PEDIATRIC OBESITY: FROM THE SPECTRUM OF CLINICAL-PHYSIOLOGY, SOCIAL-PSYCHOLOGY, AND TRANSLATIONAL RESEARCH

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PEDIATRIC OBESITY: FROM THE SPECTRUM OF CLINICAL-PHYSIOLOGY, SOCIAL-PSYCHOLOGY, AND TRANSLATIONAL RESEARCH

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EDITORIAL
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Editorial: Pediatric Obesity: From the Spectrum of Clinical-Physiology, Social-Psychology, and Translational Research

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Keywords: pediatrics and adolescents, metabolic syndrome, obesity, school children, NAFLD

Editorial on the Research Topic

Pediatric Obesity: From the Spectrum of Clinical-Physiology, Social-Psychology, and Translational Research

Obesity affects nearly one-third of children in the United States with nearly 6% of adolescents in severely obese condition and is currently a growing global epidemic that requires attention. Increasing expenses in the pediatric health care system were spent in obese children vs. children with a normal body mass index (BMI). Pediatric obesity is also noted to connect with an increased risk of many diseases such as diabetes, cardiovascular diseases, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), social and psychological problems in adolescents and young adults, and even certain types of cancer later in life. A good outline for childhood and adolescent obesity was presented by Kansra et al., spointing out that the most common cause of obesity throughout childhood and adolescence is an inequity in energy balance; that is, excess caloric intake without appropriate caloric expenditure. They give an overview from the spectrum of clinical-physiology, social-psychology, pathophysiology, and treatments options for obese pediatric and adolescent patients.

In adolescent obesity and health medicine, retrospective cohort studies in school children and anthropometric measurements affecting BMI and obesity were reported. Firstly, Hsiao et al. from Taiwan had compared the growth velocity with BMI. They found that obese and overweight girls from 9 to 13-year-old had less linear growth whereas puberty may dominate over BMI as the main contributor to high growth velocity in pre-puberty girls with underweight BMI. Secondly, dos Santos et al. from Portugal has established predictors of BMI trajectories using anthropometric measurements. They found that weight trajectories were mainly settled by early adolescence. Lack of sleep and eating routines, low emotional self-regulation, child-parent conflict, and low child-parent closeness in early childhood were significantly associated with unhealthy weight trajectories in adolescent and even adult life. Children and adolescents with obesity may also show increased lower urinary tract symptoms, such as urgency and enuresis (Wang et al.), and increased prevalence of hematuria/ proteinuria in school children (Chen et al.), although the etiology is currently unclear and may associate with sex, age, and even urbanization.

The prevalence of metabolic syndrome in children and adolescents is increasing, in parallel with the increasing trends in obesity rates. The cardinal features in pediatric metabolic syndrome included overweight and obesity, abnormal glucose metabolism, dyslipidemia, and hypertension. Other disorders associated with metabolic syndrome include fatty liver, NAFLD, and

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pro-inflammatory states. A cross-sectional study conducted by Valle-Martos et al. showed that 6–9 years-old school children with obesity, compared to children with normal weight, had higher values for liver enzymes, leptin, markers of insulin resistance, and inflammation, and endothelial dysfunction, and variables associated with metabolic syndrome.

NAFLD has become a public health issue in obese children and adolescents and the clinical course can be progressive and become chronic if not detected at an early stage. There are three reports in our current Topic covering the genetic, histology, and potential treatment in pediatric NAFLD, respectively. Recently, NAFLD was considered to be the hepatic component of metabolic syndrome, and therefore redefined as metabolic associated fatty liver disease (MAFLD). Lin et al. described the new perspectives on genetic prediction. Genetic variants including PNPLA3, TM6SF2, GCKR, MBOAT7, and HSD17B13 have been shown to confer susceptibility to MAFLD in children. Lee et al. elaborated on the relationship between histological features of NAFLD and ectopic fat on MRI in children and adolescents. The third article from Al-Baiaty et al. demonstrated possible hepatoprotective effects in using vitamin E with a high content of tocotrienol as a therapeutic alternative in treating NAFLD in obese children and adolescents.

Recent research findings has significantly increased our understanding of the mechanisms involved in white adipose tissue (WAT) browning and brown adipose tissue (BAT) activation. The discovery of metabolically active BAT in human adults, especially in lean people after cold exposure, has provoked the "thermogenic anti-obesity" idea to battle weight gain. Lu et al. clearly elaborated on the molecular mechanisms regulating UCP1 expression and discussed the potential and caution in targeting UCP1 for enhancing thermogenesis as a strategy to combat obesity.

In summary, this Research Topic has comprehensively covered important aspects of pediatric obesity research from the spectrum of clinical-physiology, social-psychology, and translational research.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Early Individual and Family Predictors of Weight Trajectories From Early Childhood to Adolescence: Results From the Millennium Cohort Study

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Background: Early infancy and childhood are critical periods in the establishment of lifelong weight trajectories. Parents and early family environment have a strong effect on children's health behaviors that track into adolescence, influencing lifelong risk of obesity.

Objective: We aimed to identify developmental trajectories of body mass index (BMI) from early childhood to adolescence and to assess their early individual and family predictors.

Methods: This was a secondary analysis of the Millennium Cohort Study and included 17,165 children. Weight trajectories were estimated using growth mixture modeling based on age- and gender-specific BMI Z-scores, followed by a bias-adjusted regression analysis.

Results: We found four BMI trajectories: Weight Loss (69%), Early Weight Gain (24%), Early Obesity (3.7%), and Late Weight Gain (3.3%). Weight trajectories were mainly settled by early adolescence. Lack of sleep and eating routines, low emotional self-regulation, child-parent conflict, and low child-parent closeness in early childhood were significantly associated with unhealthy weight trajectories, alongside poverty, low maternal education, maternal obesity, and prematurity.

Conclusions: Unhealthy BMI trajectories were defined in early and middle-childhood, and disproportionally affected children from disadvantaged families. This study further points out that household routines, self-regulation, and child-parent relationship are possible areas for family-based obesity prevention interventions.

Keywords: weight trajectories, early childhood, family context, Millennium Cohort Study, growth mixture modeling

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INTRODUCTION

Obesity is a major Public Health issue worldwide. Over the last decades, obesity has been increasing at an alarming rate and appearing at progressively younger ages (1). In fact, between 1975 and 2016, among children and adolescents aged 5–19 years, the global prevalence of overweight has increased from 4 to 18% in girls and 19% in boys, and the global prevalence of obesity has risen from 0.7 to

5.6% in girls and from 0.9 to 7.8% in boys, summing up to 340 million children and adolescents (1). Besides, 41 million children under 5 years of age were estimated to suffer from overweight or obesity in 2016 (2).

In the short term, children with overweight and obesity not only have a higher risk of hypertension, diabetes, and sleep problems, they also have a higher risk of psychological distress, such as negative body image, low self-esteem, depression, and peer problems (3, 4). Furthermore, unhealthy weight tends to persist into adolescence and adulthood, increasing the lifelong risk of these non-communicable diseases (5).

Early infancy and childhood appear to be critical periods in the establishment of lifelong weight trajectories (6–8). Therefore, it is important to understand these trajectories and their early predictors in order to inform effective public health interventions.

From an ecological perspective, family plays a major role in every aspect of a child's health and development, especially during infancy and early childhood (9). Both observational and experimental studies support the persistent effect of early family environment on health behaviors and weight status, highlighting the central role of parents in childhood obesity (10). Several studies suggest that general parenting, parenting styles and practices, and parent-child relationships can shape early eating, exercise, sleep, and screen use habits that track into adolescence (11, 12). However, its influence on weight status is still debated.

An empirical way to investigate body mass index (BMI) trajectories over time is by using Growth Mixture Modeling (GMM), an extension of Growth Curve Modeling (13). GMM is a person-oriented approach that assumes that individuals do not belong to a single homogenous population but rather to distinct unobserved subpopulations with different developmental trajectories (14). GMM focuses on longitudinal change within each subpopulation and allows the classification of individuals into latent classes based on their growth trajectories.

Investigating BMI trajectories over time requires large samples and accurate anthropometric measurements. Although several studies explored weight trajectories in childhood, some studies used categorical measures of overweight and obesity, which leads to classification bias, and others used "crude" BMI values, which do not account for growth (13). Since normal growth results in an expected increase in BMI, age- and gender-specific BMI standard deviation scores (Z-scores) are often considered the gold standard for the analysis of anthropometric data at an epidemiological level (15). In fact, the Z-score has a linear scale that allows comparison between age groups and gender. Furthermore, it can be analyzed by summary statistics: the mean Z-score reflects the nutritional status of the entire population, and the standard deviation (SD) of the Z-score reflects the quality and accuracy of the data (15). On the other hand, most of these studies explored a small subset of covariates, such as gender, ethnicity, and socioeconomic status, leaving out important factors like birthweight and breastfeeding duration that are well-known to influence weight status, and even fewer studies have included early family environment, general parenting, and parenting practices.

Thus, the main objectives of our study were (1) to identify distinct subpopulations with different developmental trajectories of BMI from early childhood to adolescence in a general prospective cohort of British children, the Millennium Cohort Study; and (2) to examine the association between early individual and family factors and subsequent BMI trajectories.

In the literature, the most commonly found trajectory is the stable normative trajectory comprising the larger portion of the sample (13). While most studies report a decreasing BMI trajectory and an increasing BMI trajectory, other studies indicate an additional stable high trajectory (13).

Therefore, we hypothesize (1) the existence of different weight trajectories, including a stable normative trajectory and a persistent high trajectory; (2) that individual and family factors influence the likelihood of belonging to a group trajectory; and (3) that an adverse early family context increases the likelihood of following an unhealthy weight trajectory.

MATERIALS AND METHODS

Participants

Data were drawn from the Millennium Cohort Study (MCS), a cohort study that follows children born between September 2000 and January 2002, and living and growing up in England, Scotland, Wales, and Northern Ireland. It also provides information about family circumstances and the broader socioeconomic context. MCS was designed to overrepresent specific subgroups of the population, namely children living in disadvantaged areas and those who are ethnic minorities. The sample is clustered by electoral wards stratified by country, ethnicity, and Child Poverty Index (16).

The study began with an original sample of 18,552 families, and at Sweep 2 (2003–04), it recruited 691 "new families" who were eligible but were missed at Sweep 1. Therefore, the total number of families ever interviewed comprises 19,243 families (19,517 children). Children were around 9 months at Sweep 1 and about 3, 5, 7, 11, and 14 years old at the subsequent sweeps. The study protocol meets the ethical requirements of the Helsinki Declaration, and it was approved by Medical Research Ethics Committee. Further information about the study design can be found in Connelly and Platt (16).

To the present study, singletons and the first-born child of the families with twins and triplets with valid data on BMI in at least one of Sweeps 2–6 were included, comprising 17,165 children.

Measures

Anthropometric Measures

Weight and height were measured by trained interviewers using standardized instruments (Tanita HD-305 scales, Tanita UK Ltd.; and portable stadiometers, Leicester Height Measure, Seca UK), with children wearing neither shoes nor outdoor clothes. Weight and height were used to calculate BMI (kg/m^2).

