Review

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Childhood obesity: an overview of laboratory medicine, exercise and microbiome

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Abstract: In the last few years, a significant increase of childhood obesity incidence unequally distributed within countries and population groups has been observed, thus representing an important public health problem associated with several health and social consequences. Obese children have more than a 50% probability of becoming obese adults, and to develop pathologies typical of obese adults, that include type 2-diabetes, dyslipidemia and hypertension. Also environmental factors, such as reduced physical activity and increased sedentary activities, may also result in increased caloric intake and/or decreased caloric expenditure. In the present review, we aimed to identify and describe a specific panel of parameters in order to evaluate and characterize the childhood obesity status useful in setting up a preventive diagnostic approach directed at improving health-related behaviors and identifying predisposing risk factors. An early identification of risk factors for childhood obesity could definitely help in setting up adequate and specific clinical treatments.

Keywords: childhood obesity; exercise; laboratory medicine; microbiota; risk factors.

Introduction

Obesity is a complex multifactorial disease characterized by irregular or excessive fat accumulation and is a result of an imbalance between energy intake and energy expenditure combined with a genetic predisposition for weight gain [1, 2].

In the last decade, a significant increase of childhood obesity incidence unequally distributed within countries and population groups has been observed. In 2010, 43 million children worldwide were obese, thus childhood obesity represents an important public health problem associated with several health and social consequences [3, 4].

The first clinical standard measure to estimate the level of adiposity in overweight children is the body mass index (BMI), that requires being adjusted for both age and gender [2].

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Obese children show over 50% probability of becoming obese adults, while non-obese children show a probability of about 10% of developing pathologies typical of obese adults, that include type 2-diabetes, dyslipidemia and hypertension, and psychosocial and emotional issues [5, 6].

Reduced physical activity and increased sedentary activities, may also result in increased caloric intake and/or decreased caloric expenditure [7, 8].

In our review, we intende to identify and describe a specific panel of parameters that allows the evaluation and characterization of childhood obesity status (Figure 1) and, therefore, set up a preventive diagnostic approach directed to ameliorate health-related behaviors and identify predisposing risk factors.

In particular, we focus our interest on epigenetic changes that may influence the process of adipogenesis and on genetic factors predisposing to obesity. Moreover, we also describe how the main biochemical parameters and cytokines levels are altered in obese children, and also identify the most specific markers of inflammatory status. We also underline the influence of obesity on the development of a number of pathological conditions including endocrine disorders and increased cardiovascular risk; in addition, we focus on how obesity modulates the gut microbiome. Finally, we described therapeutic interventions widely used to regulate body fat.

The evaluation and the understanding of the comprehensive obesity scenario and the identification of effective markers may lead to an early identification of risk factors for childhood obesity in order to set up adequate and specific clinical treatments.

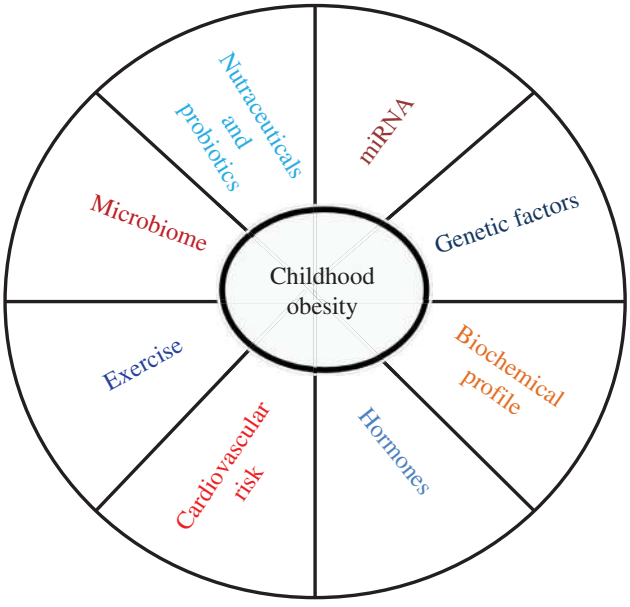

Figure 1: Scheme of diagnostic parameters aimed at evaluating childhood obesity.

miRNA

Recent studies showed that an altered regulation of microRNAs (miRNAs) causes their expression variations thus modifying the normal expression of genes involved in adipogenesis.

In particular, miRNAs represent key regulators of adipogenesis and metabolism processes and are involved in both differentiation of adipocytes and the regulation of mature adipocyte functions, including lipolysis, glucose uptake and insulin sensitivity [9, 10]. A large amount of evidence strongly suggest that dysregulation of miRNAs affects the status and function of different tissues and organs, probably contributing to metabolic abnormalities associated with obesity and obesity-related diseases [11].

MiRNAs are endogenous small non-coding RNAs 18–25 nucleotide-long that regulate gene expression through translation repression or degradation of target mRNAs at the post-transcriptional level, preferentially binding to the 3' UTR regions, therefore contributing to the regulation of many biological processes. Each mature miRNA is partially complementary to multiple target mRNAs and through RNA-induced silencing complex (RISC) may identify the target mRNAs for inactivation [12, 13].

More than 2000 different miRNAs described in humans and many studies show that miRNAs may influence the expression of 30%–50% of protein encoded by murine and human genes [14]. Some miRNAs have a tissue-specific expression and mRNA may include multiple binding sites for different miRNAs originating an intricate regulatory network. In fact, miRNAs are involved in many cellular processes such as cell proliferation, differentiation, control of stem cell self-renewal, DNA repair, apoptosis, metabolism, development and tumor metastasis [13, 15].

Recent studies underline the role of miRNAs in obese adults and children (Table 1). Several miRNAs, such as miR-130 or miR-27b, regulate adipogenic differentiation by targeting the expression of peroxisome proliferator-activated receptor-γ (*PPARγ*), the master regulator of adipogenesis [16]. Upregulation of miR-125b-5p during human adipogenesis directly inhibits this biological process, downregulating the anti-adipogenic matrix metalloproteinase 11 (*MMP-11*) [17]. In addition, Jiang et al.

Table 1: Summary of circulating miRNAs involved in childhood obesity.

miRNAs	Target/process	References
miR-130, miR-27b	Adipogenic differentiation targeting PPARγ	[16]
miR-125b-5p	MMP-11	[17]
miR-378	Adipocyte development and differentiation	[18]
miR-14	Insulin-stimulated AKT	[17]
miR-140-5p, miR-142-3p, miR-222	Morbidly obese patients	[19]
miR-532-5p, miR-125b, miR-130b, miR-221, miR-15a, miR-423-5p, miR-520c-3p	Morbidly obese patients	[19]
miR-23a, miR-27a, miR-130, miR-195, miR-197, miR-320a, and miR-509-5p	Metabolic syndrome	[11]
miR-122, miR-324e3p, miR-375, and miR-652 miR-625	Pre-gestational and gestational obesity	[20]
miR-335, miR-143, and miR-758, miR-27, miR-33, miR-378, and miR-370	Lipid metabolism	[21]
miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, miR-423-5p, miR-532-5p, miR-140-5p, miR-16-1, miR-222, miR-363, miR-122, miR-221, miR-28-3p, miR-125b and miR-328	Insulin resistance and lipid metabolism	[22]

observed that several adipokines, including interleukin (IL)-6, tumor necrosis factor (TNF)α, leptin, and free fatty acids, induce expression of miR-378 in human adipocytes [23]. miR-378 plays an important role in adipose differentiation, mitochondrial metabolism and systemic energy homeostasis. In particular, it stimulates the accumulation of triacylglycerol increasing the transcriptional activity of C/EBPα and C/EBPβ on adipocyte gene promoters; moreover, it is assumed that miR-378 being encoded within the *PGC-1β* (peroxisome proliferator-activated receptor γ coactivator 1β) gene, a transcriptional co-activator that regulates the metabolism and mitochondrial biogenesis, it can counterbalance the metabolic actions of PGC-1β [18]. Some evidence also shows that obesity induces overexpression of miR-14, which inhibits insulin-stimulated AKT activation leading to impairment of glucose metabolism [17].

