sGOT and sGPT (p=0.097 and p=0.135, respectively). However, BMI z-score was directly and positivity associated with gammaGT, sGOT and SGPT (table 8, Figure 5).

		gammaGT	sGot	sGpt
BMI z-score	Pearson's correlation	,325*	,343*	,325*
	Sign.	,031	,018	,026

Table 8. *BMI z-score and hepatic function's correlation*

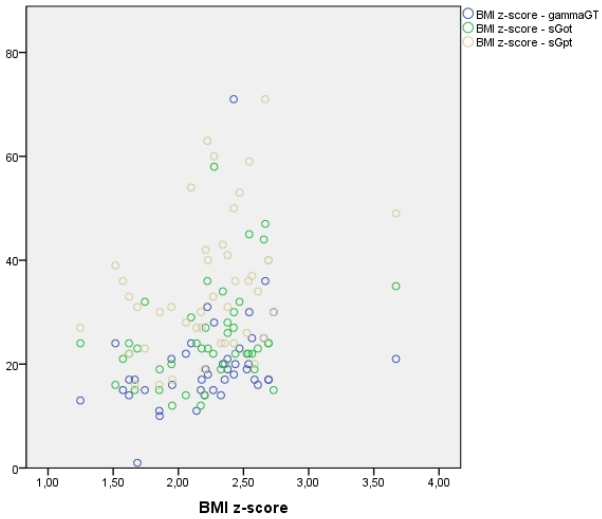


Figure 5. *Scatter plot BMI z-score and liver indexes*

Children with HS had significantly higher levels of ESR: 20 ± 9.05, vs 11.88±7.8 (p=0.044)

3.4 GH-IGF-1 axis.

Mean or median values of GH, IGF-1 (or its standard deviation, SD) and IGFBP3 are expressed in table 9.

IGF-1 basal	IGF-1 SD	GH	IGFBP3
201 (145.2-282.25)	-0.5±0.7	0.99±1.66	5.3±1.1

Table 9. *GH-IGF-1 axis in sample analyzed*

80% of our children had values of IGF-1SD <0, ie IGF-1<50°pc, but no one had IGF-1SD <-2SD (Figure 6). There was no difference in mean 1GF-1 SD values recorded in pre-pubertal e pubertal children (p=0.643). In particular, only 26.7% of boys and 15% of girls presented IGF-1 > 50°pc. No differences were recorded according IGF-1SD and pubertal stage (p=0.997)

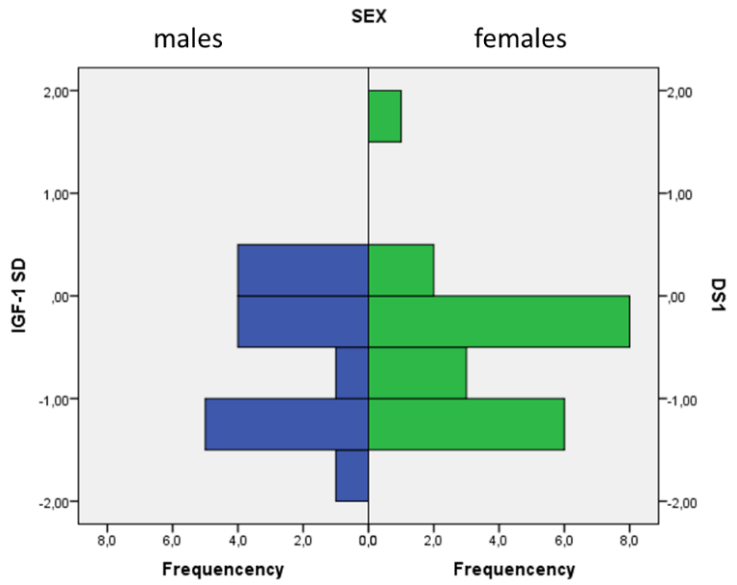


Figure 6. *Distribution of IGF-1 SD in boys and girls*

3.4.1 Somatomedin C, anthropometric and clinical values

Mean standard deviation of height was 0.47±1.2 (range -1.2-2.89). No children were affected by short stature. As expected, there was a significate positive correlation between IGF-1SD and standard deviation of height (r: 0.552, p=0.027). Surprising, instead the relation between IGF-1SD and BMI z-score (r: -0.362, p= 0.033), even after adjustment for pubertal stage (r:-0.376, p=0.044) (Figure 7).

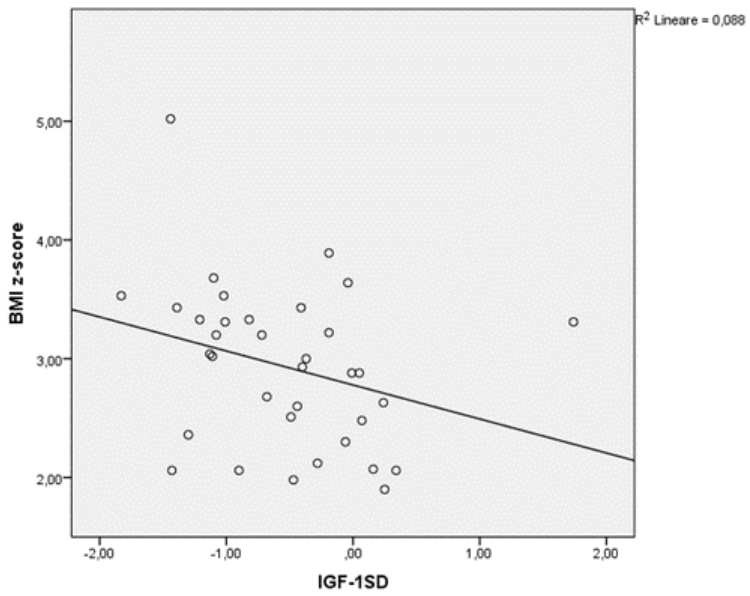


Figure 7. *Scatter plot IGF-1 SD and BMI z-score*

Children were divided according to quartile for IGF-1SD (ie, -0.47, IQR: -1.1 - -0.04).

No difference was recorded in mean age of children classified according to IGF-1SD quartile (p=0.426), and no difference also according to BMI z-score (p=0.139) and WtHr (p=0.412). However, statistical analysis showed that children with IGF-1SD <0 (i.e., IGF-1 <50°pc) had higher BMI z-score (3±0.67 vs 2.5±0.5, p=0.043) (Figure 8), but no different mean age (p=0.596).