We calculated age- and gender-specific BMI z-scores (BMIz) using World Health Organization (WHO) Anthro (17) and Anthro Plus (18) software, having as reference the WHO Multicenter Growth Reference Study population. Observations with extreme values (below −5 SD or above +5 SD) were

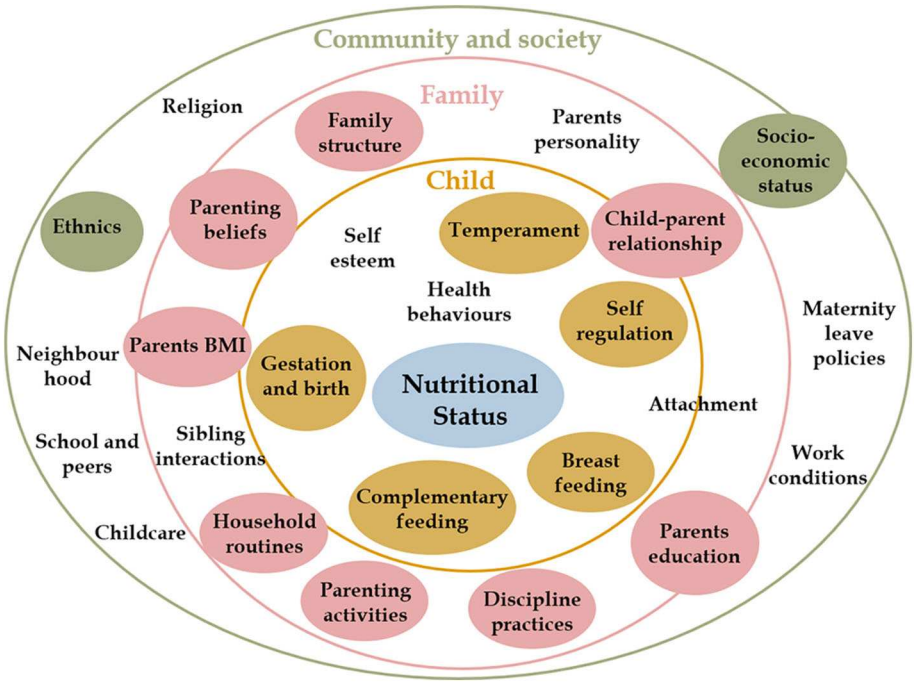

FIGURE 1 | Early life predictors of overweight and obesity. Child nutritional status is influenced by individual characteristics and behaviors (inner yellow circle) and by contextual factors at family (pink circle) and community (green circle) levels. These factors interact with each other to influence how a child grows and develops. This conceptual framework is based on Davidson and Birch's Ecological model of childhood overweight and Bronfenbrenner Ecological Systems Theory. (9) Covariates included in the analysis are presented in colored circles.

considered outliers and excluded (15). Four individuals presented BMIz values only in the first two sweeps, with borderline BMIz at age 3 (around −4 SD) corresponding to extreme thinness, and at age 5 in the overweight range (around +1.5 SD). Since there were no subsequent values to validate these observations, they were considered highly implausible and excluded.

Covariates

Predictors of nutritional status were selected based on previous research, according to Davidson and Birch's ecological model of overweight (9). A conceptual framework of early life predictors of Overweight and Obesity is presented in **Figure 1**. Further details on how covariates were categorized and coded are presented in **Supplementary Table 1**.

Sociodemographic and economic covariates were gender, ethnicity, family structure, family poverty, maternal age at birth of cohort member, maternal nutritional status, and maternal education measured at 9 months. Ethnicity was collected as a self-perceived 6-option variable including "white," "mixed," "indian," "pakistani and bangladeshi," "black or black british," and "other" (19, 20).

Perinatal and early infancy covariates

Gestational age (GA) was categorized as "extreme to moderate prematurity," "late prematurity," and "term," according to the WHO/UNICEF definition (21).

Birthweight was categorized as "low birthweight," "normal birthweight," and "high birthweight," according to the WHO definition (22).

Birthweight centiles for age and gender were calculated using Intergrowth21 software, and individuals were further classified in "Appropriate for GA," "Small for GA," and "Largefor GA" (23).

Breastfeeding duration was assessed in Sweeps 1 and 2; it was re-categorized as in Carling et al. (24), with two more categories ("6 to 12 months" and "more than 12 months"), according to its distribution.

Introduction to solid food was classified as "early introduction" if it occurred before 4 months, and "late introduction" if it occurred after 6 months, according to European Society for Pediatric Gastroenterology Hepatology and Nutrition recommendations (25).

Child temperament and self-regulation

Child temperament was assessed at 9 months by 14 items from the Carey Infant Temperament Scale, capturing three dimensions: mood, adaptability/approach-withdrawal, and regularity. We created total scores for each dimension by summing the individual responses, as in Hernández-Alava and Popli (26). High scores on the first two dimensions indicated distress and withdrawal (Cronbach's $\alpha = 0.546$ and 0.677, respectively), and high scores on the last dimension indicated regularity ($\alpha = 0.713$).

Child self-regulation was assessed at 9 months by 10 items from the Child Social Behavior Questionnaire, with two dimensions:

cognitive (α = 0.573) and emotional self-regulation (α = 0.632). Total scores for each dimension were calculated as the average of valid responses (27), so that higher scores in the first dimension indicated higher cognitive self-regulation, and higher scores in the second indicated emotional dysregulation.

Household routines, parenting beliefs, and parenting activities

Household routines were assessed at age 3, comprising sleep and feeding routines. A total sum score was created, with higher scores indicating consistent routines (α = 0.541).

Parenting beliefs were assessed at 9 months comprising three items derived from the European Longitudinal Study of Pregnancy and Childhood about the importance of stimulating, talking, and cuddling to a baby's development. We summed the responses (28), so that higher scores indicated more positive parenting beliefs (α = 0.730).

Parenting activities were assessed at age 3 and comprised five items (reading, teaching the alphabet, teaching counting, teaching songs/rhymes, drawing). A total sum score was generated, so that higher scores indicated higher involvement in these activities (α = 0.610).

Discipline practices and child-parent relationship

Discipline practices were assessed at age 3 by six items from Straus's Conflict Tactics Scale, measuring how often the mother used punishing (smack, shout, "tell off") or withdrawal tactics (ignore, take away treats, send to bedroom/naughty chair) when the child misbehaved. We created two scores: a harsh parenting score (α = 0.657) by summing the responses in the punishing items, and a positive parenting score (α = 0.556) by summing the responses in the withdrawal items (29).

Parent-child relationship was assessed at age 3 by Pianta Short Form, a 15-item self-administered scale based on attachment theory. This scale comprised two scores: closeness (α = 0.657) and conflict (α = 0.787).

Statistical Analysis

Statistical analysis was performed using IBM Statistical Package for the Social Sciences, version 24.0 (SPSS Inc., Chicago, IL) and Mplus, version 8.3 (30). We also used the packages MplusAutomation (31) and dotwhisker (32) for R version 3.5.1. (33) Statistical significance was set to $p < 0.05$.

Estimation of BMI Trajectories

Longitudinal BMI trajectories were analyzed with GMM. GMM classifies children into latent classes based on their longitudinal change, so that individuals with similar BMI trajectories are assigned to the same class, and individuals in different classes follow significantly different BMI trajectories (14).

We tested multiple GMM models with different specifications before choosing the final model. First, we performed single-group models to identify the pattern (intercept only, linear, quadratic, cubic) that represented change over time the best. We also performed a latent basis model, where the pattern of change is not predefined but driven by the data, and loadings for the *slope* factor are estimated to represent the proportion of the total amount of growth that has occurred up to that point. Next, we performed GMM with *intercept* and *slope* variances fixed at zero for each class, a subtype of GMM called Latent Class Growth Analysis (LCGA), which assumes that all individual growth trajectories within a class are homogeneous (14). Then, we performed several GMMs with different growth factor variance specifications: first, with equal variances (homoscedastic model), and then freely estimated (heteroscedastic model). To determine the optimal number of classes, we started with a one-class solution and progressively increased the number of classes.

Model Selection

The best model was chosen considering information criteria, theoretical justification, and interpretability (34, 35). First, we looked at two model fit indices: Schwarz's Bayesian Information Criterion (BIC) and the Bootstrapped Likelihood Ratio Test (BLRT) (34). BIC considers the likelihood of a model as well as the number of estimated parameters, with lower values of BIC indicating better fitting. BLRT compares a model with k classes to a model with k-1 classes, providing a *p*-value. We then looked at entropy, a measure of classification quality and separation between classes. Higher values of entropy (near 1) indicate more confidence in the classification (36). Although values above 0.8 are considered good, there is no consensual definition of what constitutes low entropy (36). Finally, we considered the interpretability of the models and their practical meaningfulness, rejecting models with clinically uninterpretable classes and with classes representing <1% of the total sample.

Association Between BMI Trajectories and Early Individual and Family Factors

The association between covariates and class membership was calculated based on crude and adjusted odds ratio (OR) using a *bias-adjusted three-step approach*, which takes into account the classification error in class assignment (37).

Missing Values

Missingness resulted from item non-response and attrition. Attrition is mainly related to sociodemographic characteristics, so the MCS study was designed to account for this bias and still provide representative information.

Missing values on BMI variables were handled with full information maximum likelihood (FIML) estimation under missing data theory. Considered the gold standard for handling missing data in latent variable indicators, FIML uses all available data points and is robust to non-normal distribution (38).

Missing values on covariates were handled using multiple imputations carried out in Mplus. We used Bayesian estimation to create 25 imputed datasets, using the Markov chain Monte Carlo algorithm, and convergence criterion was set to 0.05. We included all covariates and also BMIz variables under the missing at random assumption, accounting for the complex sample design (38). Imputed values compare reasonably to those observed.

TABLE 1 | BMI z-score summary and descriptive statistics according to WHO cut-offs.

Sweep	Age 3	Age 5	Age 7	Age 11	Age 14
n	14,176	14,922	13,603	12,864	10,958
Mean (SD)	0.88 (1.04)	0.62 (1.02)	0.48 (1.14)	0.53 (1.23)	0.39 (1.20)
Min	−4.35	−4.60	−4.94	−4.54	−4.51
Max	5.00	4.98	4.96	4.76	4.58
Range	9.35	9.58	9.90	9.30	9.09
Severe thinness	17 (0.1)	16 (0.1)	26 (0.2)	30 (0.2)	37 (0.3)
Thinness	61 (0.4)	55 (0.4)	101 (0.7)	200 (1.6)	208 (1.9)
Normal weight	7,828 (55.2)	10,160 (68.1)	9,655 (71.0)	8,018 (62.3)	7,434 (67.8)
Overweight	4,511 (31.8)	3,437 (23.0)	2,522 (18.5)	2,931 (22.8)	2,180 (19.9)
Obesity	1,759 (12.4)	1,254 (8.4)	1,299 (9.5)	1,685 (13.1)	1,099 (10)

Descriptive statistics are presented in n (%).
% Excluded extreme values (< −5 SD or > +5 SD): at Sweep 2−1%; at Sweep 3−0.4%; at Sweep 4−0.2%; at Sweep 5−0.05%; at Sweep 6−0.01%).
SD, standard deviations.

RESULTS

Body Mass Index

Table 1 shows the summary statistics of BMIz across all sweeps and the nutritional status according to WHO BMIz cut-offs.

Comparing our results to those of the WHO Multicenter Growth Reference Study, at age 3 our study sample showed a slightly higher mean BMIz, implying an upward shift of our sample distribution, with a progressive decrease in subsequent sweeps. The BMIz SD superior to 1 indicated a slightly more dispersed distribution, but in all sweeps, it was below 1.3, suggesting good quality of the data. The overall prevalence of overweight and obesity decreased from 44.2% at age 3 to 29.9% at age 14.

Covariates

Sociodemographic characteristics of the analytical sample are presented in **Table 2**, and psychosocial covariates are presented in **Supplementary Table 2**. Compared with children in the analytic sample, excluded individuals (who had no BMIzs in any of the sweeps) were more likely to be male, to be from ethnic minorities, to come from economically disadvantaged families and from single-parent families. Mothers of excluded children were younger and less educated. Complete bias analysis is available in **Supplementary Table 3**.

BMI Trajectory Estimation

The single-group models that served as a basis for subsequent GMM are presented in **Supplementary Figure 1**, showing that the quadratic and latent basis models appeared to better explain change in BMIz over time. Although the Quadratic GMM showed lower BICs, they provided uninterpretable trajectories; therefore, we decided to further analyze the latent basis GMM, presented in **Table 3**.

The *LCGA* showed much higher BICs than the other models, and since LCGA is based on the assumption that there is no within-class variability, we did not explore these models further.

Regarding the *homoscedastic GMM*, the *2-class model* showed a big heterogeneous class (95.7%) and a small well-defined class (4.3%), corresponding to an Early Obesity trajectory class.

The *3-class model* provided classes that were very disproportional (one big class comprising 91.2% and the two remaining classes comprising 4.5 and 4.3%). Furthermore, the two smaller classes were interpretable and meaningful, corresponding to an Early Obesity trajectory (4.5%) and a Late Weight Gain trajectory (4.3%); however, the bigger class (91.9%) was still heterogeneous, showing 20% of children with overweight and obesity across all sweeps, not corresponding to a normative class that we could use as a reference.

The *4-class model* showed more proportional and meaningful classes; it provided an Early Obesity trajectory (3.7%) and a Late Weight Gain trajectory (3.3%) similar to the 3-class model, and Weight Loss (69.0%) and Early Weight Gain (24%) trajectories. The *5-class model* showed an additional class that represented only <1% of the sample.