The attention of latest studies is on the identification of extracellular circulating miRNAs in blood, serum and plasma; alteration of expression of miRNAs in pathological conditions offers a great potential for the identification of novel biomarkers and candidate therapeutic targets [24]. In fact, differential profiles of circulating miRNAs are reported in subjects with obesity and different metabolic disorders [11]. A study conducted by Ortega et al. showed in obese patients a marked increase of circulating miR-140-5p, miR-142-3p, miR-222 and a decrease of miR-532-5p, miR-125b, miR-130b, miR-221, miR-15a, miR-423-5p, miR-520c-3p. In the same study changes in circulating miRNA levels in individuals undergoing bariatric surgery with subsequent weight loss were evaluated; in these subjects a significant decrease of circulating miR-140-5p, miR-122, miR-193a-5p, miR-16-1 and an increase of miR-221 and miR-199a-3p were observed [19].

In a different study altered levels in circulating miR-23a, miR-27a, miR-130, miR-195, miR-197, miR-320a and miR-509-5p have been associated with metabolic syndrome [11]. Different patterns of circulating miRNAs have also been described in pre-gestational and gestational obesity. In fact, significant reduction of miR-122, miR-324e3p, miR-375and miR-652 and increased levels of miR-625 in both pregestational obese and gestational obese pregnant women have been observed [20]. Recently, Iacomino et al. identified three differentially expressed miRNAs (miR-31-5p, miR-2355-5p and miR-206) in plasma samples of obese children compared to controls [25]. In a recent study, Can et al. reported that miR-335, miR-143, and miR-758 levels were lower in obese children, whereas miR-27, miR-33, miR-378 and miR-370 levels resulted in higher levels. The different expression of these circulating miRNAs may be responsible for the high triglycerides (TGs) and low-density lipoprotein-cholesterol (LDL-C) levels, and for the low level of high-density lipoprotein-cholesterol (HDL-C) [21]. In a study conducted by Prats-Puig et al. the changes in plasma circulating miRNA levels in prepuberal obese children were evaluated showing altered levels of specific miRNAs, i.e. an increase of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, miR-423-5p, miR-532-5p, miR-140-5p, miR-16-1, miR-222, miR-363 and miR-122 and a reduction of miR-221, miR-28-3p, miR-125b and miR-328. Moreover, the circulating levels of these miRNAs were significantly associated with BMI, percent fat mass and waist circumference (WC), parameters of fat distribution and with laboratory variables, such as homeostasis model assessment of

insulin resistance (HOMA-IR), high-molecular-weight adiponectin, C-reactive protein (CRP), and circulating lipids. In the same study, during patient follow-up, changes in plasma concentrations of the same circulating miRNAs were observed as a result of BMI increase or decrease in obese children [22].

In addition, a large number of miRNAs are associated with pathogenesis of IR, endothelial dysfunction, serum lipid level alterations, inflammation and other metabolic dysfunctions in children. The discovery that some miRNAs are enriched in the hypothalamus suggest their primary role in the hypothalamic regulation of energy intake, expenditure and body weight control [17].

Interestingly, some studies also indicate that miRNAs may be regulated by diet and lifestyle factors [26, 27]. MiRNAs therefore represent an exciting, novel and promising class of markers that could improve the early diagnosis, stratification and therapy of metabolic diseases such as obesity.

The biological role of miRNAs in obesity could be well clarified through the use of bioinformatics tools such as miRBase, miRTarbase, miRanda and others that allow predicting their involvement in important pathways such as lipid metabolism and adipocytes differentiation and to identify their target [25].

The very early detection of changes in circulating miRNA levels represent a promising strategy for characterizing obese children and evaluating how dietary factors may influence obesity through the modulation of expression of miRNAs. In fact, the presence of risk factors at an early age is associated with the subsequent development of metabolic diseases in adulthood and the prevention of obesity in children can improve their health and reduce the development of obesity-related complications in adults.

Genetics and epigenetics of obesity

Genetic factors play a relevant role in obesity risk, with percentages ranging from 6 to 85% [28]. Studies on twins have been conducted to understand the genetic component of obesity showing that monozygotic twins subjected to excessive nutrition and regular exercises present similar changes in body weight, body composition and energy expenditure compared to dizygotic twins [29]. These results put forward the inheritance of obesity and adiposity [30]. Genetic predisposition of obesity has been classified into monogenic syndromic, monogenic non-syndromic and polygenic with main effects on body fat mass [31]. Monogenic obesity is a rare condition caused by recessive mutations in a single gene. In particular, monogenic obesity is divided into non-syndromic, when it is not associated with syndromes and it involved genes encoding for enzymes and receptors with a physiologic role in the development of the hypothalamus and the leptin melanocortin system, and syndromic when it is associated with different syndromes such as Bardet-Biedl, Alström, Prader-Willi and Carpenter syndromes, and microdeletion syndromes (e.g. 1p36, 2q37, 6q16 and 9q34) [31, 32]. Polygenic forms represent the majority of cases of obesity attributable to genetic interactions with environmental, cultural and lifestyle factors. In fact, obesity is rarely caused by alterations in single genes, being the result of a complex genetic background in which the effect of genes on the pathogenesis are stronger when combined with additional genetic alterations and environmental factors [32].

Genome-wide association studies (GWAS) have allowed the identification of more than 250 genes/loci associated with obesity, discovering new alterations with previously unknown effects.

Among these, variants of the fat mass and obesity-associated gene (*FTO*) gene showed an important role in obesity and pathogenesis developing type 2-diabetes, as these variations were associated with a higher BMI, fat mass index and leptin concentrations during puberty; in addition, they have a role in the regulation of appetite, probably through the influence of nearby genes such as retinitis pigmentosa GTPase regulator-interacting protein-1 like (*RPGRIP1L*) and Iroquois homeobox 3 (*IRX3*). GWAS studies allowed the identification of a locus near the gastric inhibitory polypeptide receptor (*GIPR*), the incretin receptor, that involves a variation in postprandial insulin secretion contributing to the development of obesity and sustaining the involvement of the gut microbiome in obesity [31].

In order to identify new genetic variations, high throughput molecular diagnostics tools, such as array-comparative genomic hybridization (a-CGH) and next generation sequencing (NGS) technology, are extremely useful tools [33–37]. These approaches have allowed identifying the association of many genes with obesity that play important biological roles in this disorder. A study conducted by Locke et al. identified 97 loci associated with BMI, 56 of which were previously unknown. Many of these loci were in or near genes that play a role in different biological processes, such as neuronal development: fas apoptotic inhibitory molecule 2 (*FAIM2*), polypyrimidine tract binding protein 2 (*PTBP2*) and suppression of tumorigenicity 5 (*ST5*), hypothalamic expression and regulatory