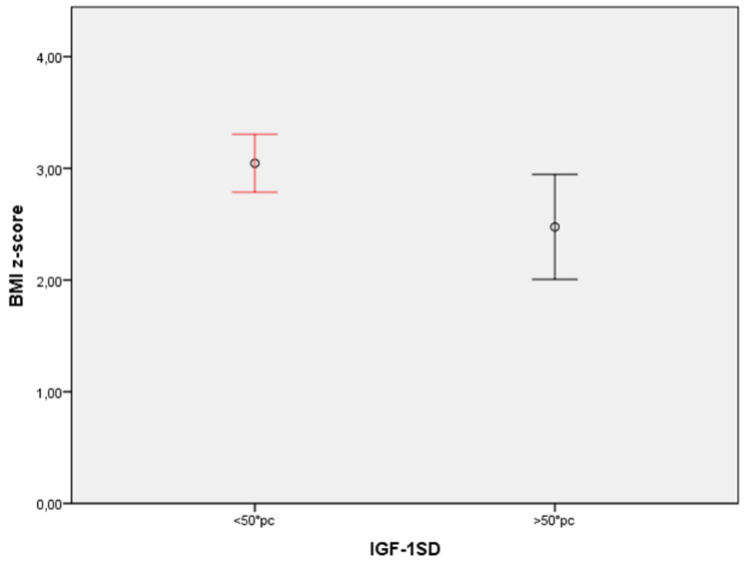


Figure 8. *Mean BMI z-score in children with IGF-1 <50° pc and > 50°pc*

An interesting association was found between IGF-1 SD and z-score of systolic blood pressure (r: -0.471, p=0.031), even after adjustment for age and sex (r:-0.460, p=0.047).

Most interesting the relation between IGF-1 and IGFBP3 (IGF-1 IGFBP3 ratio) and these parameters (Table 10):

	WtHr	BMI z-score
IGF-1/IGFBP3 ratio	R:-0.821	R:-0.518
	P=0.007	P=0.001

Table 10. *IGF-1/IGFBP3 ratio correlation with WtHr and BMI z-score*

3.4.2 IGF-1 and laboratory findings

According to lipid profile, there was statistical difference in χ^2 test according to HDL cholesterol: HDLc <10°pc was observed only in obese children with IGF-1 SD<0 (p=0.048): 37% of these children vs 0% of those with IGF-1SD≥50°pc had HDL<10°pc.

There was statistical difference in mean ESR of children with IGF-1 SD<0, vs children with IGF-1 SD ≥0: 17.42±8 vs 8.7±3 (p=0.049).

Instead, there was no statistical difference in hepatic functionality findings in children with levels of IGF-1 <50°pc (for age and sex) and IGF-1 ≥50°pc, as expressed in table 11.

	IGF-1 <50°pc	IGF-1 ≥50°pc	Total	p
sGOT	25.04±10.7	21.7±0.39	24.37±9.7	0.428
sGPT	35.6±13.7	32.43±6.6	34.97±12.6	0.558
gammaGT	20.9±12.2	16.8±4.9	20.18±11.29	0.431

Table 11. *Liver indexes in children with IGF-1<50°pc and ≥50°pc*

3.4.3 Hepatic steatosis and IGF-1

Hepatic Steatosis was more frequent in children of the first quartile for IGF-1SD: 77.8% of these had HS, vs 57.1% of the second, 50% of the third and 25% of the fourth one (p=0.186) (Figure 9). The p for the trend test was 0.033.

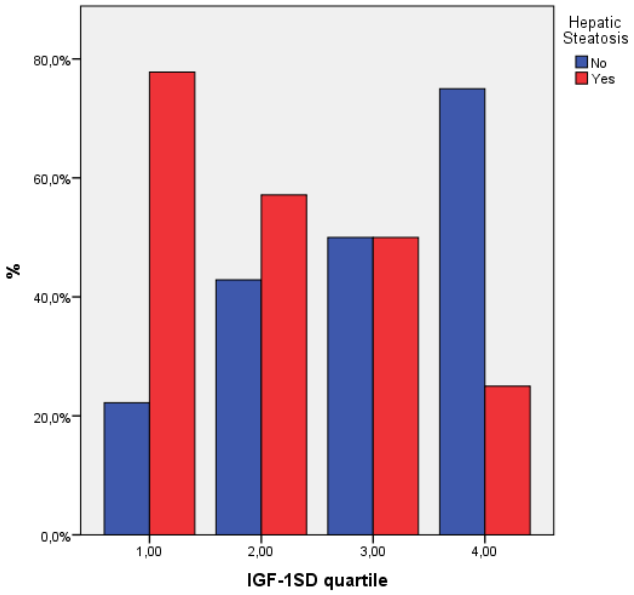


Figure 9. *HS in children divided according to IGF-1 SD quartile*

Spearman's correlation analysis confirmed this relation: HS and IGF-1SD (rho= -0.431, p=0.014), HS and IGF-1/IGFBP3 (rho: -0.409, p=0.018).

This data was also confirmed dividing children in two group: chidren with IGF-1< 50°pc and children with IGF-1 ≥50°pc. Hepatic steatosis was significantly more frequent in group IGF-1<50° (64% vs 16%, p=0.002) (Figure 10).

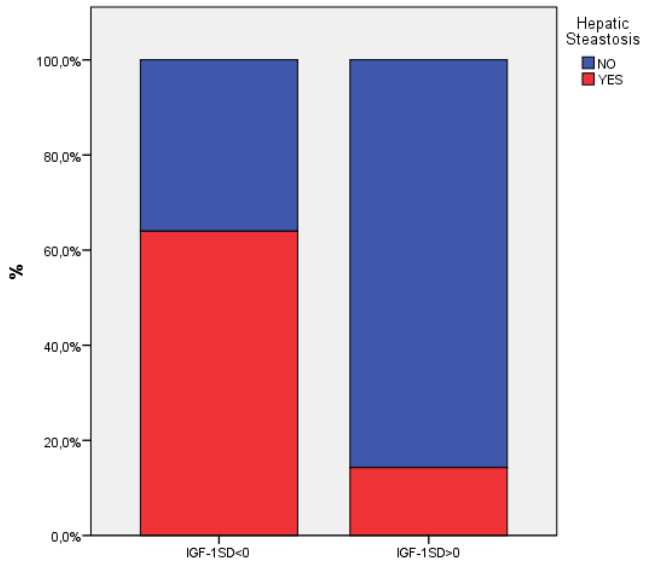


Figure 10. *HS in children with IGF-1 SD <50°pc and ≥50°pc*

Children affected by HS had lowest levels of IGF-1 SD: -0.811±0.56 vs -0.18±0.7 (p=0.012), as showed in Figure 11

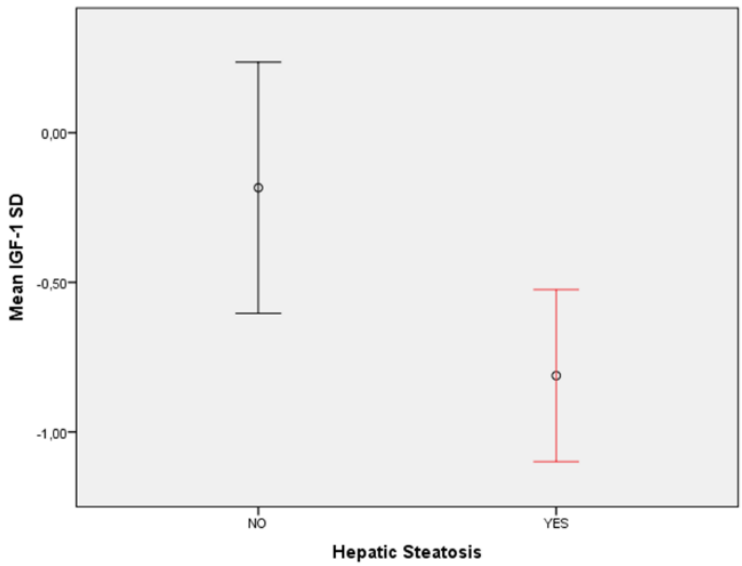


Figure 11. *IGF-1 SD in children with and without HS*

3.5 Glucose metabolism indexes.

Median HOMA index was 3.4 (IQR 2.4-5): 48.8% of children had HOMA values > 75°pc (57.1% of males and 40.9% of females).