Regarding the *heteroscedastic GMM models*, they showed lower BIC, with BLRT favoring the 2-class model. This model provided a decreasing trajectory (77.7%) and an increasing trajectory (22.3%). However, this model showed low entropy (0.49) and provided poorly defined classes, while the decreasing trajectory still comprised around 20% of children with overweight and obesity at Sweep 6.

Therefore, we considered the 4-Class Latent Basis Homoscedastic GMM the best model to explain the change in BMIz (presented in bold in **Table 3**).

BMI Trajectory Characterization

Figure 2 shows the BMIz trajectories from early childhood to adolescence. Latent growth factor means and variances are available in **Supplementary Table 4** for each class. The *intercept* and *slope* are inversely correlated ($r = -0.257$, $p < 0.001$), meaning that the higher the initial BMIz, the lower the growth. The time scores are 0, 0.326 ($p < 0.001$), 0.633 ($p < 0.001$), 0.924 ($p < 0.001$), and 1, meaning that individuals have reached 32.6% of the total change in BMIz at age 5, 63.3% at age 7, 92.4%

TABLE 2 | Characteristics of the analytical sample ($n = 17,165$).

	n	*n* (%)	Missing (%)
Gender	17,165		0
Male		8,774 (51.1)	
Female		8,391 (48.9)	
Ethnicity	17,060		0.6
White		14,045 (82.3)	
Mixed		515 (3.0)	
Indian		445 (2.6)	
Pakistani and Bangladeshi		1,179 (6.9)	
Black or Black British		628 (3.7)	
Other		248 (1.5)	
Gestational age	16,276		5.2
Extreme to moderate prematurity		319 (2.0)	
Late prematurity		892 (5.5)	
Term		15,065 (92.6)	
Birthweight	17,038		0.7
Low		1,213 (7.1)	
Normal		14,009 (82.2)	
High		1,816 (10.7)	
Birthweight for gestational age	15,958		7
Small		1,237 (7.8)	
Appropriate		11,883 (74.5)	
Large		2,838 (17.8)	
Breastfeeding duration	17,074		0.5
Never		5,705 (33.4)	
≤2 months		4,951 (29)	
2–4 months		2,067 (12.1)	
4–6 months		1,270 (7.4)	
6– 12 months		1,668 (9.8)	
≥12 months		1,413 (8.3)	
Age at complementary feeding	16,467		4.1
Before 4 months		5,145 (31.2)	
Between 4 and 6 months		10,836 (65.8)	
After 6 months		486 (3)	
Family structure (sweep 1)	16,485		4
Two parents/carers		13,765 (83.5)	
Single parenthood (one parent/carer)		2,720 (16.5)	
OECD median poverty indicator (sweep 1)	16,428		4.3
Above 60% median		10,527 (64.1)	
Below 60% median		5,901 (35.9)	
Mother age at cohort member's birth	17,100		0.4
12–19 years		1,476 (8.6)	
20–39 years		15,256 (89.2)	
40 plus		368 (2.2)	
Mother nutritional status (sweep 1)	14,296		16.7
Underweight		1,825 (12.8)	
Normal weight		6,871 (48.1)	
Overweight		3,721 (26)	
Obesity		1,879 (13.1)	
Maternal education (sweep 1)	16,431		4.3
Low (NVQ < 3/none/overseas)		11,491 (69.9)	
High (NVQ ≥ 4)		4,940 (30.1)	

Data are given as n (%).
NVQ, National Vocational Qualifications.

at age 11, and 100% at age 14. Thus, the greatest amount of change occurs during early and middle-childhood, and there is little further change (7.6%) in BMIz in early adolescence.

Observing the BMI trajectories, two clearly distinct classes are seen: the Early Obesity and the Late Weight Gain. The Early Obesity class follows a clearly distinct trajectory across all sweeps, with a significantly higher mean BMIz at the starting point (mean intercept = 3.102, $p < 0.001$) than the other classes, that then decreases until the end of the study period (mean slope = −0.935, $p < 0.001$) but is still above the obesity cut-off. Although the Late Weight Gain class shows a lower starting point than the other classes (mean intercept = −0.716, $p < 0.001$), it then shows the greatest increase (mean slope = 1.889, $p < 0.001$) throughout early and middle-childhood, reaching the cut-off of overweight by age 11.

The other two bigger classes (Weight Loss and Early Weight Gain) have close starting points at age 3 (normal-high mean intercepts of 0.723 and 0.931, respectively; Wald test $p = 0.06$), but from then forward, they follow significantly opposite trajectories during childhood (Wald test $p < 0.001$): the Weight Loss Class steadily decreases (mean slope = −0.781), remaining in the normative BMIz range, while the Early Weight Gain increases (mean slope = 0.428) and plateaus in the overweight range.

The proportions of children with overweight and obesity in each class is represented in **Figure 3**.

Association Between BMI Trajectories and Individual and Family Covariates

The association between BMI trajectories and individual and family covariates are shown in **Figure 4**, and unadjusted and adjusted OR are available in **Supplementary Table 5**.

Sociodemographics varied across classes. Males were more likely to follow the Early Obesity trajectory.

Children from single-parent families, living in poverty and with less educated mothers were more likely to follow unhealthy weight trajectories. Non-white ethnicity, in general, was associated with unhealthy weight trajectories. Indian, Pakistani, and Bangladeshi ethnicity showed a strong association with Late Weight Gain, while Black and Black British ethnicity was strongly associated with Early Obesity. Maternal overweight and obesity strongly increased the odds of Early Weight Gain and Early Obesity.

Regarding perinatal and early infancy factors, the Early Weight Gain trajectory was associated with late prematurity, high birthweight, being large for GA and with early solid introduction. Breastfeeding for more than 2 months lowered the odds of Early Weight Gain, but this association disappeared after adjusting to other covariates. The Early Obesity trajectory was associated with high birthweight and being large for GA. The Late Weight Gain trajectory was associated with extreme to moderate prematurity, low birthweight and being small for GA.

Regarding psychosocial family factors, consistent sleep and eating routines lowered the likelihood of following unhealthy weight trajectories even after adjusting for other factors. The Early Weight Gain trajectory was associated with distressed

TABLE 3 | Growth mixture models.

No of classes	1	2	3	4	5
LATENT BASIS LATENT CLASS GROWTH ANALYSIS (GROWTH FACTOR VARIANCES FIXED AT 0)					
Log-likelihood	−101683.801	−90860.327	−85782.504	−83534.897	−82288.310
AIC	203387.602	181746.654	171597.007	167107.794	164620.620
BIC	203465.108	181847.412	171721.017	167255.056	164791.134
aBIC	203433.329	181806.099	171670.170	167194.675	164721.219
Entropy	–	0.74	0.78	0.77	0.76
BLRT *p*-value	–	<0.001	<0.001	1.0000	1.0000
Parameters	10	13	16	19	22
n (%) based on estimated posterior probabilities	17165 (100)	10209 (59.5)/6955 (40.5)	9,149 (53.3)/4,992 (29)/3,023 (17.6)	7,732 (45.1)/5,292 (30.8)/2,686 (15.7)/1,453 (8.5)	6,519 (38)/5,359 (31.2)/3,117 (18.2)/1,364 (7.9)/803 (4.7)
LATENT BASIS HOMOSCEDASTIC GROWTH MIXTURE MODEL (GROWTH FACTOR VARIANCES EQUAL BETWEEN CLASSES)					
Log-likelihood	−79740.295	−79489.756	−79363.766	−79334.793	−79306.347
AIC	159506.591	159011.511	158765.531	158713.586	158662.694
BIC	159607.349	159135.521	158912.793	158884.100	158856.460
aBIC	159566.035	159084.674	158852.413	158814.185	158777.011
Entropy	–	0.88	0.82	0.58	0.65
BLRT *p*-value	–	<0.001	<0.001	<0.001	<0.001
Parameters	13	16	19	22	25
n (%) based on estimated posterior probabilities	17,165 (100)	16,412 (95.6)/752 (4.4)	15,512 (90.4)/907 (5.3)/745 (4.3)	10,983 (64)/4,825 (28.1)/716 (4.2)/638 (3.7)	11,166 (65.1)/4,675 (27.2)/641 (3.7)/628 (3.7)/53 (0.3)
LATENT BASIS HETEROSCEDASTIC GROWTH MIXTURE MODEL (GROWTH FACTOR VARIANCES VARIANT BETWEEN CLASSES)					
Log-likelihood	−79740.295	−79341.995	−79272.012	−79248.190	
AIC	159506.591	158721.990	158594.023	158558.380	
BIC	159607.349	158869.252	158787.789	158798.649	
aBIC	159566.035	158808.871	158708.341	158700.133	
Entropy	–	0.49	0.48	0.46	
BLRT *p*-value	–	0.0000	1.0000	1.0000	
Parameters	13	19	25	31	
n (%) based on estimated posterior probabilities	17,165 (100)	13,344 (77.7)/3,820 (22.3)	12,055 (70.2)/2,982 (17.4)/2,127 (12.4)	10,627 (61.9)/2,857 (16.6)/2,691 (15.7)/989 (16.6)	
LATENT BASIS HOMOSCEDASTIC GROWTH MIXTURE MODEL (GROWTH FACTOR VARIANCES EQUAL BETWEEN CLASSES)—ACCOUNTING FOR THE COMPLEX SAMPLE DESIGN					
Log-likelihood	−79081.593	−78815.046	−78677.319	**−78641.145**	−78605.349
AIC	158189.186	157662.092	157392.638	**157326.290**	157260.698
BIC	158289.944	157786.102	157539.900	**157496.804**	157454.464
aBIC	158248.631	157735.255	157479.519	**157426.890**	157375.015
Entropy	–	0.87	0.84	**0.62**	0.69
Parameters	13	16	19	**22**	25
n (%) based on estimated posterior probabilities	17,165 (100)	16,426 (95.7)/738 (4.3)	15,649 (91.2)/776 (4.5)/738 (4.3)	**11,838 (69)/4,118 (24)/636 (3.7)/571 (3.3)**	12,099 (70.5)/3,905 (22.7)/631 (3.7)/448 (2.6)/79 (0.5)

AIC, Aikaike Information Criterion; BIC, Schwarz's Bayesian Information Criterion; aBIC, sample adjusted Bayesian Information Criterion; BLRT, Bootstrapped Log-Likelihood Ratio Test. All Growth Mixture Models were estimated without covariates using MPlus, version 8.3 (ANALYSIS = MIXTURE; ALGORITHM = INTEGRATION). The number of random starts was increased to 2,000 with 500 final stage optimizations (STARTS = 2000 500). To compute the BLRT, the number of random starts for the k class was increased to 200 with 40 final stage optimizations (LRTSTARTS = 0 0 200 40). Then, we repeated the analysis accounting for the complex sample design (ANALYSIS = MIXTURE COMPLEX). The chosen model is presented in bold.

temperament and with emotional dysregulation, although these associations were attenuated after adjusting to other covariates. The Early Obesity and the Late Weight Gain trajectories were associated with emotional dysregulation and child-parent conflict, but these associations disappeared after adjusting to other covariates.