function: transmembrane protein 18 (*TMEM18*), secretogranin III (*SCG3*) and gastrin releasing peptide (*GRP*); limb development: retinoic acid receptor-β (*RARB*), transcription factor AP-2β (*TFAP2B*) and LIM homeobox transcription factor 1β (*LMX1B*), lipid biosynthesis and metabolism cytochrome P450 family 27 subfamily A member 1 (*CYP27A1*), cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*) and subfamily 1 group H member 3 nuclear receptor (*NR1H3*), cell proliferation and survival: parkin RBR E3 ubiquitin protein ligase (*PARK2*) and olfactomedin 4 (*OLFM4*) and immune system: interleukin 22 receptor subunit α2 (*IL22RA2*), mannosidase α class 1A member 1 (*MAN1A1*), interferon-γ receptor 1 (*IFNGR1*). Other genes have previously been associated with severe early-onset obesity: brain derived neurotrophic factor (*BDNF*), melanocortin 4 receptor (*MC4R*), SH2B adaptor protein 1 (*SH2B1*), TUB bipartite transcription factor (*TUB*) and proopiomelanocortin (*POMC*). It emerged that the common variants of these 97 loci affect almost 21% of cases of obesity [38]. Moreover, several studies have identified polymorphisms, within genes earlier known to be involved in monogenic obesity that also contribute to polygenic obesity. For example, mutations with homozygous loss of function in the leptin (*LEP*) gene or of its receptor LEPR are associated with hyperphagia and severe early-onset obesity in humans [39]. Proprotein convertase subtilisin/kexin type 1 gene (*PCSK1*) encodes the prohormone convertases 1 (PC1/3) expressed in neural and endocrine tissues and are highly expressed in the hypothalamus where they act on cleavage processing of POMC to α-melanocyte-stimulating hormone (α-MSH) [40]. PC1/3 deficiency is associated with hyperphagia, central diabetes insipidus, severe malabsorptive diarrhea and other endocrines dysfunctions while null mutations in *PCSK1* cause a rare non-syndromic form of obesity [41]. POMC is a pro-peptide expressed in the hypothalamus, pituitary gland and brainstem and generates a family of melanocortin peptides such as the adrenocorticotropic hormone (ACTH) and α-, β-, and γ-melanocyte-stimulating hormones (MSH), with important roles in skin pigmentation, the control of adrenal growth and energy balance. Mutations that inactivate the *POMC* gene lead to severe early-onset obesity in children, adrenal insufficiency and pigmentation abnormalities [42].

Inactivating mutations at the heterozygote status in genes such as *BDNF*, neurotrophic receptor tyrosine kinase 2 (*NTRK2*), SIM bHLH transcription factor 1 (*SIM1*), *MC4R*, *SH2B1*, melanocortin 2 receptor accessory protein 2 (*MRAP2*) and LDL receptor related protein 2 (*LRP2*) have also been associated with severe early-onset obesity [32].

A study conducted by Lee showed that genetic and epigenetic modifications of protein tyrosine phosphatase, receptor type N2 (*PTPRN2)* gene could also play an important role in the development of obesity in childhood. *PTPRN2* is localized on the membrane of insulin-containing dense-core vesicles and is involved in insulin secretion in response to glucose stimuli and in the metabolism including obesity and type 2-diabetes. Genome-wide copy number variation (CNV) analysis has led to the identification of novel candidate loci including *PTPRN2* associated in pediatric obesity. The *PTPRN2* gene is epigenetically regulated in several processes such as tumor pathogenesis and is significantly more hypermethylated in obese cases with respect to controls [43]. Chen et al. reported that the intrauterine environment in the human placenta influences the different gene expression and methylation of *PTPRN2* and consequently the prenatal growth patterns and birthweight [44]. These results showed a correlation between genetic and epigenetic events of the *PTPRN2* gene and childhood obesity. An additional array-CGH analysis of 100 children with syndromic obesity, defined the association with at least another feature such as intellectual disability (ID), facial dysmorphism or congenital malformations. Children between 1 and 18 years old all presented obesity and at least one other criterion such as ID, facial dysmorphism or a major malformation. Array-CGH performed in this cohort identified 60 CNVs in 42% of children and 22% of patients with pathogenic or potentially pathogenic CNVs. The CNVs identified in this study are: (a) deletion in 1p36.3 implicated in obesity found in the patient together with one duplication in 1q25.3 including the prostaglandin-endoperoxide synthase 2 gene (*PTGS2*), deficiency which was associated with reduced adiposity in mice; (b) 2p25.3 deletion associated with ID and obesity; (c) 1q21.1 susceptibility locus associated with several features, metabolic syndrome and obesity; (d) 16p11.2 distal deletion including *SH2B1* associated with developmental delay and obesity; (e) 17p13.3 duplication associated with developmental delay, macrosomia, overweight and characteristic facial abnormalities. Additionally, other recurrent loci not always associated with obesity were found in this cohort and considered as clinically relevant: 16p13.1 duplication associated with neuropsychiatric disorders with a small 9p21.1 deletion including the leucine rich repeat and Ig domain containing 2 gene (*LINGO2*) potentially associated with obesity in the same patient, 3q29 locus associated with mild to moderate ID and sometimes with obesity and includes the 3-hydroxybutyrate dehydrogenase 1 gene (*BDH1*) encoding a protein which catalyzes the interconversion of two major ketone bodies produced during fatty acid catabolism together with one deletion in

4q32.2q32.3 encompassing the methylsterol monooxygenase 1 gene (*MSMO1*) linked to plasma lipid concentration and cholesterol biosynthesis, the minimal critical region of the 3q13.31 microdeletion syndrome associated with ID but also with obesity, 22q11.2 "distal" deletion associated in some cases with obesity and duplication in 15q11.2q13.1 containing the imprinted genes involved in Prader-Willi and Angelman syndromes in the same patient and finally a child with Asperger syndrome that had one deletion in 7p21.3 including mitochondrial complex associated *NDUFA4* (NDUFA4), a gene highly expressed in brain and associated with autism. The following genes included in these CNVs were of particular interest: suppressor of cytokine signaling 6 (*SOCS6*), perilipin 2 (*PLIN2*), cadherin 13 (*CDH13*), contactin associated protein like 2 (*CNTNAP2*), SRY-box 3 (*SOX3*), and acyl-CoA oxidase like (*ACOXL*) [45].

These studies sustain an association between obesity and ID due to common genes involved in neurodevelopment that may affect the central circuits involved in the energy balance [45]. As obesity is characterized by genetic heterogeneity and multifactorial etiology, there are several causes that contribute to the pathogenicity of the disease, thus further studies are necessary to identify the correlation between new loci and obesity and to discover new variants. Furthermore, adequate molecular diagnosis of monogenic obesity is fundamental for patients and their relatives, while the clinicians could provide appropriate genetic counseling to the patient in order to improve their living conditions.

Biochemical profile

The evaluation of more biochemical parameters may be informative as early markers for the persistence of obesity from child to adult. Among other alterations, dyslipidemia is commonly found in children with obesity. Cook et al., reported that 45.8% of overweight children showed high levels of LDL and TGs and low levels of HDL with a higher prevalence in boys [46].

Non-HDL-C is a better indicator of persistent dyslipidemia and atherosclerosis in children and adults [47]. Typically, dyslipidemia in obesity is characterized by a high triglyceride level and low HDL level.

Obese children, who consume large amounts of sugar-sweetened beverages, show TG levels ranging from 150 to 400 mg/dL. These patients respond typically to dietary modification; if there is no response to diet, further evaluation is needed. The TG/HDL ratio is correlated to IR and early organ damage (heart, liver and carotid) [48–50].

Also, liver disease is a significant comorbidity for obesity at a young age. Elevated alanine aminotransferase (ALT) levels have been considered a marker for non-alcoholic fatty liver disease (NAFLD) in obese children. The prevalence of NAFLD in obese children is around 40% [51]. Bright liver on ultrasound examination, with or without elevation of ALT (>26 U/L in boys and >22 U/L in girls), suggests NAFLD [52–54]. However, determination of ALT alone may underestimate the extent of liver injury. Elevated levels of liver enzymes appear to be seen with increased age and WC. NAFLD is more prevalent in children with a higher WC and is directly proportional to age, weight and height [55, 56].

The patients' family history is a significant predictor for a number of co-morbidities. Parental obesity could be considered a critical factor in the development of obesity due to genetic factors and family food choices. Evaluation of transaminases is suggested in all children and adolescents with obesity starting at the age of 6 years [57–60]. NAFLD may also be screened in overweight children presenting the waist-to-height ratio >0.5, and the assessment yearly repeated [61].