In particular, 14% of overall population had HOMA levels > 97° for age and sex.

Homa index was strictly associated with BMI z-score (r: 0.328, p=0.032), even after adjustment for age and sex (r: 0.393, p=0.011) (Figure 12).

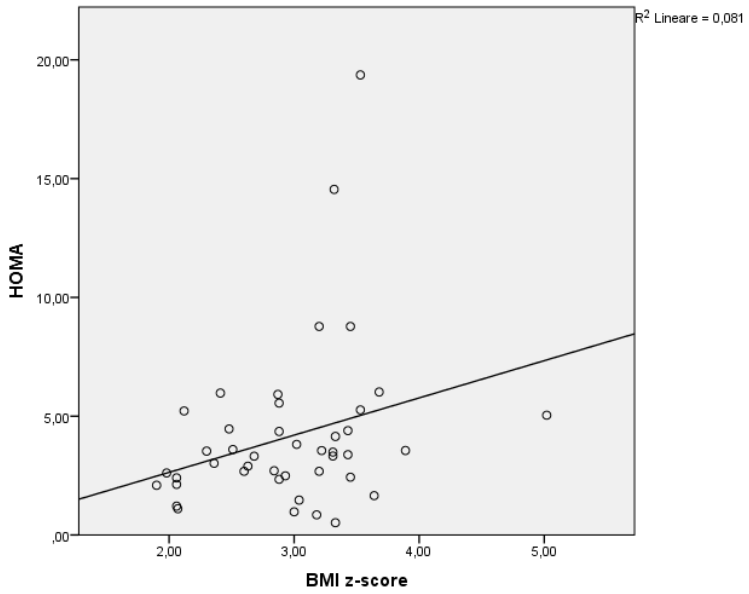


Figure 12. *Scatter plot Homa and BMI z-score*

Moreover, children with Homa index > 75°pc ha higher levels of BMI z-score (3.2±0.6 vs 2.7±0.5; p=0.003) (Figure 13).

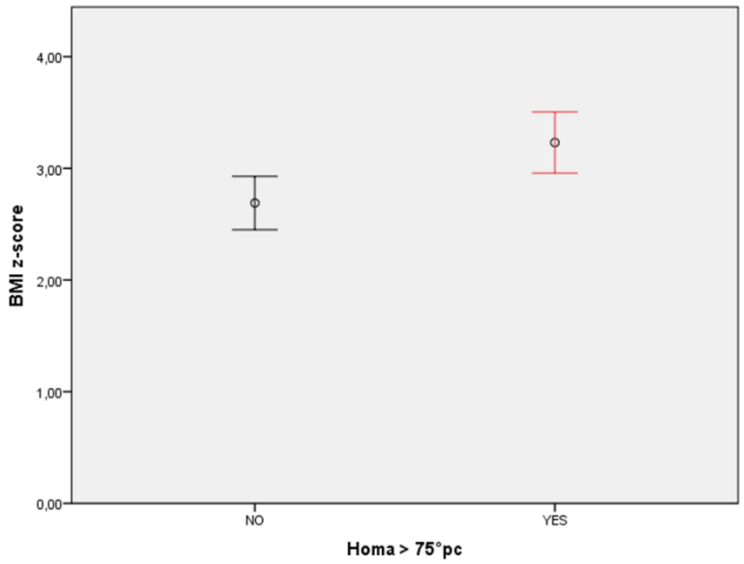


Figure 13. *BMI z-score in children with HOMA ≤75°pc and >75°pc*

No apparent relation was observed between HOMA and WtHr (p=0.224), but statistical significance was reached in partial correlation analysis, correcting data for age and sex (r:0.422, p=0.029).
Homa index did not seem related to IGF-1 SD levels (p=0.085), but even if not statistical significance was reached, the sign of the relation was negative (r:-0.296).

Mean HbA1c was 5.4 (36 mmol/mol) ±0.4 (CI 95% 5.3-5.6%, range 4.7-6.3): 34.8% of males and 17.4% of females had values ≥5.8% (40 mmol/mol), 26.1% of the whole sample (Figure 14).

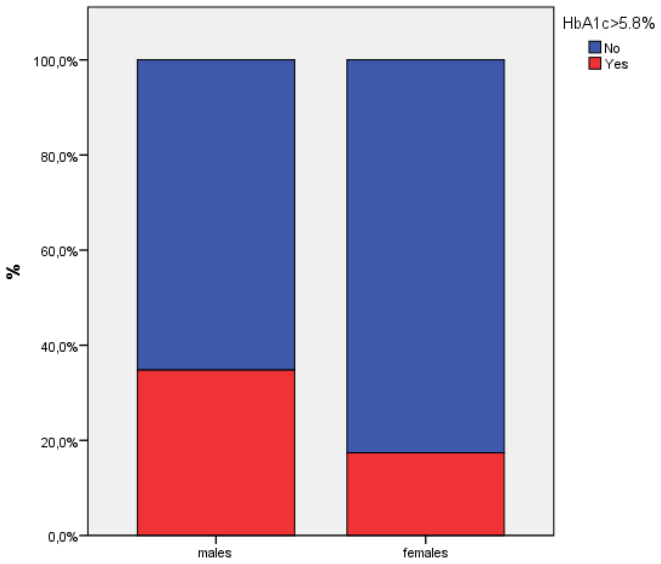


Figure 14. *Percentage of children with HbA1c >5.8%*

HbA1c levels were correlated with BMI z-score (r:0.318, p=0.031), but no with IGF-1 SD (p=0.730). Data on glycaemia and insulinemia after glycemic load are expressed in table 12.

	Basal	30'	60'	90'	120'
Glycemia mg/dl	83.1 ±8.04	131.07 ±23.2	110.5 (100-129.2)	110 (99-117.5)	99.5 (89.5-113)
Insulinemia uUI/ml	16.3 (12-21.5)	123.1 (91.9-200.6)	119.2 ±101.7	114.8 ±73.8	68.7 (42.8-135.7)

Table 12. *Glycemia and Insulinemia levels during OGTT*

Two children had glycemic pathological values after 2h-glycemic load (>140 mg/dl, ie 145 mg/dl); moreover 14 children (7 boys, 7 females) had 1h glycemia ≥ 123 mg/dl and 9 children (5 males and 4 females) had glycaemia at 1h >130 mg/dl.[163]

Basal Glyaemia was also correlated with HbA1c levels: r:0.418, p=0.005 (Figure 15).