DISCUSSION

We used GMM to capture the developmental change in BMI from early childhood to adolescence. We found four weight trajectories: Weight Loss (69%), Early Weight Gain (24%), Early Obesity (3.7%), and Late Weight Gain (3.3%). Using data

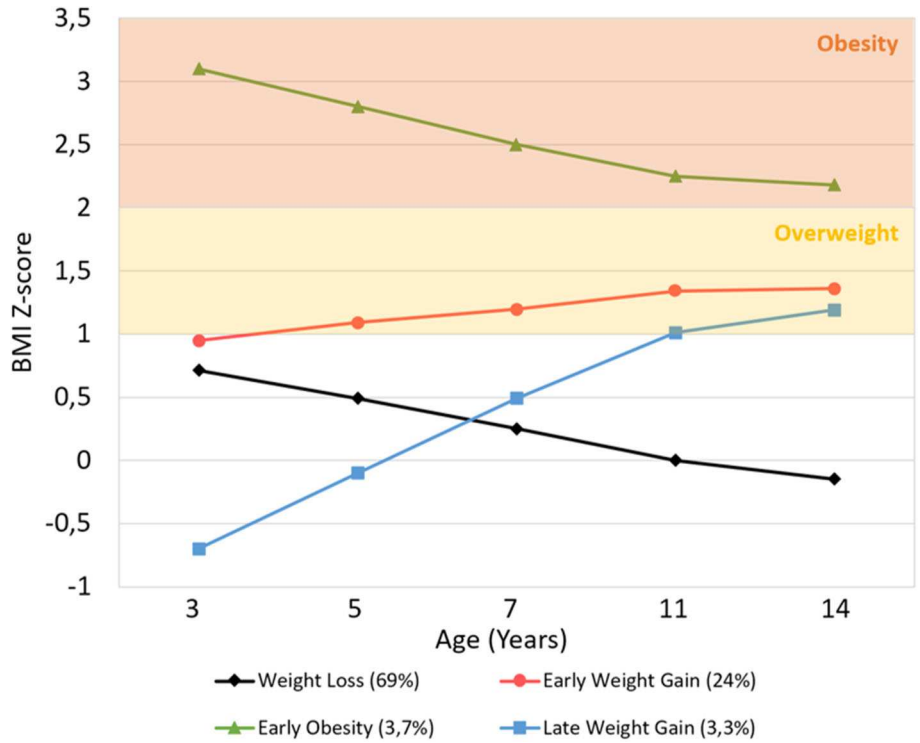

FIGURE 2 | Average BMI Z-score trajectory for each class, from age 3 to 14 years. Overweight and obesity ranges were based on WHO Multicenter Growth Reference Study age- and gender-specific cutoffs.

from the MCS, a recent study applying LCGA to raw BMI data from 3 to 11 years also found four trajectories: *Stable* (83.8%), *Moderate Increasing* (13.1%), *High Increasing* (2.5%), and *Decreasing* (0.6%) (39). In addition, a similar study using the same period and that applied GMM instead of LCGA to raw BMI values found four trajectories, but with a significantly different interpretation: *Low normal* (boys, 49%; girls, 42%), *Mid normal* (boys, 36%; girls, 38%), *Overweight* (boys, 12%; girls, 16%), and *Obesity* (boys, 2%; girls, 3%) (19). In the latter study, there were no increasing or decreasing trajectories, meaning that children did not change to different weight categories during the study period (no mobility from normal to overweight/obesity categories or vice-versa) (19). Based on a methodologically different approach, our study further builds on the contrasting results of these previous studies, applying a homoscedastic latent basis GMM on age- and gender-specific BMIz to better capture BMI variation with growth and expanding the analysis from age 3 to age 14.

BMI Trajectories Are Mainly Settled by Early Adolescence

The greatest amount of change in BMIz occurred during early and middle-childhood, and there was little further change after age 11. In our study sample, by the time children enter school, they have reached one third of the total amount of change in their BMIz; further, 59.8% of the total amount of change occurs between ages 5 and 11, suggesting that early and middle-childhood are two different critical periods to intervene in weight trajectories. The importance of preschool years in weight gain has been established in other studies (6). In contrast, in one study using data from the Avon Longitudinal Study of Parents and Children (7) and in another in the USA (8), BMIzs steadily increased during childhood, with the greatest change occurring after school entry, suggesting that excess weight gain and obesity also develop in middle-childhood. In both studies, there was little further change in early adolescence.

BMI Trajectories Are Influenced by Different Early Childhood Factors

The Early Obesity and Late Weight Gain classes follow clearly distinct and extreme trajectories across all sweeps and represent smaller groups of individuals (3.7 and 3.3%, respectively). The other two classes, Weight Loss and Early Weight Gain, have close starting points at age 3 and then follow less extreme but opposite trajectories during childhood, which represent the majority of individuals (69 and 24%, respectively). Although poverty, ethnic minority, single-parenthood, and low maternal education formed a common core of predictors of unhealthy weight trajectories, we found different associations with early biological, psychological, and family factors.

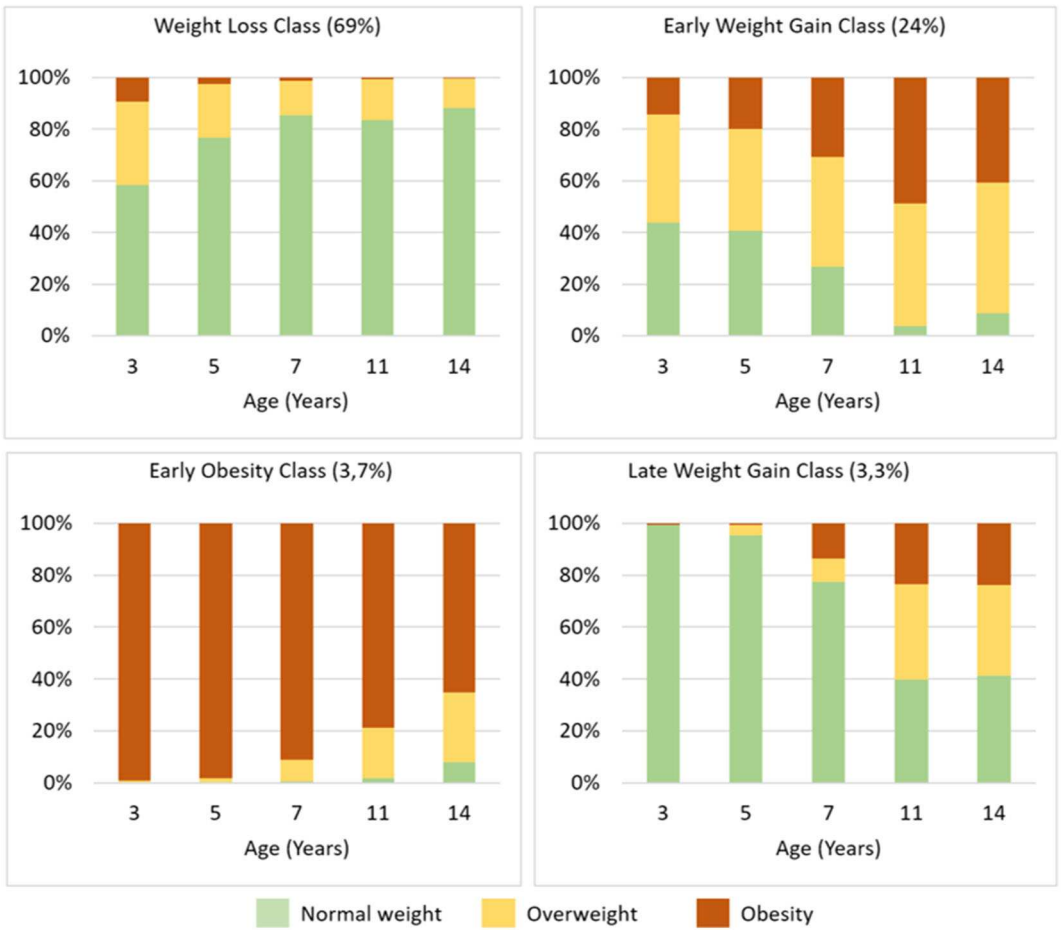

FIGURE 3 | BMI Z-score trajectory class characterization, showing the proportions of children with overweight and obesity in each class according to WHO Multicenter Growth Reference Study age- and gender-specific cutoffs.

Early Socioeconomic Context

In our study, children living in economically disadvantaged, ethnic, and single-parent families at 9 months of age were at greater odds of following unhealthy weight trajectories compared to their peers living in white advantaged families. Poverty has been consistently associated with obesity in both children and adults. Indeed, several studies support that early childhood poverty has an enduring association with obesity (40) and that its effect persists despite subsequent improvement in socioeconomic status (41).

In our study, we found a strong association between ethnicity and unhealthy weight trajectories, that persisted even after adjusting for other factors. Several studies support that ethnic minority children have a significantly higher risk of obesity (42, 43). There may be multiple mechanisms underlying the relationship between ethnicity and obesity, including genetic and metabolic differences, alongside differences in lifestyle, eating and exercise habits (44). In a recent study in the USA, although African American and Hispanic children showed higher rates of overweight and obesity, this relationship disappeared after controlling for family income (42). Also, children from ethnic minorities tend to live in deprived neighborhoods, with lower access to healthy food options and to parks and playgrounds, and are at greater risk of poverty and segregation (43, 45), suggesting there is a complex interplay between ethnicity, socioeconomic status and obesity. In our study, the association between socioeconomic status and unhealthy weight trajectories was attenuated by other factors like maternal nutritional status and maternal education. In fact, maternal education appears to play a major role in childhood obesity (46). A large prospective study including data from 11 European cohorts concluded that low maternal education substantially increased the risk of early childhood adiposity in all countries (46). Therefore, maternal education might moderate the effect of poverty on obesity.

Child Factors

Gestational age, birthweight, breastfeeding, and complementary feeding

In our study, children with high birthweight and who were large for GA were at greater odds of Early Weight Gain and Early Obesity. Interestingly, late prematurity was associated with the Early Weight Gain trajectory, but extreme to moderate