Moreover, in obesity, the expanded and inflamed adipose tissue causes an imbalance in the secretion of cytokines, adipokines, growth factors and other biological mediators that have a particular role in liver fibrosis [62–64]. In particular, TGF-β1, PDGF-BB, leptin, and ferritin induce the production and accumulation of extracellular matrix (ECM) which has a crucial role in liver fibrogenesis [65] involving three major signaling pathways including MAP kinase, PI3K/Akt, and NF-κB. Liver fibrosis can be modulated by positively promoting resolution. The primary target for therapeutic strategy is to reduce chronic parenchymal injury induced by the primary etiology. For this purpose, several antioxidants and hepatoprotective agents have been reported to significantly increase liver fibrosis in rodent models (vitamin E, glutathione, N-acetylcysteine, S-adenosyl-methionine, resveratrol, curcumin, herbal supplements, inhibitors of NADPH oxidase isoforms and many others) [66, 67]. Several non-invasive tests have been made available for the assessment of liver fibrosis, such as the aspartate transaminase to platelet ratio index, enhanced liver fibrosis, FIB-4, FibroTest, Forns index and FibroScan [68–70].

Therefore, the identification of targets for anti-fibrotic therapy and non-invasive methodologies and biomarkers could be useful in monitoring the fibrotic progression of liver in obesity, and potentially the response to treatment [65, 71].

It is essential to evaluate a possible impairment in glucose metabolism in children with obesity. Fasting

blood glucose determination is recommended in all overweight children and adolescents starting at the age of 6, as the first step for the identification of prediabetic conditions and type 2-diabetes. The oral glucose tolerance test (OGTT) is indicated after the age of 10 or at the onset of puberty. Impaired fasting glucose is determined as a fasting plasma glucose of ≥100 mg/dL but <126 mg/dL on repeated determinations. Impairment in glucose tolerance is defined as plasma glucose ≥140 mg/dL but <200 mg/dL on an OGTT at a 2-h timepoint [72].

Type 2-diabetes is the endpoint of metabolic decompensation that may evolve over months to years. In IR, the body can produce insulin but the muscle, fat and liver do not respond appropriately and thus cannot easily absorb glucose from the bloodstream, with progression from IR to prediabetes with impaired glucose tolerance (IGT) and impaired fasting glucose over time [73].

NAFLD, in combination with a high value of TGs, fasting blood glucose or TG to HDL-C ratio (TG/HDL-C) is associated with an increased risk of IGT and therefore, an OGTT may be considered in the latter cases [72, 74]. Besides, markers of hepatic fat content (serum γ-glutamyltransferase [GGT] activity and other liver enzymes) have been shown to predict the incidence of type 2-diabetes, IR and cardiovascular disease independently of obesity [75, 76].

Screening for a kidney profile is not recommended in non-diabetic and non-hypertensive children and adolescents with obesity. However, in adults, obesity is an independent risk factor for chronic kidney disease. Indeed, obesity complications (i.e. hypertension, dyslipidemia, IR, type 2-diabetes, inflammatory state, autonomous system dysfunction) can alter kidney function. The obesity-related glomerulopathy takes place in obese patients, and ameliorates after weight loss [77, 78]. Therefore, obesity is likely to be a risk factor for chronic renal disease in children too. Indeed, children with the renal disease have BMIs higher than the healthy population and kidneys transplanted from obese donors have reduced glomerular filtration and a higher rate of dysfunction than the kidneys obtained from regular weight donors [79, 80]. In the light of current evidence, assessment of microalbuminuria is not recommended in non-diabetic and non-hypertensive obese children. Cases of severe obesity that may be associated with proteinuria remain to be evaluated individually [81–85].

The most common metabolic complication in children with excess weight is IR. An association between obesity and IR and between obesity and diabetes has also been demonstrated in children, also IR has been recognized as a condition preceding the onset of type 2 diabetes (in adults). IR in humans does not seem to depend on obesity, as severe IR also characterizes patients lacking subcutaneous fat, such as those with lipodystrophy [86]. A linear correlation has been found between liver fat content and direct measures of hepatic IR independent of obesity. Thus, fatty liver might help to explain why some, but not all, obese individuals have IR and why even lean individuals may be IR and thereby at risk of developing diabetes-related metabolic disorders. Growing evidence suggests that GGT is not only a marker of fatty liver but also a marker of oxidative stress. Probably the occurrence of GGT-mediated redox reactions plays a direct role in the pathogenesis of atherogenic dyslipidemia and poor glycemic control, independently of the presence of fatty liver, possibly through the induction of chronic inflammation and IR. It is therefore possible that obesity itself may not be a sufficient risk factor for atherogenic dyslipidemia or poor glycemic control in type 2 diabetes [75, 87].

Insulin metabolism is tightly linked with the insulin-like growth factor (IGF) system, an evolutionarily conserved group of factors exerting long-term effects on growth. IGF-1 regulates cell proliferation, differentiation, migration and survival in cells.

Hyperinsulinemia leads to higher bioavailability of free, active IGF-1 by downregulating the synthesis of IGF binding proteins (IGFBP-1, IGFBP-2) and by upregulating hepatic IGF-1 synthesis. Different biomarkers, including fasting insulin, C-peptide, which is cleaved from proinsulin and is considered an indicator of endogenous insulin secretion with a longer half-life than insulin itself and IGF-1 as well as IGF-binding proteins have been investigated. Fasting insulin and C-peptide have been shown to correlate positively with BMI. However, the relationship of IGF-1 with obesity is less evident because of the above-described effects of obesity on IGF-1 synthesis on the one hand, and IGF-1 bioavailability on the other hand. Studies relating to total IGF-1 to obesity found inverse associations with BMI. Few studies investigated obesity or BMI as a determinant of free IGF-1, but a small cross-sectional study showed that free IGF-1 but not total IGF-1 was higher in obese than in normal-weight individuals. In a meta-analysis of prospective cohort studies, higher fasting insulin concentrations were associated with higher risk of hypertension and coronary heart disease (CHD) but not with stroke. C-peptide has been shown to predict total and cardiovascular mortality in non-diabetic individuals better than other measures of IR including fasting insulin, blood glucose and the HOMA-IR [88]. The biochemical parameters mentioned should be useful to characterize the childhood condition, to evaluate the risk of the disease and also follow-up the pathology progression.