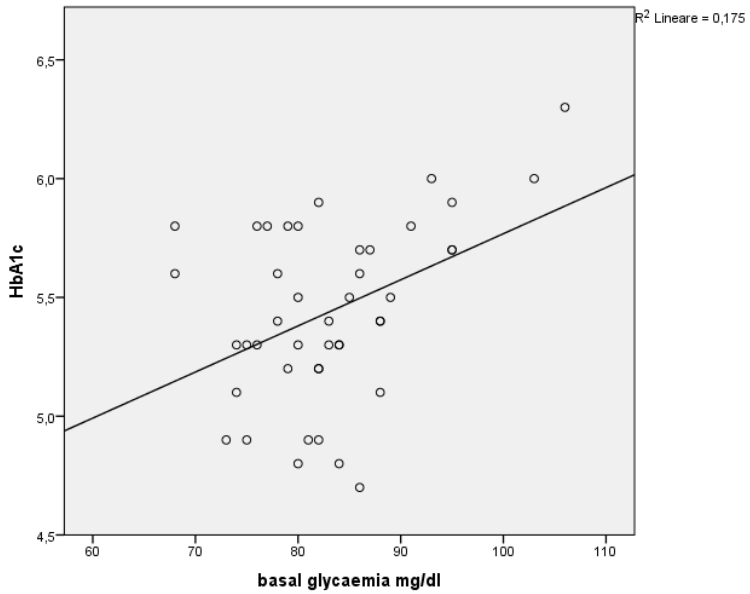


Figure 15. *Scatter plot glycemia and HbA1c levels*

Mean or median, where appropriate, values of glycaemia and blood insulin during OGTT are expressed in table 11. Index of insulin resistance and insulin sensitivity were calculated.[150,151,152,153]

Median WIBSI value was 3.1 (IQR 1.9-4.5): 75% of male group and 76.2% of females had Matsuda index <4.3 (p=0.929).

No relation was observed between WIBSI and IGF-1SD (p=0.690)

Median IGI was 2.5 (IQR 1.8-3.9) uUI/ml x mg/dl^{-1}. IGI was correlated IGF-1 SD (r: 0.464, p=0.006)

Median ODI was 7.6 (IQR 5.5-12.1).

Oral glucose effectiveness was calculated (OGE), median value was 7.6 mg/dl/min (5.5- 12). OGE was correlated with age: r: -0.761, p<0.001. (Figure 16)

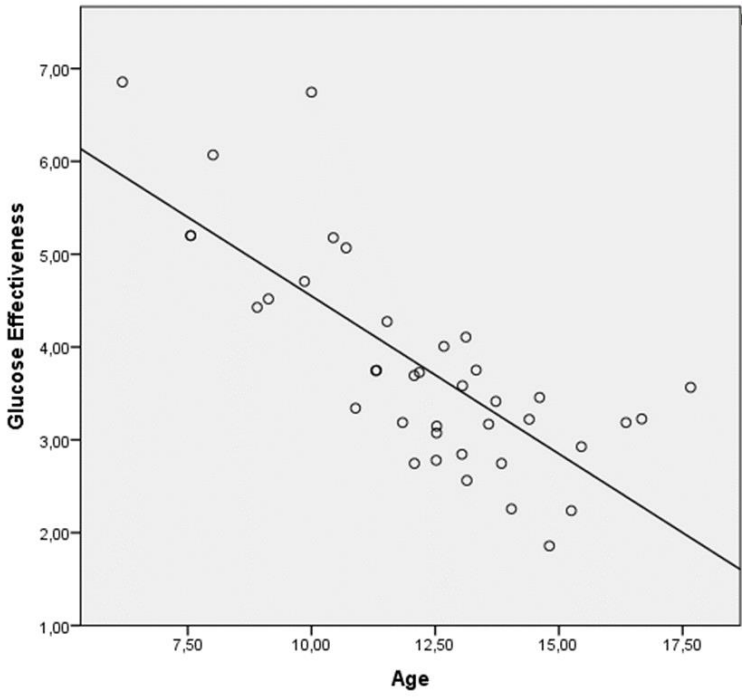


Figure 16. *Scatter plot glucose effectiveness and age*

The area under the glucose and insulin curves (AUC_G and AUC_I) were computed from 0 to 30 (AUC30) minutes and from 0 to 120 minutes (AUC120). Ratios were calculated. Results are expressed in table 13.

AUCG120	AUCG30	AUCI120	AUCI30	AUCG/I 120	AUCGI30
583.5 ±76	212.38 ±32	487.9±272.6	137.2	1.3	1.5
			(106.7-29.3)	(0.88-1.5)	(1-2.09)

Table 13. *AUC for insulin and glucose during OGTT*

We analyzed the curve morphology according to glucose and insulin concentrations during OGTT: 59.5% of children had a monophasic curve, 31% biphasic, 7.1% triphasic curve and 2.4% a monotone curve for glucose, while 46.2% had monophasic curve, 43.6% biphasic curve, 7.7% triphasic curve and 2.6% a monotone curve according to insulin determinations.

HS was detected in 42.9% of children with monophasic curve, 60% in biphasic and 100% of children with triphasic curve according to insulin during OGTT (p=0.199).

3.5.1 Somatotropic axis and glucose metabolism.

IGF-1 and IGF-1SD were analyzed during OGTT: mean (or median) values are expressed in table 14.

IGF-1 basal	IGF-1 60'	IGF-1 120'	IGF-1 SD 0'	IGF-1 SD 60'	IGF-1 SD 120'
201 (145.25-282.25)	211.34±94.8	209.69±87.1	-0.53±0.7	-0.57±0.7	-0.6±0.66

Table 14. *IGF-1 and IGF-1 SD mean levels during OGTT*

T student test for paired sample showed a significant reduction in IGF-1SD from 0' to 60' ($p<0.001$, mean - 0.2 ±0.2), and from 0' to 120' ($p<0.001$, mean - 0.17 ±0.2) but no statistical difference between 60' and 120' ($p=0.602$). The same modification was recorded for IGFBP3 between 0' and 60' (mean -0.36± 0.5, $p=0.001$), and 0' -120' (mean -0.33±0.46, $p=0.001$).

Correlation analysis showed significance between IGF-1SD and blood insulin during OGTT as showed in table 15.