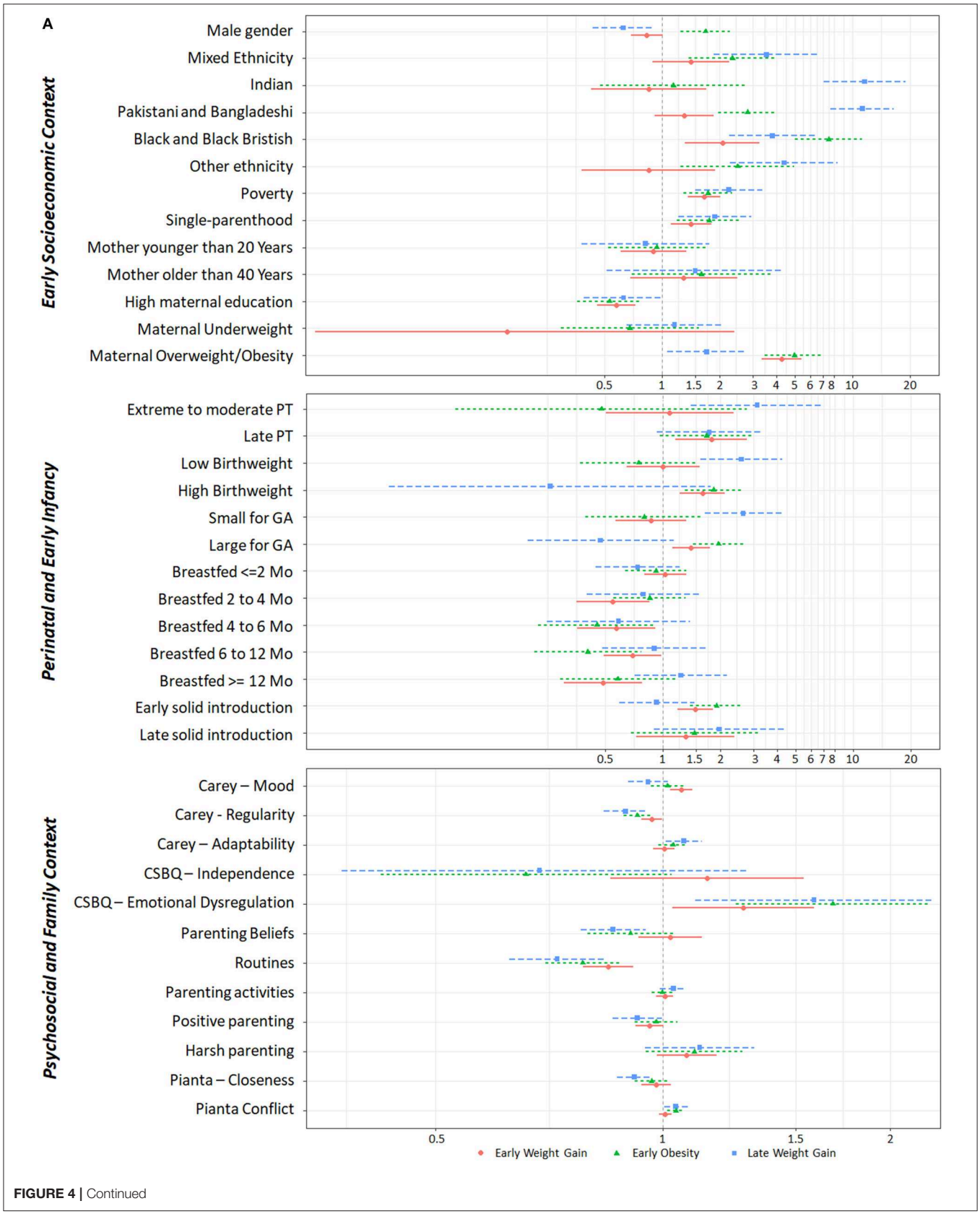

FIGURE 4 | Continued

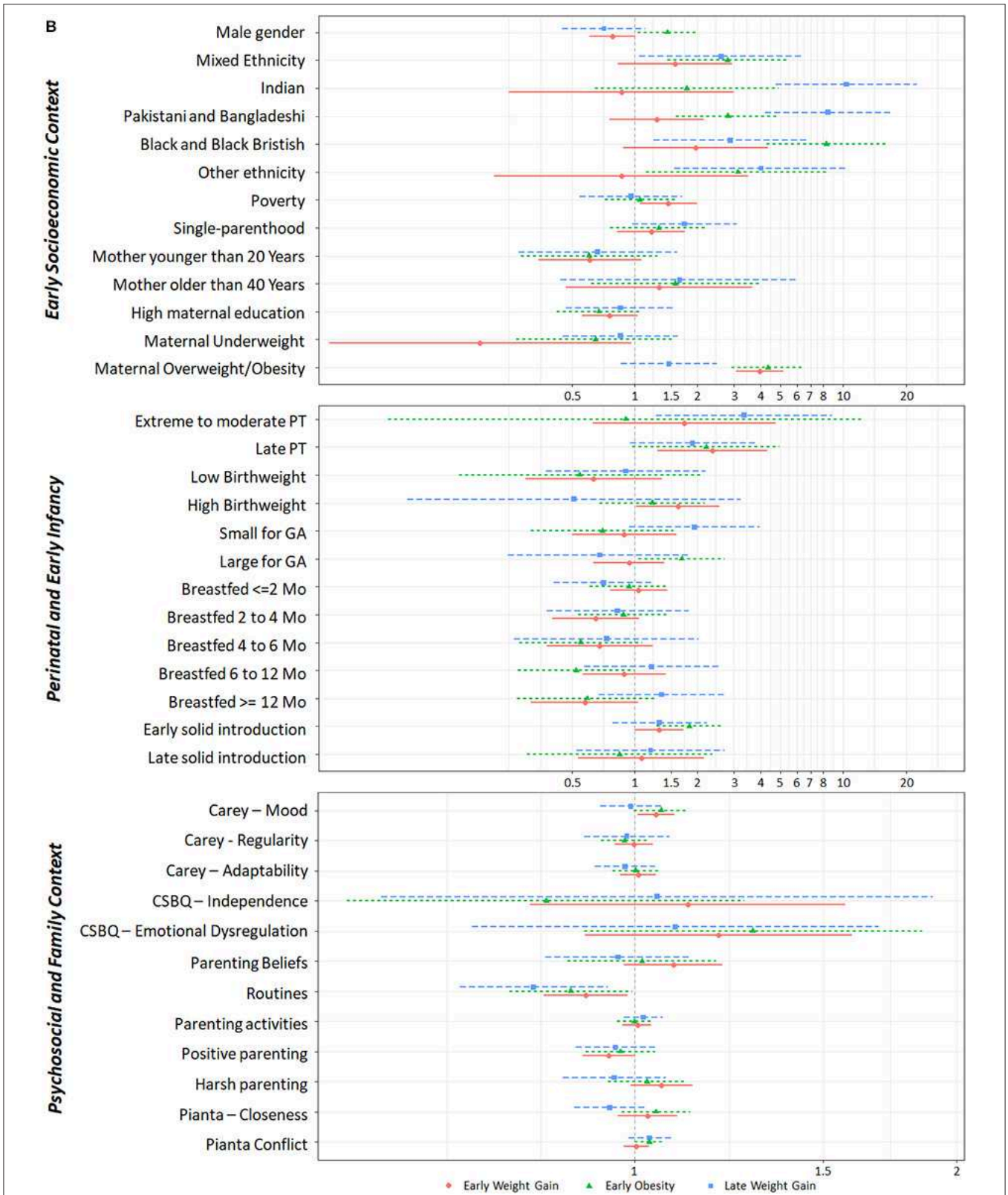

FIGURE 4 | Early life predictors of weight trajectories. Association between early life individual and contextual factors and BMIz trajectory classes, using the Weight Loss class as the reference class. **(A)** Unadjusted Odds Ratio; **(B)** Adjusted Odds Ratio. CSBQ, Child Social Behavioral Questionaire; GA, Gestational Age; PT, prematurity.

prematurity and being small for GA increased the likelihood of Late Weight Gain.

Early life context, including pregnancy, has a strong effect on later risk of obesity. Two cross-sectional studies of young adults found that those who had been born before 33 weeks GA had higher adiposity and cardiometabolic risk than those born at term (47, 48). Moreover, it has been proposed that both nutrient overabundance and scarcity during pregnancy and infancy lead to a metabolic programming that results in an increased obesity risk throughout the lifespan (49). In fact, in a longitudinal cohort in Rotterdam, individuals with fetal growth restriction followed by infant weight acceleration had higher visceral and liver adiposity than those with normal fetal and infant growth (50).

In our study, being breastfed for more than 2 months lowered the likelihood of Early Weight Gain and being breastfed for more than 4 months lowered the likelihood of Early Obesity, but not of Late Weight Gain. Early solid food introduction had an opposite association. These associations were moderated by socioeconomic and biological factors. Breastfeeding has many demonstrated benefits for both mother and child (51). The mechanisms underlying the relationship between breastfeeding and obesity are still debated and include human milk polysaccharides, modulation of gut microbiota, and promotion of sensitive feeding and self-regulation (52, 53). In the literature, the protective effect of exclusive breastfeeding on rapid weight gain is seen mainly in early childhood, but its long-lasting effect has been debated (54). Other authors even argue that this relationship may stem from the more favorable socioeconomic and educational background of breastfeeding mothers (55).

Child temperament and self-regulation

In our study, emotional dysregulation in infancy was associated with Early Obesity, but this association disappeared after adjusting for other factors.

The association between infant self-regulation and adolescent obesity has already been demonstrated in a study using the same MCS cohort (27). A growing body of evidence indicates that self-regulation has an important role in eating behavior, with children who show a lower capacity to self-sooth and to self-regulate being at greater risk of using food as a consolation tool and of obesity (56). The ability to regulate emotions begins in infancy and develops in the context of early mother-child reciprocal interactions. Breastfeeding has been shown to promote a mother's sensitivity and capability of attributing mental states to their babies, and to predict more positive and sensitive behaviors during feeding at 12 months (53).

Family Factors

Household routines, parenting beliefs, and parenting activities

In our study, children with consistent sleep and eating routines in early childhood were less likely to follow unhealthy weight trajectories, even after adjusting for socioeconomic and biological factors. The effect of household routines on obesity has already been consistently demonstrated in a study using the MCS and in the USA (27, 57).

In our study, more positive parenting beliefs at age 9 months decreased the odds of Late Weight Gain, but this association disappeared after adjusting to other covariates. Although parental engagement in children's daily activities has been previously associated with lower obesity risk (58), we found no association between parenting activities and weight trajectories.

Discipline practices and child-parent relationship

Children who had a close relationship with their mothers in early childhood were less likely to follow the Late Weight Gain trajectory, and child-parent conflict in early childhood was associated with Early Obesity and Late Weight Gain. Previous research shows a strong association between parent-child relationship and unhealthy eating and sedentary behaviors, factors well-known to promote weight gain. A recent review highlights that a secure parent-child bond and high parental connectedness are associated with better eating and general health behaviors, while an insecure attachment and difficult parent-child relationship were associated with disordered eating and sedentary behaviors, mediated by temperament and self-regulation (59). Regarding weight status, in a longitudinal study, individuals with poor mother-child relationships in early infancy, assessed by maternal sensitivity and attachment, were at greater odds of obesity during adolescence (60).

In our study, positive parenting showed a small protective association with Late Weight Gain that disappeared after adjusting to other factors. Harsh parenting has been demonstrated to increase the risk of obesity, while the appropriate use of family rules has been associated with a deceleration of obesity risk in adolescence (58, 61).

Strengths and Limitations

This study provided new evidence about the relationship between nutritional status trajectories and child and family factors. Its prospective longitudinal design allowed the exploration of BMI trajectories from early childhood to adolescence, and its large sample size allowed the exploration of multiple important individual and family covariates. To date, this is one of few studies that comprehensively included psychological factors like child temperament, self-regulation, parenting, and child-parent relationship alongside socioeconomic and biological factors.

Also, this study further explored the methodological aspects of investigating BMI trajectories. Anthropometric measures were directly collected according to the best standards, providing reliable and accurate data for subsequent analysis. Furthermore, trajectories were based on a continuous measure of BMI rather than on categorical measures of overweight and obesity. Especially, trajectories were based on BMI Z-scores, considered the gold standard for evaluating anthropometric measures. Moreover, it included 5 repeated measures of BMI over time and explored different GMM specifications.

Nevertheless, this study is not without limitations. We found it challenging to choose the best model. Although there is a common understanding on how model selection should be

guided, there is some debate about the best indicators and their cut-offs. Furthermore, we opted for a homoscedastic GMM because the heteroscedastic GMM provided less substantive and poorly defined classes. Nevertheless, we must acknowledge that homoscedasticity might influence our results. Also, non-invariant models, which are more flexible and which have more parameters to estimate, tend to have superior information criteria at the expense of decreased interpretability and entropy, and one might question what would be the use of a better fitting model if it had lost its classification accuracy. Therefore, meaningfulness has a significant role in model selection. Although the entropy of our final model is not ideal, the subsequent regression analysis was adjusted to this classification bias.

Attrition is a major problem in longitudinal studies. GMM uses FIML to estimate the BMI trajectories, including all available data in the analysis, and therefore minimizes the bias effect of attrition.

Child and family covariates showed different percentages of missing values, and a complete case analysis would have substantially reduced the available sample; thus, we needed to impute missing values, which might influence our associations. Multiple imputation was performed according to best practice, and analysis in imputed and non-imputed data yielded concordant results. Although we recategorized covariates according to their distributions, previous research, and, whenever possible, international standards and recommendations, this recategorization might affect the association between BMI trajectories and covariates.

Conclusion

This study focused on BMI trajectories and looked at their early childhood predictors. In our study, 31% of the children followed an unhealthy weight trajectory that was mainly set by the time they reached early adolescence. Therefore, obesity prevention interventions should target children in early and middle-childhood, particularly those living in disadvantaged families. This study further points out that the lack of routines, low emotional self-regulation, low child-parent closeness, and child-parent conflict are significantly associated with unhealthy BMI trajectories; therefore, these are possible areas for family-based, health-promotion interventions. Further studies should focus on how different family and parenting factors interplay and influence weight trajectories, and on possible short- and long-term consequences on health status and well-being, following a developmental perspective.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available through the UK Data Service [https://beta.ukdataservice.ac.uk/datacatalogue/series/series?id=2000031], but restrictions apply to the availability of these data, which were used under Special License for the current study. Data are available through the UK Data Service after approval by the CLS Data Access Committee.

ETHICS STATEMENT

The Millennium Cohort Study protocol meets the ethical requirements of the Helsinki Declaration and ethical approval has been sought for all sweeps: Sweep 1 - South West MREC (MREC/01/6/19), Sweep 2 - London MREC (MREC/03/2/022), Sweep 3 - London MREC (05/MRE02/46), Sweep 4 - Yorkshire MREC (07/MRE03/32), Sweep 5 - Yorkshire and the Humber - Leeds East MREC (11/YH/0203), and Sweep 6 - London Central MREC (12/LO/1786). The present analyses did not require additional ethical approval. Written informed consent to participate in each sweep was provided by the participants' parents/ legal guardian.