Cytokines

Adipose tissue is a complex and dynamic endocrine organ active in cellular reactions and metabolic homeostasis that synthesize and release hormones, cytokines, extracellular matrix proteins and growth and vasoactive factors, collectively termed adipokines. Mechanisms underlying adipose tissue dysfunction include: adipocyte hyperplasia and hypertrophy with adipose tissue expansion, immune cells infiltration into the adipose tissue and ECM remodeling allowing adequate tissue expansion to adapt the excessive caloric intake [89]. The adipose tissue expansion induces activation of the immune response both innate and adaptive. Several studies revealed an infiltration of different immune cells such as lymphocytes, eosinophils, neutrophils, mast cells, foam cells [90] and in particular an increase in the number of macrophages in adipose tissue of obese subjects [91]. These changes in number and function of immune cells promote adipose tissue inflammation in obesity. The innate immune system is the first line of defense in response to injury or pathogens and comprises principally monocytes and macrophages [92]. In obese subjects, macrophages are increased in adipose tissues; in these cells a polarization towards M1-type macrophages with a pro-inflammatory phenotype is observed together with the secretion of pro-inflammatory cytokines. Adipose tissue macrophages are classified into two types: M1 or "classically activated" macrophages and M2 or "alternatively activated" macrophages. M1 macrophages are the first line of defense against intracellular pathogens and secretes inflammatory cytokines including interleukins IL-1, IL-6, IL-12, IL-1β, monocyte chemoattractant protein (MCP)-1, inducible NOS (iNOS) and TNF-α. M2 macrophages are implicated in inflammation resolution and secretes interleukins IL-4, IL-10, and transforming growth factor-α (TGF-α). Multiple studies have shown that inflammation in obesity is associated with an increase of M1 macrophages and a decrease of M2 macrophages in adipose tissue. Macrophages are characterized by plasticity and change rapidly in response to external stimuli. In fact, humans and murine models with a dominant M2 phenotype show a "phenotypic switch" towards an M1 phenotype under the stress of obesity [89, 93]. The adaptive immune system, that include T cells, B cells, natural killer cells (NK), natural killer T cells (NKT) and type 2 innate lymphoid cells (ILC2), plays an important role in the obesity, and is critical for specific immune responses and the development of immune response memory. T lymphocytes consist mainly of CD4+ T cells and CD8+ T cells. In adipose tissue, CD4+ T cells are generally classified into the T helper 1 (Th1) and T helper 2 (Th2) but also include T helper 17 (Th17), induced T regulatory cells (iTreg) and regulatory type 1 cells (Tr1). Th1 and Th17 cells release interferon-γ (IFN-γ) and IL-17 stimulating pro-inflammatory M1 macrophage functions and the release of IL-6 and TNF-α. On the other hand, anti-inflammatory Th2 cells and Treg cells produce IL-4, IL-10 and IL-13 promoting macrophage differentiation towards an M2 phenotype. During adipose tissue expansion the imbalance between the relations of Th1:Treg and Th1:Th2 cells are responsible for a shift of macrophages to M1 macrophage phenotype; this contributes to start and perpetuate adipose tissue inflammation [89, 94]. Secretion of proinflammatory cytokines from adipose tissue causes a persistent inflammatory state associate with the risk of developing adverse outcomes in obesity-linked complications. Studies conducted on obese people and animals show a higher level of serum TNF-α, IL-1β and IL-6 produced by macrophages of adipose tissue. These cytokines regulate proliferation and apoptosis of adipocytes, promote lipolysis, inhibit lipid synthesis and decrease blood lipids through autocrine and paracrine mechanisms. The mentioned mechanisms will be predisposed to decrease lipid accumulation in the adipose tissue. Circulating concentrations of TNF-α are greater in obese subjects while concentrations of TNF-α and IL-6 are reduced following weight loss. TNF-α induce apoptosis in preadipocytes and adipocytes, block differentiation and induce de-differentiation in preadipocytes reducing adiposity [95]. On the other hand, an increase of IL-6 in obese subjects stimulates liver secretion of CRP, an acute phase protein of inflammation that increases in obese individuals. CRP represents a marker for the early diagnosis of metabolic syndrome and cardiovascular risk in obese children [96]. However, studies investigating the links between TNF-α, IL-6 and obesity in children, have shown inconsistent findings. For example, a study conducted on obese Caucasian subjects showed that IL-6 levels were not increased [97] and another study in overweight/obese Hispanic children reported raised levels of CRP and TNF-α, but not IL-6. Being that IL-6 is secreted from adipose tissue and induces the hepatic production of CRP, both values should increase in obese children [98]. Moreover, high levels of IL-6 have been found in overweight/obese 15-year-old girls compared with healthy weight girls, but there was no differences observed in 8-year-old subjects and in boys [99]. Discrepancies between CRP and IL-6 might originate from a non-strictly rigorous pre-analytical phase or inaccurate analytical phase, thus underestimating the measurement of IL-6.

The evaluation of different cytokine levels in obese children considering age, sex and ethnicity could better define the relationship between obesity and inflammation.

Furthermore, it has been shown that adipokines and myokine hormones, such as irisine, produced by adipose tissue and skeletal muscle have a critical role in the regulation of energy metabolism and inflammation [100]. In fact, the adiponectin Acrp30 has anti-diabetic, anti-atherogenic and anti-inflammatory properties [101, 102] while irisin plays a role in the maintenance of energy balance increasing energy expenditure and improving glucose homeostasis [103]. Dysregulation of Acrp30, its high molecular weight (HMW) oligomers, and irisin may be involved in the development and progression of obesity and IR in adults and children.

Moreover, as in adults, concentrations of leptin results are higher in the serum of obese children and its increase is related to the body fat and BMI. Leptin is a hormone of a protein nature that acts on receptors present at the hypothalamic level by regulating body weight; in fact, it causes a decrease in appetite and an increase in energy expenditure. It was hypothesized that there is a leptin resistance in prepubertal children due to an higher energy requirements and only at the end of puberty phase the leptin resistance return to normal level. This data makes leptin resistance in the prepubertal era, necessary for normal growth and development, allowing greater energy storage essential for growth. Elevated leptin levels in obese children could represent a consequence of a leptin resistance alteration necessary for a normal development [104].

All these data could improve our knowledge of the physiopathology of this complex and multifactorial disease thus leading to a new scenario for a more accurate diagnostic approach.

Hormones

In a small number of children showing a sudden onset of obesity, an endocrinopathy should be suspected [105, 106]. In particular, hypothalamic obesity, hypothyroidism, hypercortisolism, growth hormone deficiency (GHD) or pseudohypoparathyroidism could be hypothesized. Early recognition of an endocrine disease allows physicians to start effective therapies with relevant benefits for the patients. The following clinical scenarios are commonly found [107]:

(a) Hypothalamic obesity. Congenital defects or injury to the hypothalamus (i.e. surgery for craniopharyngioma, trauma, pituitary tumors) which damage ventromedial, arcuate, paraventricular and dorsomedial nuclei of the hypothalamus can cause obesity. These hypothalamic nuclei release neuropeptides which regulate appetite and energy consumption. In these patients, other endocrine diseases are commonly found such as GHD, hypothyroidism and precocious or late puberty [106, 108].

(b) Hypothyroidism. Mild BMI gain and linear growth decrease associated with high thyroid-stimulating hormone (TSH) and low thyroxine (T4) levels are suggestive of hypothyroidism. In such a case diminished energy consumption and water retention lead to weight increase [109]. Moreover, TSH is able to bind its receptor on fat tissue, promoting adipogenesis and increase in size of adipose tissue [110, 111]. In obese children, higher triiodothyronine (T3), but not T4 circulating levels were found. Free T4 and free T3 were normal or slightly elevated, suggesting an adaptive response of thyroid function to increased BMI in order to reduce further weight gain [112, 113]. However, T3 increase was not associated with pathological conditions such as hypothyroidism, iodine deficiency and autoimmune thyroiditis [114].

(c) Hypercortisolism. Children that present central obesity with growth deficiency, hirsutism and hypertension are suspected of hypercortisolism. An endogenous increase of cortisol in children is frequently a consequence of pituitary microadenoma in children over 6 years of age and of adrenal hyperplasia or tumor in younger patients. Moreover, long-term treatments with cortisone (as in the case of autoimmune, dermatological or respiratory diseases and, rarely, in pediatric cancer) may lead to iatrogenic hypercortisolism [115]. In children, high glucocorticoids levels have a significant impact on bone, leading to a decrease in linear growth [116]. This clinical feature allows early suspicion of the underlying disorder.

(d) GHD. Children with GHD exhibit mild truncal obesity, decreased growth rate and short stature. To confirm GHD, provocative tests have to be performed, after exclusion of thyroid dysfunction, sex steroid alterations and hypercortisolism [117]. Because of their low specificity, these tests have to be performed only in those children where the probability of finding GHD is high [118]. Low circulating levels of IGF-1 and insulin-like growth factor binding protein-3 (IGFBP3) are strongly suggestive of GHD [119, 120].

(e) Albright hereditary osteodystrophy (AHO). Mutations inactivating the guanine nucleotide-binding protein G(s) subunit (GNAS), the gene coding for α chain of Gs, cause AHO. These patients present short stature, round facies, early-onset obesity, developmental delay and mental disabilities, multihormone resistance (e.g. PTH, TSH, gonadotropins, and glucagon)

[121] and pseudohypoparathyroidism types 1a [106, 108, 122–124]. The unexpected weight gain in these children seems to be caused by mild hyperphagia and increased food intake [125]. In addition, some authors reported that these patients have a reduced energy consumption [126, 127].

Furthermore, GHD (caused by GHRH resistance) [128] and hypothyroidism (from TSH resistance) [129] also contribute to weight gain in PHP1a. Laboratory tests in these patients show a picture of PTH resistance: elevated PTH and phosphate circulating level and hypocalcemia [130, 131]. Collectively, there are several alerts that have to be taken into account to hypothesize an endocrine cause of childhood obesity: in fact, stature and height increase are slowed down and bone age is delayed [132]. A regular growth rate generally is not related to endocrine causes [133, 134]. A short stature suggests GHD, central obesity arising in early childhood (<6 years) associated with growth deficiency may indicate hypercortisolism, mild hyperphagia associated with short stature, and early-onset obesity, may be associated with AHO [135].