	INSULIN 30'	60'	90'	120'
IGF-1 SD0	r: 0.391	NS	NS	NS
	p: 0.025			
IGF-1SD60	r: 0.490	r: 0.411	NS	NS
	p: 0.01	p: 0.033		
IGF-1SD120	r: 0.601	r: 0.399	r: 0.396	NS
	p: 0.001	p: 0.048	p: 0.05	

Table 15. *IGF-1 SD correlation with insulin during OGTT*

An interesting relation was observed between IGF-1SD and AUC for insulin secretion during OGTT, the relation is expressed in table 16. Insulinogenic index was positively related with IGF-1SD 0' (r: 0.464, $p=0.006$), 60' ($r=0.524$, $p=0.005$), 120' ($r=0.538$, $p=0.06$) (Figure 17). These data was also confirmed for IGF-1/IGFBP3 ratio.

	AUCI30	AUCI120
IGF-1 SD0	NS	NS
IGF-1 SD60	R:0.455	R:0.431
	P: 0.017	P:0.025
IGF-1 SD 120	R:0.566	R:0.495
	P:0.003	P:0.012

Table 16. *Correlation between IGF-1 SD and AUCI*

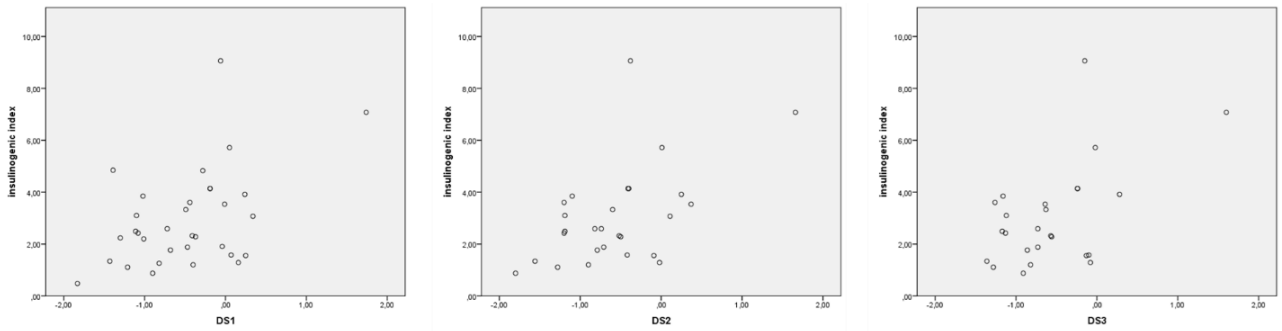


Figure 17. IGI and IGF-1 SD scatter plot

Hepatic insulin resistance index was associated with IGF-1SD at 60'-120' (r=0.395, p=0.041; r=0.516, p=0.008, respectively).

No significant modifications on GH levels during OGTT were observed, but there was a correlation between GH120' and insulinogenic index (r= 0.439, p=0.028), GH0' and HOMA index (r:0.6, p=0.01) and percentile of HOMA index (r:0.414, p=0.032).

We also analyzed hepatic insulin sensivity index (HISI) as marker of liver sensibility to insulin action. HISI was direct correlated with IGF-1 SD 0' (r:0.551, p= 0.027), but not relation was observed between HISI and IGF-1 SD 60' and IGF-1 SD 120'.

Children with HS had also lower IGF-1SD 0' and 120' during OGTT (-0.8±0.5 vs -0.18±0.7, p=0.012 basal, -0.8±0.4 vs -0.36±0.76 at 120'). No difference was recorded according age in children affected by HS.

There was no difference in IGF-1SD e IGF-1/IGFBP3 in children divided according the type of glucose o insulin curve.

3.5.2 GH-somatomedin axis and lipid profile

According to lipid levels, 16.3% of our sample had total cholesterol > 90°pc, 28.6% HDL cholesterol <5°pc, 36.7% triglycerides > 90°pc.[19] Correlation analysis showed a positive association between IGF-1SD and HDL cholesterol (r: 0.373, p= 0.03)(Figure 18).

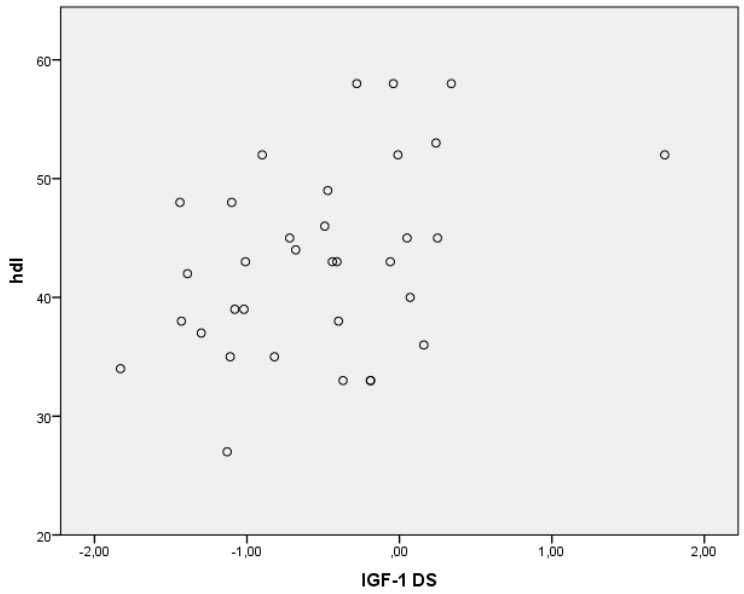


Figure 18. *HDL and IGF-1 SD scatter plot*

3.6 Cardio-metabolic risk score.

According to cardio-metabolic cluster: 25% of children had one risk factor, 50% two risk factors, 18.8% three and 6.3% four risk factors. No differences were recorded according to IGF-1 SD of children divided according to metabolic risk score, even if children with four risk factors had lower levels of IGF-1SD (<-1SD), Figure 19. Correlation analysis showed a strong inverse correlation between cardio-metabolic risk score and IGF-1 SD0' after adjustment of data for age and pubertal stage, age and sex (r:-0.629, p=0.029).

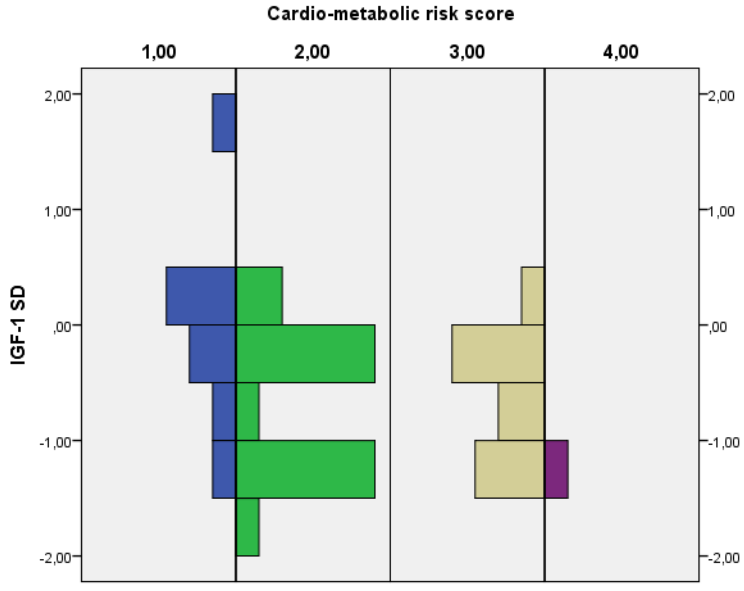


Figure 19. *Cardiometabolic risk score and IGF-1 SD*

3.7 Regression analysis

In our study, the most important finding related to obesity comorbidities was IGF-1 SD, in particular with Liver Steatosis. Multiple Linear regression analyses was adopted to assess the role of clinical and metabolic parameters on prediction of HS. Variables used for analysis had no problem of collinearity. We used HS as dependent variable and IGF-1 SD, BMI z-score, WtHr as independent variables in a stepwise analysis. IGF-1 SD was the only predictor on HS prevision with statistical significance (Table 17).