AUTHOR CONTRIBUTIONS

Data analysis was performed by CS. The first draft of the manuscript was written by CS and all authors commented on previous versions of the manuscript and revised it critically for important intellectual content. All authors contributed to the study conception and design, read and approved the final manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGMENTS

The Millennium Cohort Study (MCS), which began in 2000, was conducted by the Centre for Longitudinal Studies (CLS). It aims to chart the conditions of social, economic, and health advantages and disadvantages facing children born at the start of the twenty-first century. The Principal Investigator and Director of the MCS is Prof. Emla Fitzsimons, UCL Institute of Education, University College London. For details, see https://cls.ucl.ac.uk/cls-studies/millennium-cohort-study/. MCS was commissioned by the Economic and Social Research Council (ESRC - http://www.esrc.ac.uk/), whose funding has been supplemented by a consortium of Government departments and the Wellcome Trust (http://www.wellcome.ac.uk/).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00417/full#supplementary-material

REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, adults. *Lancet*. (2017). 390:2627–42. doi: 10.1016/S0140-6736(17)32129-3
2. de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr*. (2010) 92:1257–64. doi: 10.3945/ajcn.2010.29786
3. Rodriguez-Ayllon M, Cadenas-Sanchez C, Esteban-Cornejo I, Migueles JH, Mora-Gonzalez J, Henriksson P, et al. Physical fitness and psychological health in overweight/obese children: a cross-sectional study from the active brains project. *J Sci Med Sport*. (2017) 21:179–84. doi: 10.1016/j.jsams.2017.09.019
4. Bacchini D, Licenziati MR, Garrasi A, Corciulo N, Driul D, Tanas R, et al. Bullying and victimization in overweight and obese outpatient children and adolescents: an Italian multicentric study. *PLoS ONE*. (2015) 10:e0142715. doi: 10.1371/journal.pone.0142715
5. Patton GC, Coffey C, Carlin JB, Sawyer SM, Williams J, Olsson CA, et al. Overweight and obesity between adolescence and young adulthood: a 10-year prospective cohort study. *J Adolesc Health*. (2011) 48:275–80. doi: 10.1016/j.jadohealth.2010.06.019
6. Gardner DS, Hosking J, Metcalf BS, Jeffery AN, Voss LD, Wilkin TJ. Contribution of early weight gain to childhood overweight and metabolic health: a longitudinal study (EarlyBird 36). *Pediatrics*. (2009) 123:e67–73. doi: 10.1542/peds.2008-1292
7. Hughes AR, Sherriff A, Lawlor DA, Ness AR, Reilly JJ. Timing of excess weight gain in the Avon Longitudinal Study of Parents and Children (ALSPAC). *Pediatrics*. (2011) 127:e730–6. doi: 10.1542/peds.2010-0959
8. Datar A, Shier V, Sturm R. Changes in body mass during elementary and middle school in a national cohort of kindergarteners. *Pediatrics*. (2011) 128:e1411–7. doi: 10.1542/peds.2011-0114
9. Birch LL, Davison KK. Family environmental factors influencing the developing behavioral controls of food intake and childhood overweight. *Pediatr Clin North Am*. (2001) 48:893–907. doi: 10.1016/S0031-395570347-3
10. Vollmer RL, Mobley AR. Parenting styles, feeding styles, and their influence on child obesogenic behaviors and body weight. A review. *Appetite*. (2013) 71:232–41. doi: 10.1016/j.appet.2013.08.015
11. Sleddens EFC, Gerards SMPL, Thijs C, de Vries NK, Kremers SPJ. General parenting, childhood overweight and obesity-inducing behaviors: a review. *Int J Pediatr Obes*. (2011) 6:e12–27. doi: 10.3109/17477166.2011.566339
12. Halliday JA, Palma CL, Mellor D, Green J, Renzaho AMN. The relationship between family functioning and child and adolescent overweight and obesity: a systematic review. *Int J Obes*. (2014) 38:480–93. doi: 10.1038/ijo.2013.213
13. Mattsson M, Maher GM, Boland F, Fitzgerald AP, Murray DM, Biesma R. Group-based trajectory modelling for BMI trajectories in childhood: a systematic review. *Obes Rev*. (2019) 20:998–1015. doi: 10.1111/obr.12842
14. Muthén B, Muthén LK. Integrating person-centered and variable-centered analyses: growth mixture modeling with latent trajectory classes. *Alcohol Clin Exp Res*. (2000) 24:882–91. doi: 10.1111/j.1530-0277.2000.tb02070.x
15. Mei Z, Grummer-Strawn LM. Standard deviation of anthropometric Z-scores as a data quality assessment tool using the 2006. WHO growth standards: a cross country analysis. *Bull World Health Organ*. (2007) 85:441–8. doi: 10.2471/BLT.06.034421
16. Connelly R, Platt L. Cohort profile: UK Millennium Cohort Study (MCS). *Int J Epidemiol*. (2014) 43:1719–25. doi: 10.1093/ije/dyu001
17. Department of Nutrition for Health and Development at the World Health Organization (WHO). *WHO Anthro Survey Analyser: Software for Analysing Survey Anthropometric Data for Children Under 5 years of Age*. Geneva: World Health Organization (2018). Available online at: https://whonutrition.shinyapps.io/anthro/ (accessed October 25, 2019).
18. WHO. *WHO AnthroPlus for Personal Computers Manual: Software for Assessing Growth of the World's Children and Adolescents*. Geneva: WHO (2019). Available online at: https://www.who.int/growthref/tools/en/ (accessed October 25, 2019).
19. Stuart B, Panico L. Early-childhood BMI trajectories: evidence from a prospective, nationally representative British cohort study. *Nutr Diabetes*. (2016) 6:e198. doi: 10.1038/nutd.2016.6
20. Flouri E, Ioakeimidi S, Midouhas E, Ploubidis GB. Maternal psychological distress and child decision-making. *J Affect Disord*. (2017) 218:35–40. doi: 10.1016/j.jad.2017.04.034
21. March of Dimes, PMNCH, Save the Children, WHO. Born too soon: the global action report on preterm birth. In: Howson CP, Kinney MV, Jelawn, editors. *Maternal, Newborn, Child and Adolescent Health*. Geneva: World Health Organization (2012). Available online at: https://www.who.int/maternal_child_adolescent/documents/born_too_soon/en/ (accessed October 25, 2019).
22. World Health Organization. *ICD-10: International Statistical Classification of Diseases and Related Health Problems: Tenth Revision*. Geneva: World Health Organization (2004).
23. Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the newborn cross-sectional study of the INTERGROWTH-21st Project. *Lancet*. (2014) 384:857–68. doi: 10.1016/S0140-673660932-6
24. Carling SJ, Demment MM, Kjolhede CL, Olson CM. Breastfeeding duration and weight gain trajectory in infancy. *Pediatrics*. (2015) 135:111–9. doi: 10.1542/peds.2014-1392
25. Fewtrell M, Bronsky J, Campoy C, Domellöf M, Embleton N, Fidler Mis N, et al. Complementary feeding: a position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) committee on nutrition. *J Pediatr Gastroenterol Nutr*. (2017) 64:119–32. doi: 10.1097/MPG.0000000000001454
26. Hernández-Alava M, Popli G. Children's development and parental input: evidence from the UK millennium cohort study. *Demography*. (2017) 54:485–511. doi: 10.1007/s13524-017-0554-6
27. Anderson SE, Sacker A, Whitaker RC, Kelly Y. Self-regulation and household routines at age three and obesity at age eleven: longitudinal analysis of the UK millennium cohort study. *Int J Obes*. (2017) 41:1459–66. doi: 10.1038/ijo.2017.94
28. Kroll ME, Carson C, Redshaw M, Quigley MA. Early father involvement and subsequent child behaviour at ages 3, 5 and 7 years: prospective analysis of the UK millennium cohort study. *PLoS ONE*. (2016) 11:e0162339. doi: 10.1371/journal.pone.0162339
29. Rajyaguru P, Moran P, Cordero M, Pearson R. Disciplinary parenting practice and child mental health: evidence from the UK millennium cohort study. *J Am Acad Child Adolesc Psychiatry*. (2019) 58:108–16.e2. doi: 10.1016/j.jaac.2018.06.033
30. Muthen LK, Muthén BO. *Mplus Version 8 User's Guide*. Los Angeles, CA: Muthen & Muthen (2017).
31. Hallquist MN, Wiley JF. MplusAutomation: an R package for facilitating large-scale latent variable analyses in mplus. *Struct Equat Model*. (2018) 25:621–38. doi: 10.1080/10705511.2017.1402334
32. Solt F, Hu Y. *Dotwhisker: Dot-and-Whisker Plots of Regression Results*. The Comprehensive R Archive Network (CRAN) (2015). Available online at: https://cran.r-project.org/web/packages/dotwhisker/index.html (accessed December 5, 2019).
33. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2019).
34. Nylund KL, Asparouhov T, Muthén BO. Deciding on the number of classes in latent class analysis and growth mixture modeling: a Monte Carlo simulation study. *Struct Equat Model*. (2007) 14:535–69. doi: 10.1080/10705510701575396
35. Todo N, Usami S. Fitting unstructured finite mixture models in longitudinal design: a recommendation for model selection and estimation of the number of classes. *Struct Equat Model*. (2016) 23:695–712. doi: 10.1080/10705511.2016.1205444
36. Celeux G, Soromenho G. An entropy criterion for assessing the number of clusters in a mixture model. *J Classif*. (1996) 13:195–212. doi: 10.1007/BF01246098
37. Vermunt JK. Latent class modeling with covariates: two improved three-step approaches. *Pol Anal*. (2010) 18:450–69. doi: 10.1093/pan/mpq025
38. Lang KM, Little TD. Principled missing data treatments. *Prev Sci*. (2016) 19:1–11. doi: 10.1007/s11121-016-0644-5
39. Kelly Y, Patalay P, Montgomery S, Sacker A. BMI development and early adolescent psychosocial well-being: UK millennium cohort

study. *Pediatrics.* (2016) 138:e20160967. doi: 10.1542/peds.2016-0967
40. Lee H, Andrew M, Gebremariam A, Lumeng JC, Lee JM. Longitudinal associations between poverty and obesity from birth through adolescence. *Am J Public Health.* (2014) 104:e70–6. doi: 10.2105/AJPH.2013.301806
41. Li M, Mustillo S, Anderson J. Childhood poverty dynamics and adulthood overweight/obesity: Unpacking the black box of childhood. *Soc Sci Res.* (2018) 76:92–104. doi: 10.1016/j.ssresearch.2018.05.009
42. Rogers R, Eagle TF, Sheetz A, Woodward A, Leibowitz R, Song M, et al. The relationship between childhood obesity, low socioeconomic status, and race/ethnicity: lessons from Massachusetts. *Child Obes.* (2015) 11:691–5. doi: 10.1089/chi.2015.0029
43. Wong MS, Chan KS, Jones-Smith JC, Colantuoni E, Thorpe RJ, Bleich SN. The neighborhood environment and obesity: understanding variation by race/ethnicity. *Prev Med.* (2018) 111:371–7. doi: 10.1016/j.ypmed.2017.11.029
44. Stryjecki C, Alyass A, Meyre D. Ethnic and population differences in the genetic predisposition to human obesity. *Obes Rev.* (2018) 19:62–80. doi: 10.1111/obr.12604
45. Morgan Hughey S, Kaczynski AT, Child S, Moore JB, Porter D, Hibbert J. Green and lean: is neighborhood park and playground availability associated with youth obesity? Variations by gender, socioeconomic status, and race/ethnicity. *Prev Med.* (2017) 95:S101–8. doi: 10.1016/j.ypmed.2016.11.024
46. Ruiz M, Goldblatt P, Morrison J, Porta D, Forastiere F, Hryhorczuk D, et al. Impact of low maternal education on early childhood overweight and obesity in Europe. *Paediatr Perinat Epidemiol.* (2016) 30:274–84. doi: 10.1111/ppe.12285
47. Thomas EL, Parkinson JR, Hyde MJ, Yap IKS, Holmes E, Doré CJ, et al. Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. *Pediatr Res.* (2011) 70:507–12. doi: 10.1203/PDR.0b013e31822d7860
48. Sipola-Leppänen M, Vääräsmäki M, Tikanmäki M, Matinolli HM, Miettola S, Hovi P, et al. Cardiometabolic risk factors in young adults who were born preterm. *Am J Epidemiol.* (2015) 181:861–73. doi: 10.1093/aje/kwu443
49. Koletzko B, Brands B, Grote V, Kirchberg FF, Prell C, Rzehak P, et al. Long-term health impact of early nutrition: the power of programming. *Ann Nutr Metab.* (2017) 70:161–9. doi: 10.1159/000477781
50. Vogelezang S, Santos S, Toemen L, Oei EHG, Felix JF, Jaddoe VWV. Associations of fetal and infant weight change with general, visceral, and organ adiposity at school age. *JAMA Netw Open.* (2019) 2:e192843. doi: 10.1001/jamanetworkopen.2019.2843
51. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet.* (2016) 387:475–90. doi: 10.1016/S0140-6736(15)01024-7
52. Alderete TL, Autran C, Brekke BE, Knight R, Bode L, Goran MI, et al. Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. *Am J Clin Nutr.* (2015) 102:1381–8. doi: 10.3945/ajcn.115.115451
53. Farrow C, Blissett J. Maternal mind-mindedness during infancy, general parenting sensitivity and observed child feeding behavior: a longitudinal study. *Attach Hum Dev.* (2014) 16:230–41. doi: 10.1080/14616734.2014.898158
54. Liu JX, Xu X, Liu JH, Hardin JW, Li R. Association of maternal gestational weight gain with their offspring's anthropometric outcomes at late infancy and 6 years old: mediating roles of birth weight and breastfeeding duration. *Int J Obes.* (2017) 42:8–14. doi: 10.1038/ijo.2017.183
55. Coduti N, Gregoire M, Sowa D, Diakakis GM, Chen Y. Characteristics of exclusively breastfeeding mothers. *Top Clin Nutr.* (2015) 30:174–83. doi: 10.1097/TIN.0000000000000033
56. Graziano PA, Kelleher R, Calkins SD, Keane SP, Brien MO. Predicting weight outcomes in preadolescence: the role of toddlers' self-regulation skills and the temperament dimension of pleasure. *Int J Obes.* (2013) 37:937–42. doi: 10.1038/ijo.2012.165
57. Anderson SE, Whitaker RC. Household routines and obesity in US preschool-aged children. *Pediatrics.* (2010) 125:420–8. doi: 10.1542/peds.2009-0417
58. Huang DYC, Lanza HI, Anglin MD. Trajectory of adolescent obesity: exploring the impact of prenatal to childhood experiences. *J Child Fam Stud.* (2014) 23:1090–101. doi: 10.1007/s10826-013-9766-6
59. Blewitt C, Bergmeier H, Macdonald JA, Olsson CA, Skouteris H. Associations between parent-child relationship quality and obesogenic risk in adolescence: a systematic review of recent literature. *Obes Rev.* (2016) 17:612–22. doi: 10.1111/obr.12413
60. Anderson SE, Gooze RA, Lemeshow S, Whitaker RC. Quality of early maternal-child relationship and risk of adolescent obesity. *Pediatrics.* (2012) 129:132–40. doi: 10.1542/peds.2011-0972
61. Lohman BJ, Gillette MT, Neppl TK. Harsh parenting and food insecurity in adolescence: the association with emerging adult obesity. *J Adolesc Health.* (2016) 59:123–7. doi: 10.1016/j.jadohealth.2016.03.024
62. dos Santos CS, Picoito J, Nunes C, Loureiro I. Early individual and family predictors of weight trajectories from early childhood to adolescence: results from the Millennium Cohort Study. *medRxiv [Preprint].* (2020). doi: 10.1101/2020.02.26.20027409