In obese children presenting these clinical features, an appropriate laboratory work-up including thyroid function evaluation, ACTH stimulation testing, dexamethasone stimulus and serum, salivary, 24-h urine free cortisol need to be performed [136]. This kind of approach can identify specific endocrine disorders. Consequently, an adequate treatment strategy can be adopted and a physiological condition including normal growth can be restored.

Cardiovascular risk

There is robust evidence that adult obesity is a risk factor for atherosclerotic diseases and cardiovascular events [137, 138], mainly as a result of its strong association with the risk of developing arterial hypertension, dyslipidemia and type 2-diabetes [139–142]. Obesity, indeed, is also associated with morbidity during developmental age: arterial hypertension, dyslipidemia, glucose intolerance and diabetes; they are not exclusive for adult obesity, but can also begin to increase cardiovascular risk from childhood in children with severe obesity [2, 143].

The combination of obesity and hypertension is a complex and multifactorial condition that seems to involve IR, activation of the sympathetic nervous system, the renin-angiotensin system, abnormal renal sodium retention, possible resistance to leptin and altered vascular reactivity, and alterations of the hypothalamus-pituitary-adrenal axis [140, 144, 145]. The most common metabolic complication in children with excess weight is IR [141]. An association between obesity and IR and between obesity and diabetes has also been demonstrated in children, IR has also been recognized as a condition preceding the onset of type 2 diabetes (in adults).

To date, a great deal of scientific evidence supports the close association between childhood obesity and the development of increased cardiovascular risk, with long-term follow-up [146–149]. Data from a broad meta-analysis [146], show that childhood obesity is significantly and positively associated with adult systolic blood pressure (Zr = 0.11; 95% CI: 0.07, 0.14), diastolic blood pressure (Zr = 0.11; 95% CI: 0.07, 0.14), high TG levels (Zr = 0.08; 95% CI: 0.03, 0.13), and significantly and inversely associated with adult HDL (Zr = −0.06; 95% CI: −0.10, −0.02) [146]. Juonala et al. [150] analyzed data from four prospective cohorts (two North Americans, one Australian and one Finnish). They measured BMI both in childhood and in adulthood, to establish whether a change from obesity during childhood to a non-obese BMI in adulthood, as compared with obesity during childhood that persists into adulthood, would be correlated to a reduced risk of cardiovascular and diabetes diseases. The study cohort consisted of 6328 subjects, and the mean length of follow-up was 23.1 ± 3.3 years. Only 14.6% of children with normal weight have developed adult obesity, while 65% of overweight children and 82% of obese children have developed adult obesity. Patients with a persistent high adiposity status from childhood to adulthood, showed an increased risk of type 2 diabetes (DM2), as compared with normal BMI subjects both in childhood and in adulthood (relative risk [RR]: 5.4; range 95% confidence interval [CI]: 3.4–8.5, $p < 0.001$). They showed an increase risk of arterial hypertension (RR 2.7, 95% CI 2.2–3.3, $p < 0.001$), high LDL-C (RR 1.8, 95% CI 1.4–2.3, $p < 0.001$), low HDL-C (RR 2.1, 95% CI 1.8–2.5, $p < 0.001$), elevated TG levels (RR 3.0, 95% CI 2.4–3.8, $p < 0.001$), and increased carotid-artery intima-media thickness (RR 1.7, 95% CI 1.4–2.2, $p = 0.002$). Adult subjects with normal BMI but childhood obesity, had a similar cardiovascular risk as compared with subjects with normal BMI in both childhood and adulthood [150]. The increased incidence of development of cardiovascular risk factors is expressed in an increased incidence of cardiovascular events, mainly concerning CHD [149]. Ohlsson et al. [148] conducted a study exclusively on male subjects; overweight boys during adolescence and overweight boys during childhood and youth, which showed they had an increased risk of cardiovascular mortality compared with normal-weight subjects (hazard ratio [HR] 2.39, 95% CI 1.86–3.09; HR 1.85, 95% CI 1.28–2.67; respectively).

Childhood obesity involves an increased risk of CHD, which may be mediated by cardiovascular risk factors [151]; in fact, the cumulative burden of this conventional risk factors in this setting patients may alter premature steps in atherosclerosis [152]. Moreover, the development of these risk factors begins very early and also in the initial degrees of obesity. In a large prospective cohort study, data from 5235 children aged 9–12 years at baseline, were analyzed [153]. The prevalence of cardiovascular risk factors at age 15–16 were higher in obese subjects group at baseline [153]. De Kroon et al. [154], have analyzed data from 642 subjects from the Terneuzen Birth Cohort. Results showed that BMI change from 2 to 18 years are related to increased cardiometabolic risk at young adulthood, the age interval 2–6 year being the most predictive [154]. Zabarsky et al. [155], have investigated obesity-associated morbidity incidence in severely obese youth. They divided a childhood obesity cohort into degrees of obesity (classes I, II, III and IV), based on the percentiles of BMI by age and sex. Baseline classes III and IV obesity compared with overweight were related with increased risk of having obesity-associated morbidity at follow-up (OR = 5.76, p = 0.001; OR = 5.36, p = 0.001, respectively). The results show that the metabolic risk associated with childhood obesity precedes advanced obesity degree, suggesting to use a complication-oriented approach and management [155]. Even though there is evidence in obese children of a worse outcome in adulthood, a mild reduction in body weight before the adolescence, can considerably reduce the risk for cardiovascular and metabolic disease later in life [150].

The relationship between obesity, hypercholesterolemia and lipoprotein profile modifications is well known [156–158]. Additional and independent risk factor for CHD is represented by high lipoprotein (a) [Lp(a)] levels, studied in several patient groups [159–161]. The association between its plasma levels and the condition of childhood obesity is unclear [162]. Glowinska et al. [163] studied lipid profiles in obese, hypertensive and diabetic young patients showing significant changes in lipid metabolism, including Lp(a), apolipoprotein A (Apo A) and Apo B levels. The association between Lp(a) levels and obesity is unclear probably because Lp(a) circulating levels are mainly genetically determined, but there are several studies that show a significant decrease in Lp(a) levels proportional to weight loss and Lp(a) levels reduction with physical activity [161, 164].

In conclusion, childhood obesity has both significant short-term and long-term consequences on the cardiovascular system, which may be mediated through the development of cardiovascular risk factors and circulating mediators produced by adipose tissue [152, 165]. Appropriate strategies should be implemented to counteract the progress of childhood obesity, considering that its cardiovascular effects may be reversible if children normalize their weight before adulthood.

Exercise

It has been suggested that exercise plays a pivotal role in treating overweight and obese children and adolescents [8, 166]. Exercise seems to favor a reduction in body weight, BMI, central obesity, fat mass percentage, TGs, IR markers, and cardiorespiratory fitness. Exercise interventions help to regulate body fat; however, in the young population, it is unknown to what extent more significant volumes of exercise influence body fat. Current findings showed overall a significant medium reduction in BMI and a small effect size in fat mass percentage and a small but significant reduction in central obesity [167, 168]. Regarding the dose-response effects of exercise on measures of adiposity, a meta-analysis conducted by Atlantis et al. including 481 overweight boys and girls (aged ~12 years) concluded that 155–180 min per week of moderate to high-intensity exercise was effective in reducing body fat and central obesity in overweight and obese children and adolescents [169]. Besides, studies with overweight and obese participants concluded that aerobic plus resistance exercise interventions (8–24 weeks' duration) produced decreases in body weight, BMI and fat mass, but no changes in fat-free mass and WC [170, 171]. Stoner et al. found that exercise intervention reduced BMI (moderate effect), body weight and body fat percentage (all with small effects) and noted that central obesity reduction in response to weight loss might be conditioned by obesity phenotype (BMI vs. fat mass) [172]. Exercise can be therapeutic in reducing body fat; in fact, it is possible to observe an increase of energy expenditure, lipid oxidation and lipid synthesis inhibition by the activation of the AMP-activated protein kinase pathway and free fatty acid flux to the liver [173]. Mainly abdominal obesity excess is linked to a raised lipid profile and a risk of developing IR and metabolic syndrome, constituting the basis for impaired vascular function [174].