Coefficients[a]

Model		Understandardized Coefficents		Standardized coefficents	t	Sign.
		T	Std. Error	Beta		
1	(Costant)	1,459	,282		5,182	,001
	IGF-1 SD	-,004	,001	-,849	-4,243	,004
2	(Costant)	,583	,678		,859	,423
	IGF-1 SD	-,003	,001	-,652	-2,784	,032
	BMI ZSCORE	,299	,213	,328	1,402	,211
3	(Costante)	1,093	,954		1,145	,304
	IGF-1 SD	-,003	,001	-,668	-2,753	,040
	BMI ZSCORE	,355	,231	,390	1,534	,186
	WtHr	-,007	,009	-,169	-,788	,466

Table 17. *Regression analysis, Y: Hepatic Steatpsis; X: IGF-1 SD, BMI z-score, WtHr*

3.8 Genetic analysis

All children analyzed were homozygous for the A→G variant of IGF-1 receptor.

4 Discussion

Pediatric obesity is a persistent, epidemic problem, and preventing pediatric obesity and its comorbidities is of paramount importance. In fact, obesity related comorbidities could affect every physiological setting and begin early in life.

The relation between obesity and cardiovascular risk can be inferred by our data too. Even if or study was not focalized on blood hypertension evaluation, 46.2% and 16% of sample presented high systolic blood pressure and high diastolic one, respectively. In particular, we found a negative relation between systolic blood pressure and IGF-1 SD, even after adjustment for age and sex.

Cardiovascular disease (CVD) is the major cause of death all over the world and one of the most important modifiable factors in CVD is Hypertension. Chronic inflammation described in obesity status and autonomic dysfunction are one of the most important aspects involved in hypertension pathophysiology.[157,159] Solid evidence connect IGF-1 with endothelial function too: vascular endothelial and smooth muscle cells express IGF-1 receptor which could mediate angio-protective effect of somatomedin C.[164] Circulating IGF-1 is also known to induce vasodilatation, which contributes to the regulation of blood pressure.[164] Naturally, the white coat effects should be taken into account in our sample; but, studies demonstrate that the same with coat effect on blood pressure should be not considered only a simple benign phenomenon, because endothelial dysfunction was described also in children with white coat hypertension based on mechanism similar to essential hypertension.[165]

The cardio-metabolic score confirmed these explanations: obesity is a condition strictly related to cardiovascular risk. In fact, only 25% of our sample had only one risk score, while 50% presented two risk factors, 18.8% three and 6.3%, really four risk factors, configuring an high-risk cardio-metabolic condition. This score could be considered a surrogate of MS. The lack of consensus for the optimal diagnostic criteria for the pediatric metabolic syndrome, efforts have shifted from defining metabolic syndrome towards identifying youth with a cardio-metabolic risk clustering factor, who are at increased cumulative risk.[162] The score was also negative related with IGF-1, confirming a role of GH/IGF-1 axis in metabolic disruption.

The development of obesity is characterized by dysregulation of many pathways involved in metabolic processes and gene expression, as previously discussed. Secondary form of obesity are very rare, some of these are related to endocrine diseases; but essential obesity itself is an endocrine disruptor, determining lot of endocrine disturbances.[13] The altered secretory profile of adipose tissue and also the single fat cell in the obese state is responsible for various metabolic and endocrine alterations resulting in clinical end points such as type 2 diabetes, liver disease, atherosclerosis and

certain types of cancer. It should be stressed that almost all endocrine parameters which are affected by increased body fat mass can be normalized by caloric restriction, increased physical activity, and weight loss.[96]

Decreased insulin sensitivity and compensatory hyperinsulinemia are features of obesity, likely leading to modification in GH secretion. Growing evidence underlines that IGF-1 is a very important hormone involved in cardiovascular physiology, affecting metabolic homeostasis, vasodilatation, cardiac contractility and hypertrophy, authophagy, apoptosis and antioxidative processes. [166]

In our sample, almost 50% of children had HOMA index >75°pc for age and sex, condition related to IR. The index was strictly related with BMI z-score, individuating the role of adiposity on IR state, in particular the role of visceral adiposity if we consider the relation between HOMA and WtHr, reached after correction of data for age and sex.

So, we can confirm that insulin resistance was recorded in the majority of our sample and, in particular, acanthosis nigricans, a relatively common skin lesion observed in obese patients characterized by thickened and rough skin, irregular wrinkles, and brown pigmentation, was frequently observed in obese children enrolled in the study.[167]

We did not observe relation between Homa and IGF-1, but we underline that the Pearson's correlation index was negative.

When we focalize the attention to the topic of this thesis, data are very interesting.

We found a global reduction in IGF-1 levels: 80% of children had values of IGF-1 <50° for age and sex. In particular, our results showed that children with IGF-1 SD <0 had higher BMI z-score and WtHr. This result is supported by the literature: Iranmanesh et al, showed that the severity of the functional GH-IGF-1 defect is proportional to the degree of obesity.[97]

Viesti et al, described a different tissue expression of IGF-1 in the adipose tissue of obese subjects compared to controls, in particular in normal-weight individuals the expression of Somatomedin C would be greater precisely in visceral adipose tissue than in subjects with excess weight.[168] Thus, visceral adiposity could also affect the share of bioavailable IGF-1.[168]

This observation was confirmed by our results, showing a direct invers correlation between the IGF-1/IGFBP3 and WtHR and BMI z-score. So the availability of free IGF-1 seems to be reduced in visceral obesity.

Abnormalities in the GH-IGF-1 axis are common among obese children and adolescents. As we discussed above, in obese children several aspects of the GH secretory patterns are altered, with decreased GH half-life, frequency of secretory episodes and daily production rate.[80,169]

On the other hand, IGF-1 deficiency increases IR, alters lipid metabolism, promotes oxidative damage and dysregulates the neuro-hormonal axis, creating a vicious circle. [99,101]

In particular, lower levels of IGF-1 SD seemed to be associated with worst metabolic profile of our children and, on contrary, IGF-1 levels were positive associated with HDL cholesterol. This positive association is in line with published reports that provide the evidence that somatomedin C may be an independent modulator for HDL. IGF-1 may influence HDL cholesterol by modulating insulin sensitivity, but an alternative mechanism involves an IGF-1 dependent regulation of hepatic scavenger receptor of class B1 (SR-B1).[170] This receptor mediates the binding of HDL and the selective uptake into cells. Studies in vitro showed that IGF-1 downregulates the expression of SRB-1.[170] So, a reduction in IGF-1 may result in upregulation of SRB-1 and an increased liver uptake of HDL with decreasing HDL cholesterol in blood.