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DATA REFERENCES

1. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: Longitudinal Family File, 2001-2015. [data collection]. 2nd Edition. UK Data Service. SN:8172, doi: 10.5255/UKDA-SN-8172-2

2. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: First Survey, 2001-2003. [data collection]. 12th Edition. UK Data Service. SN: 4683, doi: 10.5255/UKDA-SN-4683-4

3. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: Second Survey, 2003-2005. [data collection]. 9th Edition. UK Data Service. SN: 5350, doi: 10.5255/UKDA-SN-5350-4

4. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: Third Survey, 2006. [data collection]. 7th Edition. UK Data Service. SN: 5795, doi: 10.5255/UKDA-SN-5795-4

5. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: Fourth Survey, 2008. [data collection]. 7th Edition. UK Data Service. SN: 6411, doi: 10.5255/UKDA-SN-6411-7

6. University of London, Institute of Education, Centre for Longitudinal Studies. (2017). Millennium Cohort Study: Fifth Survey, 2012. [data collection]. 4th Edition. UK Data Service. SN: 7464, doi: 10.5255/UKDA-SN-7464-4

7. University of London, Institute of Education, Centre for Longitudinal Studies. (2019). Millennium Cohort Study: Sixth Survey, 2015. [data collection]. 4th Edition. UK Data Service. SN: 8156, doi: 10.5255/UKDA-SN-8156-4

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New Perspectives on Genetic Prediction for Pediatric Metabolic Associated Fatty Liver Disease

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Non-alcoholic or recently re-defined metabolic associated fatty liver disease (MAFLD), a spectrum of progressive hepatic disease, has become a public health issue in obese children and adolescents. MAFLD is a complex metabolic disease strongly associated with obesity and insulin resistance. It is not known why not every obese subject will develop MAFLD. Different ethnic/racial groups display differences in MAFLD prevalence, indicating genetic factor plays a role. In the past two decades, sequence variations in genetic loci, including *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, *HSD17B13*, etc. have been shown to confer susceptibility to MAFLD in children and adults. This review article provides an updated viewpoint of genetic predictors related to pediatric MAFLD. We discuss whether these susceptible genes can be clinically used for risk stratification and personalized care. Understanding human genetics and molecular mechanisms can give important information not only for prediction of risk but also on how to design drugs. In view of current epidemic of MAFLD worldwide, it is necessary to identify which children with MAFLD progress rapidly and need earlier intervention. In the future, a comprehensive analysis of individualized genetic and environmental factors may help assess the risk of children with MAFLD and personalize their treatment.

Keywords: fatty liver, genetics, sequence variation, precision medicine, pediatric, children, obesity

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INTRODUCTION

There is increasing interest in metabolic associated fatty liver disease (MAFLD), defined as excessive deposition of fat in the liver in the absence of significant alcohol consumption. The progression of MAFLD encompasses a spectrum of conditions ranging from fat in the liver—simple steatosis, fat with inflammation and/or fibrosis-steatohepatitis to advanced fibrosis and cirrhosis over time (1). MAFLD is one of the most common chronic liver disease in the whole world (2, 3), becoming a global health burden (4). The long-term follow-up study revealed adults with MAFLD had increased liver related and non-liver related mortalities (5). MAFLD is now a serious health condition not only for adults, but also for children (6).

Pathogenesis of MAFLD is complicated, multifactorial (7), and strongly associated with obesity related comorbidities such as insulin resistance, cardiac dysfunction, and kidney disease, etc. (8–11). Nowadays, MAFLD is no longer regarded as a primary hepatic disease, but rather a component of metabolic syndrome. Therefore, a recent expert consensus group suggested the metabolic associated fatty liver disease "MAFLD" as a more appropriate term than the

nomenclature of non-alcoholic fatty liver disease (NAFLD). Since children drink less alcohol, we believe that the term MAFLD is more suitable for children and should replace NAFLD (12).

Due to a continuum from obesity to metabolic syndrome, patients with MAFLD may benefit from early identification of the disease risk and individually targeted treatment (13). Establishing clinical predictors is necessary for MAFLD diagnosis and risk stratification. Previously reported biochemical factors include elevated total cholesterol, triglycerides, fasting insulin, increased fasting glucose and insulin concentrations, homeostatic model assessment for insulin resistance (HOMA-IR) index, and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (14, 15).

There are several differences between pediatric and adult MAFLD in prevalence, risk propensity, and liver histology (**Table 1**) (20, 21). Risk factors such as alcohol abuse, drug abuse and comorbidities among children are much less than those in adults. Understanding the differences between pediatric and adult MAFLD can help assess how NAFLD progresses from childhood toward adulthood. The histological pattern of pediatric MAFLD is different from that of adults. The classic histological findings that represent MAFLD are: steatosis, swelling, inflammation, and fibrosis. In adults, steatosis, inflammation, and accumulation of collagen start in the perivenular area (zone 3), while in children, it usually starts in the periportal area (zone 1) with lack of ballooning (22). However, it is not clear whether patients with pediatric MAFLD pattern differ in pathogenesis, prognosis, or response to treatment (23). In addition, due to lack of long-term follow-up from children to adults, the natural history and prognosis of MAFLD in children are still uncertain. Compared with adults with MAFLD, children with MAFLD have a much longer course of disease. Reversing the course of MAFLD in childhood is indeed an unmet need.

EPIDEMIOLOGY

The increasing epidemic of obesity and sedentary lifestyle continues to raise the prevalence of MAFLD (24). As the global obesity epidemic worsens metabolic disorders, the health burden of children's MAFLD has become huge (16, 25). Recent research indicated in obese children, MAFLD is present in nearly one-third of boys and one-fourth of girls (26). Not every obese subject will suffer from MAFLD, which suggests that genetic and/or environmental factors contribute to each individual's susceptibility. In fact, MAFLD can occur in non-obese individuals (27, 28).

Different ethnic/racial groups display differences in MAFLD prevalence (29). It is recognized the highest prevalence is in the American Hispanic population followed by the Caucasian and the African-American (30). In pediatric population, obese Hispanic adolescents are more likely to develop MAFLD than obese non-Hispanic adolescents (31). Compared with the West, the East has a lower MAFLD incidence and prevalence (32).

Different lifestyle and nutrition status may partially account for the differences among ethnic groups. However, the Western diet and sedentary lifestyle have led to the emergence of obesity and MAFLD in Asia over the last decade (33). On the other hand, growing evidence reveals the importance of genetic factors in the development and progression of MAFLD. Since the frequency of genetic variants differs among ethnic groups, increasing understanding of the genetic predisposition to MAFLD may help us to decipher the reasons for its occurrence (34).

HERITABILITY OF MAFLD

There is growing awareness of the role of genetic factors in the etiology and prognosis of MAFLD. The differences in disease distribution observed in adults and children with MAFLD indicate genetic susceptibility plays a crucial role in the development of MAFLD (6). In a twin study, Loomba et al. revealed that MAFLD-associated hepatic steatosis and fibrosis are heritable traits (35). Genome-wide association studies (GWAS) have identified several important single nucleotide polymorphisms (SNPs) affecting the severity and progression of MAFLD (36). Overall, the dynamic interactions between genetic and environmental factors further modulate the disease phenotype, susceptibility, development, and progression (37–39).

Till now, MAFLD susceptible genes have been reported to be involved in a wide spectrum of pathogenic mechanisms, including lipid metabolism, insulin signaling, oxidative stress, inflammation and fibrogenesis, etc. Genetic factors do not have the same effects across studies due to different study populations and designs. For example, people with *PNPLA3* variant are usually considered to have more hepatic steatosis, inflammation, and fibrosis. However, Kotronen et al. reported *PNPLA3* SNP did not improve the prediction of liver fat content by using their liver fat score equation (40). In fact, MAFLD is a polygenetic disease and we need more genetic information, rather than just one SNP, to establish the predictive model.

MAJOR COMMON GENETIC VARIATIONS OF MAFLD

Herein, we provide a comprehensive update on genetic variations related to pediatric MAFLD (**Table 2**). The genetic determinants of MAFLD not only can predict the progression of MAFLD, but also are the possible targets for therapy.

To date, several major common MAFLD susceptible genes reported in adults have been replicated in pediatric studies, such as *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, and *HSD17B13*. The effects and presumptive functions of these susceptible genes are discussed as follows:

PNPLA3

Patatin-like phospholipase domain containing 3 (*PNPLA3*) gene encodes a transmembrane protein "adiponutrin," which is expressed predominantly in the liver, retina, skin, and adipose tissue (68, 69). In 2008, Romeo et al. first identified the association of a *PNPLA3* gene I148M variant with hepatic fat content in adults by GWAS (70). Subsequently, Romeo et al. reported this variant was associated with increased levels of ALT/AST in obese children, which suggests that it confers a genetic susceptibility

TABLE 1 | Differences between adult and pediatric MAFLD.

Clinical Features	Children	Adults
Prevalence (4, 16)	7.6% overall, and 34.2% obese	24% overall, and 45–70% obese
Histology (17)		
Steatosis	Typically zone 1	Typically zone 3
Inflammation	Mainly portal	Mainly lobular
Ballooning	Rale	Common
Fibrosis	Predominantly portal-periportal	Perisinusoidal chicken wire
Cirrhosis (18)	1–2%	5–10%
Prognosis marker (19)	Unknown (lack of long-term longitudinal studies)	Degree of fibrosis

to liver damage since childhood (71). Furthermore, *PNPLA3* gene I148M variant modulates the progression and liver-related outcomes in patients with MAFLD (72). The effects of *PNPLA3* variants on pediatric MAFLD have been validated in different ethnicities, including Han Chinese, Hispanic, and Caucasian (41–44). Till now, *PNPLA3* is regarded as the most robust susceptible gene for MAFLD across different ethnicities.