Supervised training is a more potent stimulus than non-supervised training in enhancing (by ~1.5%) flow-mediated dilation (FMD). Previous prognostic studies had suggested the clinical relevance of this improvement in FMD. A meta-analysis of 5547 adults was associated with a 1% increase in FMD with a 13% decrease in cardiometabolic events, so that the magnitude of FMD improvement

found following exercise can favorably affect endothelial function in healthy young adults, indicative of another cardioprotective effect of exercise against the progression of atherosclerosis. In this context, several biologically plausible mechanisms could explain the effects of exercise in modulating endothelial function and arterial stiffness [175]. The primary physiological mechanisms involved upregulate endothelial nitric oxide synthase activity with a subsequent decreased expression of nicotinamide adenine dinucleotide phosphate oxidase (NADPH), and stimulation of radical scavenging systems that include copper/zinc-containing superoxide dismutase (Cu/ZnSOD), extracellular superoxide dismutase, glutathione peroxidase, and glutathione [175]. Also, aerobic exercise performed for 60 min, thrice a week, at ≤75% maximum heart rate improves LDL-C and TG concentrations in obese children, and combined exercise increases HDL-C concentrations [176]. Marson et al. demonstrated that in overweight adolescents the improvements in the insulin sensitivity index (IR, HOMA) were similar for aerobic and for strength training, and neither were there differences in the improvements between the exercise training groups and those with combined training. The findings overall showed a significant reduction in fasting glucose and fasting insulin, and these findings have important health implications and provide health care professionals with therapeutic strategies for the treatment of childhood obesity and the reduction of IR in the young population [177]. There is evidence [178] that exercise improves various parameters related to health (body mass, BMI, fat mass, TGs, fasting glucose, fasting insulin). Surprisingly, relative to those of over 12 weeks, the 2- to 4-week programs showed more significant improvements in BMI, visceral fat, and subcutaneous fat. Probably, the shorter programs do not need the strict control of the progression of exercise intensity that the more extended programs do so that their results would be more pronounced. However, in general, for the cardiometabolic and vascular parameters it seems programs of 4–12 weeks, or of more than 60 min per session, or with a total exercise time of 1500 min or more were effective in improving HDL-C levels, fasting glucose, fasting insulin, HOMA-IR, intrahepatic fat, systolic blood pressure and carotid intima-media thickness. This phenomenon may be due to the organism's rapid adaptation to the load represented by the physical exercise program, so that an increase in the intensity may be needed to produce a new adaptation. Although in the field of sports the precise quantification of training programs is the norm, in the field of health, quantification of exercise programs is at best only very general in form, and sometimes almost non-existent [179]. A dose-response relationship for the different health parameters could be applied to obtain benefits in health and to develop more effective lifestyle interventions in the obese young population. Giving the positive effects of physical exercise, efforts should be made to increase physical activity adherence in overweight children. Indeed, current adherence of children to lifestyle recommendations to prevent childhood obesity is low. In the Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and infants (IDEFICS) study only 1.1% of children observed five out of six recommendations, with differences by country and age (children in northern countries and younger children showed better adherence to the recommendations) [180].

Microbiota

The intestinal microbiota is composed of many different microorganisms mostly from the Bacteria kingdom [181]. About 90% belong to the phyla *Firmicutes*, and *Bacteroidetes* and the most abundant genera are *Bacteroides*, *Faecalibacterium* and *Bifidobacterium* the proportions of which vary between individuals. At the species level, there is a great variety which can originate a unique profile for each host [182, 183].

Gut microbiotas in childhood are affected by geography and food culture, in fact in children living in advanced countries their guts are mainly constituted by *Bacteroides-Bifidobacterium*-dominate microbiotas (BB-type) whereas children in developing countries are represented by *Prevotella*-dominated microbiotas (P-type) [184–186].

Furthermore, these two microbiota-types are often observed within the same country but are associated with the level of development in an area. Nakayama et al. recently found that P- and BB-type microbiotas were represented in children living in rural and urban sites and that a type shift was associated with modernization of consumed foods, and notably a change to high-fat diets. These studies suggest that diet had a more significant influence on gut microbial communities than host genetics. Indeed, the microbiotas were enriched in bacteria with genes encoding bile acids that aid in lipid absorption or oligosaccharide-degrading enzymes involved in plant digestion in BB-type and P-type children, respectively [187].

The composition of gut microbiota is defined at birth and evolves until about 3 years old, then it remains constant during the lifetime; however, a number of factors, such as antibiotics and diet, can change it [188, 189]. In human twins, the similarity of the microbiota is within the same family members and not only in monozygotic twins,

indicating that environmental exposure has more impact than genotype in microbiota development [190, 191].

Cho et al. demonstrated that low dose antibiotic exposure in young mice led to increased adiposity, metabolic hormone levels, and short-chain fatty acids (SCFA) levels, as well as changes to the hepatic metabolism of lipids and cholesterol [192]. The antibiotic exposition has an overpowering impact on gut microbiota [193]. Epidemiological studies have demonstrated that the administration of antibiotics during childhood is correlated with a higher risk of obesity [194, 195]. Early life exposition to antibiotics has also been proposed to drive to obesity [196]. In neonates, after repeated exposure to antibiotics, has been shown a decrease of anti-obesogenic bacteria (*bifidobacteria* and *Bacteroides*) [197]. The common exposure to antibiotics, in the perinatal period, exerts a wide effect on the gut microbiota. After the neonatal period, diet is the most important factor regulating the gut microbiota with breast milk promoting the ascendancy of *bifidobacteria* in the infant gut [198].

In addition, Cox et al. found that low dose penicillin given at birth can induce sustained effects on body composition and enhance high fat diet-induced obesity in mice. Furthermore, the obese phenotype was transferable to germ-free mice by transfer of low-dose penicillin microbiome, implicating the microbiome as the driver of this phenotype as opposed to antibiotics [199]. High fat and protein diets significantly reduced SCFA and altered intestinal bacteria composition. The supplementation with prebiotics promotes the growth of specific gut microbiota species, and the use of probiotics has benefits in host health, but the impact on large-term gut microbiota remains to be proven [200–202].

In obesity, gut microbiota is known to be altered by a decreased ratio of *Bacteroidetes* to *Firmicutes*, with a superior capacity to harvest energy from a diet [203–205].

Besides, long-term diet habits influence the composition of gut microbiota. Intestinal bacteria react to daily dietary fat and carbohydrates and change its metabolic pattern [206–208].

A very recent study explored the impact of gut microbiota and diet on visceral fat mass accumulation (VFM) in 1760 older female twins [209].

In this large study it was shown that gut microbiota composition and diet are related to VFM accumulation and that these two factors are closely linked. Moreover, operational taxonomic units (OUTs) relative abundance, even after revision for nutrient inlet, is strongly associated with VFM indicating that bacteria may not require the presence of nutrients to affect host phenotypes. However, the influence of nutrients in improving the growth of beneficial microbes and their consequence on host health is not ruled out [209].

In this same twins UK cohort, Le roy et al., demonstrated novel evidence of association between six adiposity phenotypes and fecal microbial OTUs. Most of these results involve visceral fat associations, with the strongest associations between visceral fat and *Oscillospira* members suggesting that this genus if replicated could be a key player of the host-GM mutualism in relation to fat deposition. While several genera appear as protective factors in VFM, only *Blautia* stood out as a risk factor that is in part heritable [210]. When neonatal microbiota starts is until now a matter of great debate. The idea that, under physiological status, the human fetal environment is sterile and microbial colonization develops with birth, has been in dispute for decades. Recently, there has been different evidence that have described a lowly bacterial colonization in the placenta, endometrium, amniotic fluid and meconium in healthy, full-term pregnancies [211]. These findings influenced some researchers about the seeding of the microbiota before birth. Moreover, different scientists have pointed out the necessary to lend part of attention when working on samples with a low microbial biomass due to the contamination issues with such samples, when using PCR amplification and NGS techniques [211–213].