Insulin resistance and the compensatory hyperinsulinemia could mediate the reduction of somatotropic hormone secretion through the release of somatostatin, although at present the data on IGF-1 concentrations in the different IR conditions are conflicting and unclear.[85,94] The link between nutrition and growth is probably due to a complex interaction, not entirely defined, involving insulin, GH, IGF-1 and IGFBPs.[80,169]

The interactions between GH and insulin are the basis of many endocrine processes and metabolic disorders. Furthermore, a cross talk between IGF-1 and insulin pathways at the receptor and downstream levels of signaling way is assumable.[171]

Reduction in expression of IGF-1 gene was described in obese adolescents, and low levels of IGF-1 are associated with diabetes among adults.[88],[107]

In particular, IGF-1 SD basal and during OGTT levels were correlated with insulin secretion and, moreover, IGI (insulinogenic index) was directly correlated with IGF-1SD. The Insulinogenic Index is a valid marker of Beta Cell Function, it, in fact, strongly correlates with a direct assessment of beta cells activity. This measure defines the response of beta cell to glucose. Beta-cell function and insulin sensitivity are strictly linked processes in the government of glucose homeostasis.[172]

These results suggest a relationship between IGF-1 and insulin secretion under basal condition and during glycemic load as well, indicating a possible role in definition of insulin secretion and beta cell function.

Insulin plays a central role on blood glucose homeostasis, but the same GH-IGF-1 system exerts complementary effect on glucose regulation. As we discussed above, Insulin and IGF-1 share structural homology, which interact with the same membrane receptors by different affinities to mediate a wide range of metabolic and growth-promoting functions. Studies have shown that insulin was likely to decrease IGF-1 through differential modulation of IGF-binding proteins.[86,111] To reinforce this hypothesis, we found a good relation between IGF-1SD and AUCINS and insulinogenic index.

So, we can speculate that serum IGF-1 levels could be an important marker of βcell function and glucose metabolism as well as lipid metabolic responses.

Hepatic Steatosis is considered liver manifestation of metabolic syndrome and represents one of the major co-morbidities obesity related, frequently associated with impaired glucose metabolism. Studies have underlined that GH an IGF-1 concentrations are decreased in patients with HS and there is a relationship between low IGF-1 and hepatic dysfunction, as observed in our data.[136,173]

Over 50% of children presented ultrasonographic signs of liver disease. This condition was also characterized by higher inflammatory state. In fact, Hepatic Steatosis discovered in enrolled children was also characterized by higher levels of ESR vs not affected children. However, one of the most important findings was the relation between IGF-1 and HS, providing, once again, indications on the role of GH/IGF-1 axis. HS is often observed in patients with endocrine disease and particularly in the impairment of hypothalamic-pituitary axes.[174] Hepatic complications in adult GHD have emerged and replacement therapy drastically reversed this hepatic condition.[175]

Moreover, Liver-specific deletion of the GH receptor in mice resulted in a 90% reduction in serum IGF-I levels.[176] These evidences confirm the intricate relation between liver and somatotropic pathway. Restoration of IGF-1 through transgene into the hepatocytes improved overall insulin sensitivity and lipid profile, and reduced body adiposity, but was insufficient to protect against steatosis-induced hepatic inflammation and oxidative stress, suggesting a presence of direct and direct action of GH in hepatocytes.[177] So, the cross-talk is very complicated, but this point could be the start for new therapies for liver diseases.

All children with HS presented acanthosis nigricans. This simple data is an exhortation to observe always carefully the skin, as marker of obese-related metabolic complications. As known, acanthosis nigricans occurs when the concentration of insulin-like growth factor receptors in the skin is too low relative to the amount of insulin present, causing accumulation of insulin in the skin, proliferation of epidermal cells, and thickening of keratocytes.[178]

In connection with these observations, result on Hepatic Insulin Sensitivity Index (HISI). We focused on liver our attention because before diabetes is manifest, insulin resistance can occur separately and/or with different degrees in liver versus skeletal muscles as illustrated by different contributions. Hepatic insulin sensitivity and peripheral insulin sensitivity are strongly interrelated, but hepatic insulin sensitivity explains only about half of the variation in peripheral insulin sensitivity. IGF-1 was related to HISI and inversely correlated with insulin resistance indexes. The possible reasons of this correlation may be related to the structural homology between insulin and IGF-1, which interact with the same membrane receptors with different affinities to mediate a wide range of metabolic and

growth-promoting functions. Studies have also shown that insulin was likely to decrease IGF-1 through differential modulation of IGF-binding proteins.[114,179]

Another possible explanation for the association between IGF-1 and insulin pathway is driven by chronic inflammatory. Low levels of IGF-1 in the C3H.6T mice and in the liver-specific IGF-1 knockout mouse were associated with enhanced inflammatory phenotype.[180,181] Furthermore, we previously discussed about the relationship between IGF-1 and adiponectin and other cytokines, another link in this complex connection.

Obesity is characterized by low grade inflammation, this condition could drive insulin resistance by production of cytokines and by macrophage activation, involving also interaction between IGF-1 and insulin. Insulin has been considered to be pro-inflammatory in macrophages and has been reported to promote foam cell formation. By contrast, recent research showed that IGF-1 itself might have a fundamental role in macrophage activation and that somatomedin C has anti-inflammatory and antifoam cell formation effects.[182]

According to these, our results showed that levels of ESR, index of inflammation, were not only correlated with liver function indexes, but also that subjects with IGF-1SD <0 had higher levels of ESR, indicating a major inflammatory state. Even if the variables involved in this complex mechanism are really manful, we can postulate that low grade inflammation has an important role in pathogenesis of comorbidities obesity-related and in turn on GH/IGF-1 axis..

According to GH levels, even if no significant modifications were recorded in GH during OGTT, there was a correlation between GH120' and insulinogenic index, GH0' and HOMA index and its percentile. Hyperinsulinemia leads to change in GH secretion and GH levels, mostly by increasing the hypothalamic somatostatin release.[80] These aspects could be an important point of view in growing physiology of obese children.

Several cross-sectional studies have shown that obese children tend to be taller and to present an acceleration of pubertal and skeletal maturation compared to normal weight youth. Specifically, they have shown that during prepubertal years obese children have higher height velocity and accelerated bone age compared to their lean peers. However, this prepubertal advantage in growth tends to gradually decrease during puberty, when obese children show a reduced growth spurt compared with lean subjects. 80, [169] The higher growth in obese children appears to be mainly GH independent, but the reduction of puberal spurt is partially related to reduced GH secretion. GH secretion is, in fact, impaired in response to all traditional pharmacological stimuli acting at the hypothalamus. Thus, the evaluation of the axis is an important tool to define the growth trend of obese children. So, we can assert that the relation between anthropometric data and somatomedin C is so not less important.

Published data have shown a negative correlation between the increased BMI and peak height velocity, this is in line with our analysis.[96]

BMI z-score was negatively correlated with IGF-1 SD and this condition could partially explain the negative effect of adiposity on growth pattern. Limitation of this observation is the lack of bone age and the lack of a follow up period to calculate height velocity of enrolled children.

Thus, our data suggest that the assessment of IGF-1 might provide useful clinical information on severity and complications of obesity in children, as confirmed by HS and cardio-metabolic risk score. The GH/IGF-1 axis plays a central role in adapting to fasting by directing the substrate metabolism away from carbohydrate utilization to mobilization of lipids from adipose tissue and oxidation, particularly in skeletal muscles. Both genetic factors and environmental influences such as diet are associated with variations in IGF-1 levels in adults. [183] Moreover, GH and IGF-1 regulate metabolic, immune, and hepatic stellate cell function, and alterations in the production and function of GH is associated with obesity and HS.[184]

These results therefore suggest a complex and not fully defined relationship between the various elements of the metabolic syndrome and the somatomedin axis.

The observations presented so far lead to hypothesize a multifaceted, and still unclear, mechanism underlying visceral adipose tissue - metabolic syndrome - somatomedin GH axis, which includes the intervention of hyperinsulinism and relative insulin resistance, of low-grade inflammation and cytokines involved in obesity-related chronic inflammation. The functional reduction of the GH / IGF-1 axis, in turn, could act on body composition, causing unfavorable changes similar to those found in real GH deficiency (reduced muscle mass and increased visceral adipose tissue), contributing to worsening insulin resistance and its metabolic sequelae and fueling this complex vicious circle and maybe modifying also physical fitness. Status of GH deficiency is, in fact, known as condition of reduced aerobic capacity and physical performance.[185] If this status is transferred in obese children, we can postulate that the functional deficit of GH/IGF-1 axis should get worse physical activity and physical competence, increasing sedentary.

Thus, reassuming, GH secretion is consistently reduced in obesity, as a consequence, low GH secretion could further contribute to accumulation of abdominal fat. Increased circulating Free Fatty Acids levels and hyperinsulinemia in obesity may also have a suppressive effect on GH secretion, worsening the condition. The same low-grade inflammation, characterized by elevated circulating levels of TNF-α, IL-6, and other cytokines, could reduce GHR expression in the adipose tissue of obese subjects. [186,187]

The effect on IGF-1 production, instead, is not only influenced by GH. IGF-1 is produced in large amounts in the liver as well as in adipose tissue and a great part of its production is GH independent.

We so can assert that the liver, insulin secretion and low grade inflammation, once again could be an explanation of IGF-1 secretion pattern observed in obese. These clarifications, confirmed by several reports, are sustained by our results: children with lower levels of IGF-1 presented higher levels of ESR. It's very interesting to think about anorexia nervosa, condition opposite to obesity but in the same way inducing alteration in the axis. Subjects affected by anorexia has an increased production of GH and decreased levels of IGF-1, similar to obesity, condition induced probably by a GH resistance in peripheral tissues. These subjects, moreover, generally present higher levels of ghrelin. So, two very apparently different conditions are characterized by an overlapping state, confirming the role of adipose tissue as an endocrine driver in pathophysiology and endocrine disrupting.

Another state that explains the role of IGF-1 in insulin pathway is the leprechaunism, a rare genetic condition of extreme insulin-resistance: the treatment with recombinant IGF-1 improve metabolic and clinical parameters, preventing fatal outcomes.[188]

So our analysis showed that multiple mechanisms may explain GH-IGF-1 alterations in obesity: hyperinsulinemia, one of the stronger inhibitors of GH secretion by peripheral and central actions; free fatty acids (FFA) and leptin, both of which are increased in obesity. In the central nervous system, moreover, an important role is related to ghrelin that stimulates appetite. Ghrelin is also an important link between the regulation of energy homeostasis and the activity of the GH/IGF-1 axis. Ghrelin is a strong GH secretagogue. Plasma ghrelin levels inversely correlate with body mass index, thus, ghrelin levels are reduced in obese children. In the same way, leptin has pleiotropic activities, influencing pituitary hormones, such as thyroid- stimulating hormone (TSH) and GH. The hormone leptin (from the Greek word "leptos" meaning "thin") is a 167-amino acid peptide hormone encoded by the ob (obesity) gene and secreted by white adipocytes. Its discovery in 1994, has greatly improved our understanding of how the adipose tissue "communicates" with other systems in the body, in particular with the central nervous system.[189] Leptin not only links fat tissue with the CNS, but also to other tissues in the body.

To sum up, growth hormone secretion is decreased in obesity, and impaired somatotropic function in obesity is functional and may be reversed by weight reduction.There is an adipo-cerebral dialogue in pediatric obesity; adipocytes, far from being only fat deposits, are capable of endocrine functions, which assign them a leading role in neurohormones regulation involved in different processes. The plasticity of adipocytes and their dialogue with the central nervous system are so attractive fields of investigation and are a part of the concept of the "brain–adipose axis", introduced by Shimizu.[190]

We now know that both very low and high IGF-1 levels are related to increased cardiovascular and metabolic risk. Our hypothesis is that this system works in different levels according to physiological

or not physiological state, maybe involving also different affinity to IGF-1R according to IGF-1 and insulin levels.

The evaluation of IGF-1R polymorphism did not show differences in our sample: all selected children were wide type, so our findings suggest this kind of IGF-1R polymorphism do not contribute to the genetic susceptibility to insulin-resistance and metabolic disarrangement of glucose metabolism in enrolled obese children. Other IGF-1R polymorphism, probably, should be taken into account to better explain this pathway.

So, finally, we can assert that the evaluation of obesity, insulin sensibility and insulin resistance indexes in our childen should taken into account IGF-1 SD as additive marker of metabolic profile. In age of personalization of medicine and treatment, and precision medicine, we can suppose that IGF-1 could be an additive marker and, maybe, a decision tool for prevention and treatment of pediatric obesity.

The road is still open and leaves room for a myriad of speculations, so further studies are needed to better define this fascinating, how complex, path that connects somatomedins and obesity.

5 Conclusions

Experimental data indicate that IGF-1 could be implicated in the pathogenesis of MS and in the distribution of adipose tissue. Several studies have also correlated IGF-1 with cardiovascular risk. Therefore our thesis opens the way to new perspectives, in which visceral adipose tissue plays a key role by mediating, in an unclear way, its cardio-metabolic effects through the somatomedin system. The role of IGF-1 needs further examination whether it is a potential therapeutic target in obese patients of increased cardiometabolic risk or only an indicator of metabolic disorders. Further analysis are needed to clarify the main mechanism of this complex relation.

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