Preterm birth can be considered a polymicrobial disease [214] and it has been reported that adults who were born prematurely present increased risk for obesity [215, 216] in comparison with individuals who were born after a full-term pregnancy. Moreover, the incidence of several prenatal and early postnatal factors associated with the development of infant adiposity (such as prematurity and low birth weight, gestational diabetes, excess body mass gain during gestation and infant formula feeding) has also increased [217]. In this scenario, preterm birth can be considered a further risk factor for obesity.

As mentioned earlier, the intestinal microbiota colonization occurs at birth, or before in uterus, and it is the perinatal period that is the most important one to define gut microbiota in later ages. Early life environment factors involved are:

(a) host genetics [218, 219];
(b) *in uterus* colonization [191, 220, 221];
(c) maternal lifestyle [222];
(d) birth delivery mode [223–225];
(e) breastfeeding vs. formula [225];
(f) antibiotics exposure [226, 227];
(g) hygiene level [228, 229];
(h) prebiotics and probiotics administration to pregnant women and neonates [230, 231].

At 3–5 years old, the gut microbiota composition is similar to adults and remains very stable [220]. A large amount of studies reveals differences between gut microbiota of obese and lean children. In obese children with ages between 4 and 5 years old, Karlsson et al. found significant differences in the abundance of bacteria but only a tendency in their diversity. The abundance of *Enterobacteriaceae* was significantly higher in obese children, whereas there was a significantly lower amount of *Desulfovibrio* and *Akkermansia muciniphila*-like bacteria. No substantial differences were found in the content of *Lactobacillus*, *Bifidobacterium* or the *Bacteroides fragilis* spp. [232]. In another study comparing children between 6 and 16 years of age, the authors found elevated *Firmicutes*-to-*Bacteroidetes* ratio in obese children compared with thin ones. Additionally, low relative proportions of *Bacteroides vulgatus* and high levels of *Lactobacillus* spp. were observed in the obese children [233]. Also, gut microbiota changes were found in obese adolescents when they altered their lifestyle, mainly diet and exercise, suggesting interactions between diet, gut microbiota and host metabolism and immunity in obesity [234]. Several recent studies suggest the positive role of gut microbiota modulation in adult obesity treatment. However, there are no sufficient data to state that child dysbiosis increases the risk of subsequent obesity. Primary prevention strategies may include modeling maternal and infants gut microbiota to break the obesity cycle. The use of prebiotics, probiotics and changes in maternal lifestyle may be successful interventions to avoid childhood dysbiosis and consequent obesity [235].

Nutraceuticals and probiotics

Pharmacological therapy should only be used after failure of multidisciplinary lifestyle interventions. When substantial weight loss cannot be achieved through lifestyle-based interventions, the use of drugs is justified, mainly in obese individual affected by serious different disorders [236–240]. Orlistat (tetrahydro-lipstinate) is the single drug available for the treatment of children and adolescents with severe obesity. Few studies are available on the effects of anti-obesity pharmacological therapy in pediatric ages [241–244]; it seems to produce significant weight loss and favoring behavioral changes. There is no implications on the mineral balance if a low-calorie diet is associated with usual mineral intake; in contrast, it must prevent liposoluble vitamins deficiency [245–248].

Obesity has been correlated with a specific profile of the gut microbiota characterized by lower levels of bacteria belonging to the *Bacteroides* and *Bifidobacterium* genera compared to that of lean individuals [249]. Besides, bifidobacteria have been shown to be higher in children maintaining normal weight at 7 years old than in children developing overweight, and their administration was able to reduce serum and liver TG levels and to decrease hepatic adiposity [250, 251]. The mixture of *B. breve* (BR03 and B632) was used in a cross-over double-blind, randomized controlled trial to re-establish metabolic homeostasis and reduce chronic inflammation in obese children. Preliminary results demonstrated that a *B. breve* administration in obese children is promising: 8 weeks of treatment seems to ameliorate glucose metabolism and could help in weight management by reducing BMI, waist to height ratio and WC [252].

Intake of fiber supplements is inversely associated with body weight, body fat and BMI [253], thus promoting satiation, reducing macronutrients absorption, and modifying the production of gut hormones. Although increasing consumption of fibers (e.g. fruits, vegetables, grains, legumes) with diet is a crucial step to curb the obesity, fiber addiction should also be considered. Glucomannan, a soluble and viscous dietary fiber derived from the konjac plant, can cause weight loss and improve lipid and lipoprotein parameters and glycemic status with minimal gastrointestinal side effects [254]. Even though the weight reduction by *psyllium* is weakly supported, it has been showed to be able to improve glucose homeostasis, and the lipid and lipoprotein profile in obese children. Inulin (from chicory root) is being sold in many mainstream stores as a fiber supplement, including for use in children; however, its weight-lowering effect has not been studied [255]. Additional studies are necessary to gain definitive conclusions.

Conclusions

Childhood obesity represents one of the most pressing medical and public health problems of our day. The prevalence is unacceptably high, and the rate of increase in severe obesity continues to climb. Obesity is classified into three classes: class I (95th percentile to <120% of the 95th percentile), class II (120%– <140% of the 95th percentile), and class III (≥140% of the 95th percentile).

The multifactorial etiology of obesity is related to genetics and epigenetic predisposition, nutrition, environment, lifestyle and hormone dysfunction. This review intended to underline the role of the main factors influenced by the gain weight and also those that may modulate and predispose to childhood obesity.

There is increasing evidence for the role of epigenetic factors in the development of obesity. In particular, miRNAs represent relevant regulators of processes related to adipogenesis contributing to metabolic abnormalities associated with childhood obesity and obesity-related diseases.

Heritable factors appear to be responsible for 30%–50% of the variation in adiposity. It was well demonstrated that obese children with genetic syndromes typically show early-onset obesity and characteristics and also pathological phenotypes.

The most common single gene defects currently identified in children with obesity are mutations in the melanocortin 4 receptor. Other gene defects include those in leptin, leptin receptor, proopiomelanocortin and proprotein convertase.

Furthermore, the screening of biochemical parameters can well define the organ's metabolic status and characterize any modification and/or organ damage. We also focused on the regulation of different inflammatory obesity-related cytokines that modulate the proliferation and apoptosis of adipocytes, promoting lipolysis and inhibit lipid synthesis through autocrine and paracrine mechanisms.

Most children with endocrine disorders resulting in weight gain show poor linear growth, short stature and/or hypogonadism. This status includes either endogenous or exogenous glucocorticoid excess, hypothyroidism, growth hormone deficiency, and pseudohypoparathyroidism type 1a.

In addition, we underlined the increased risk of hyperinsulinemia, IR, prediabetes, and also the high prevalence of other cardiometabolic risk factors including elevated blood pressure, low levels of HDL-C, and elevated levels of TGs. In particular, Lp(a) evaluation is strongly recommended as an independent causal risk factor for cardiovascular disease.

Moreover, we also considered the benefits of physical activity necessary to avoid the energy imbalances strictly related to overweight and to contribute to achieve healthy habits and adequate life style that improves lower levels of overweight. In this contest gut microbiota may also play a pivotal role in the early diagnosis of childhood obesity as it has been correlated with a specific profile of the gut microbiota. Furthermore, a brief outline was made on the possibility of designing a therapy intervention strategy by using the intake of fiber supplements together with life style interventions.

Each factor described in this review can contribute to define an early diagnostic tool and highlight the importance to adopt early interventions that target both weight and mental health in childhood to minimize negative outcomes later in adulthood. The design of specific and individualized diagnostic models, based on the degree of obesity, is strictly necessary to devise a suitable multidisciplinary medical intervention.

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