The wild type PNPLA3 protein facilitates triglyceride hydrolysis. Impaired hydrolysis of triglycerides/lipid droplet (LD) remodeling in hepatocytes leads to hepatic steatosis (73, 74). PNPLA3 I148M promotes the accumulation of intracellular lipids in the liver by reducing the lipidation and secretion of low-density lipoprotein (VLDL) particles (75). Further studies showed the *PNPLA3* variant not only increases the odds of developing fatty liver itself, but it also determines the degree of hepatic injury and the full spectrum of histopathologic consequences of MAFLD (76). Several studies have shown that PNPLA3 increases the stimulation of hepatic stellate cells by affecting the metabolism of retinoids, leading to liver fibrosis (77–79). Hence, *PNPLA3* SNP has emerged as the key genetic determinant of MAFLD severity in both adults and pediatric patients (80). A recent study showed that silencing *Pnpla3* with antisense oligonucleotides improved liver steatosis and fibrosis in *Pnpla3* I148M knock-in mice, indicating that PNPAL3 could be a potential target for treatment in human (81).

In addition to the effect on liver, *PNPLA3* rs738409 variant has been reported to be associate with reduced glomerular filtration rate (GFR) in children with obesity, indicating the variant *PNPLA3* genotype may be related to kidney dysfunction in children independent of MAFLD status (82, 83).

TM6SF2

The human transmembrane 6 superfamily member 2 (*TM6SF2*) gene encodes a protein of 351 amino acids with 7–10 predicted transmembrane domains. TM6SF2 protein facilitates the transfer of neutral lipids from cytoplasmic to luminal LDs and VLDL particles. Overexpression of *TM6SF2* decreases the number and size of LDs (84).

In 2014, Kozlitina et al. reported that the *TM6SF2* rs58542926 variant, a C-to-T substitution, encoding a glutamate to lysine change at codon 167 (E167K) and associated with high hepatic triglyceride content and elevated liver serum enzymes levels in adults enrolled in the Dallas Heart Study (85). *TM6SF2* E167K variant carriers with MAFLD have impaired hepatic lipid synthesis from polyunsaturated fatty acids (86). Further studies reported the *TM6SF2* rs58542926 variant also influences hepatic fibrosis and metabolic homeostasis (87, 88). In children, the association between *TM6SF2* rs58542926 variant and MAFLD has been replicated in different ethnicities (45–47). Interestingly, the plasma levels of triglycerides are lower in *TM6SF2* E167K variant carriers than in the non-carriers (89). The increase in hepatic steatosis for loss of function mutation in *TM6SF2* is due to a double mechanism, namely a reduction in the lipidation of VLDL particles (84) and in the number of the secreted apolipoprotein B100 particles (90). In addition to its effects on the liver, it has been reported that *TM6SF2* variants also affected the renal function in children (91) and adults (92) independently of MAFLD.

GCKR

Glucokinase regulator protein (GCKR) is an inhibitor of glucokinase which regulates glucose storage and disposal and controls *de novo* lipogenesis by regulating the flux of glucose into hepatocytes (93). In 2011, Speliotes et al. reported variants in or near *GCKR* are associated with liver fat content and histopathologic phenotypes at genome-wide significance levels (94). Subsequent meta-analysis provides evidence of significant association between *GCKR* rs780094 (an intronic variant) and risk of MAFLD (95). Silva et al. measured metabolites by mass spectrometry and found novel associations of the *GCKR* rs780094 variant with amino acids and their downstream metabolites, especially lipids (96). In addition, the progression of fibrosis in MAFLD could be influenced by the *GCKR* genotype (97). The effect of *GCKR* genotype on pediatric MAFLD have been reported. The variant *GCKR* rs780094 has been reported to confer susceptibility to MAFLD in obese school children and adolescents in Taiwan (48).

Another common variant in *GCKR* rs1260326, which is in linkage disequilibrium with rs780094, was associated with hepatic triglyceride content in the Dallas Heart Study (98). This variant encodes for a proline to leucine substitution at the 446 position (P446L), resulting in a loss of the affinity of the GCKR protein for the glucokinase. Consequently, more glucokinase is available in the cytoplasm to convert glucose into glucose-6-phosphate. The increased production of glucose-6-phosphate results in an increased rate of glycolysis, leading to increased production of malonyl-CoA, the precursor of *de novo* lipogenesis (99). The rs1260326 in *GCKR* gene is also linked to fatty liver in obese youths (49).

MBOAT7

The membrane-bound O-acyltransferase domain-containing protein 7 (*MBOAT7*) is a 6 transmembrane domain (100). Hepatocyte specific inactivation of this gene caused an increase in hepatic fat content due to a non-canonical triglyceride synthesis pathway related to a high turnover of phosphatidyl inositol

TABLE 2 | Genetic variations associated with pediatric MAFLD.

Gene	Variant	Chr.	Population (ethnicity)	Subjects (n)	Function	Consequence	Phenotype	References
PNPLA3	rs738409 C>G	22q13.31	Mexican	1,037	Lipid droplets remodeling	Ile148Met	↑MAFLD, Fibrosis	(41)
			Taiwanese	520				(42)
			Hispanic	327				(43)
			Caucasian	149				(44)
TM6SF2	rs58542926 C>T	19p13.11	Italian	1,010	Modulate hepatic VLDL secretion	Glu167Lys	↑MAFLD, Fibrosis	(45)
			402 Caucasians 266 African Americans 289 Hispanics	957				(46)
			Taiwanese	831				(47)
*GCKR**	rs780094 C>T	2p23	Taiwanese	797	Modulate hepatic lipogenesis	Intronic variant	↑MAFLD, Fibrosis	(48)
	rs1260326 C>T		181 Caucasians 139 African Americans 135 Hispanics	455		Leu446Pro		(49)
*MBOAT7***	rs641738 C>T	19q12.42	Italian	1,002	Remodeling of phosphatidylinositol	Glu17Val	↑MAFLD, Fibrosis	(50)
			Caucasian	467				(51)
			Taiwanese	831			No effect	(47)
	rs626283 G>C		Caucasian	860		Intron variant	↑MAFLD	(52)
HSD17B13	rs72613567: TA	4q22.1	Italian	685	Retinol dehydrogenase activity	Splice donor variant	↓MAFLD, Fibrosis	(53)
IRGM	rs10065172 C>T	5q33.1	Taiwanese	832	Alter hepatic lipophagy	Leu105=	↑MAFLD	(54)
			Italian	613				(55)
MTTP	rs2306986 G>C	4q23	Han Chinese	368	VLDL secretion	Glu98Asp	↑MAFLD, Fibrosis	(56)
LPIN1	rs13412852 C>T	2p25.1	Italian	142	Lipogenesis	Intronic variant	↑MAFLD, Fibrosis	(57)
IRS-1	rs1801278 A>G	2q36.3	Italian	71	Impair insulin signaling	Gly971Arg	↑Fibrosis	(58)
ENPP1	rs1044498 A>C	6q23.2	Italian	71	Inhibit insulin signaling	Lys173Glu	↑MAFLD, Fibrosis	(58)
			German	70				(59)
GPR120	rs116454156 G>A	10q23.33	Italian	581	Modulate inflammation response	Arg270His	↑MAFLD	(60)
UGT1A1	rs4148323 G>A	2q37.1	Taiwanese	234	Increase bilirubin with anti-oxidant activity	Gly71Arg	↓MAFLD	(61)
PPARGC1A	rs8192678 G>A	4p15.1	Taiwanese	781	Regulate cellular energy metabolism	Gly487Ser	↑MAFLD	(62)
HO-1	(GT)n repeat	22q12	Taiwanese	101	Anti-oxidative stress	Promoter activity	↑MAFLD	(63)
CNR2	rs35761398 A>G	1p36.11	Italian	118	Modulate inflammation response	Gln63Arg	↑MAFLD	(64)
KLB	rs17618244 G>A	4p14	Italian	249	Upregulate lipotoxic and proinflammatory genes	Arg728Gln	↑MAFLD	(65)
KLF6	rs3750861 G>A	10p15.2	Italian	152	Regulate hepatic stellate cell activation and fibrogenesis,	IVS1-27A	↓Fibrosis	(66)
FDFT1	rs2645424 A>G	8p23.1	87 Caucasians 61 African Americans 81 Hispanics	229	Modulate intrahepatic cholesterol biosynthesis	Intronic variant	↑MAFLD	(67)

Chr, chromosome; MAFLD, metabolic associated fatty liver disease; VLDL, very low-density lipoprotein.

**GCKR rs780094 and rs1260326 are in strong linkage disequilibrium.*

***MBOAT7 rs641738 and rs626283 are in strong linkage disequilibrium.*

(101). Down-regulation of *MBOAT7* predisposes subjects to MAFLD (102).

In 2016, Mancina et al. first reported the rs641738 C>T variant in the *MBOAT7* gene was associated with increased risk of MAFLD in adults of European descent (103). Subsequently, an association between *MBOAT7* variant and liver fibrosis severity was confirmed by Krawczyk et al. (104). In an animal study, *MBOAT7* over-expression was negatively correlated with obesity and insulin sensitive, driving the progression of MAFLD (105). A pediatric study showed the *MBOAT7* rs641738 variant was associated with plasma concentrations of ALT in obese children (50, 51). Umano et al. reported that another variant (rs626283) in *MBOAT7* gene was associated with MAFLD in Caucasian obese children (52). Notably, conflicting data has reported that *MBOAT7* rs641738 polymorphism does not influence hepatic steatosis and liver injury as determined by serum levels of CK-18 fragment in obese Taiwanese children of Han Chinese ethnicity (47). The difference in the effect of *MBOAT7* variant on MAFLD might be due to different ethnicities.

HSD17B13

Increasing data demonstrate hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) acts a pivotal part in hepatic lipid homeostasis and the pathogenesis of MAFLD (106). In 2018, Abul-Husn et al. reported that a loss-of function splice variant (rs72613567:TA) of *HSD17B13* gene was associated with a reduced risk of chronic liver disease and of MAFLD progression (107). More recently, an exome-wide association study confirmed the *HSD17B13* rs72613567 variant influenced the susceptibility and histological severity of MAFLD (108). A pediatric study in Italy, reported obese children carrying the *HSD17B13* variant had lower hepatic steatosis and pediatric MAFLD fibrosis index than non-carriers (53). A recent work also showed that this genetic variation provided kidney protection for children (109).

The HSD17B13 protein is a liver-specific LD-associated protein and exhibits retinol dehydrogenase activity. The *HSD17B13* variant is supposed to alter mRNA splicing, yielding a truncated protein with reduced enzymatic activity. However, the exact role and function of HSD17B13 in MAFLD pathophysiology remains largely uncharacterized. Over-expression of *HSD17B13* in human hepatoma cell lines or C57BL/6 mice leads to excessive lipid accumulation. *HSD17B13* gene encodes a LD-associated protein, which is involved in regulating lipogenesis (110). Recently, the Rotman group published interesting studies on the inactivation of *Hsd17b13* in mice (111) and the identification of an enzymatic active site metabolizing retinol (112).

HEPATIC LIPID METABOLISM

Excessive fat accumulation damages hepatocytes and leads to inflammatory response, cytokine production, oxidative stress, abnormal cellular signaling, and activation of stellate cells. The intracellular storage and utilization of lipids play an important role in supporting cellular energy homeostasis. In addition to the major MAFLD susceptible genes aforementioned, several important genetic variants have been identified to affect hepatic lipid metabolism.

IRGM

In 2009, Singh et al. first reported that LDs can be degraded in hepatocytes by a specific autophagy-related process "lipophagy" (113). The immunity-related GTPase family M protein encoded by the *IRGM* gene controls autophagy activation (114). Our previous study found obese children with the variant *IRGM* rs10065172 TT genotype have a higher risk of MAFLD and elevated ALT levels compared with subjects with wild type (54). A downregulation of *IRGM* in HepG2 cells decreased autophagic flux accompanied by an increased lipid accumulation. In contrast, overexpression of *IRGM* decreased LD content in HepG2 cells. This genetic association has been replicated in a cohort of obese Italian children (55). A recent murine study revealed liver specific suppression of *Ifgga2* (the mice ortholog of human *IRGM*) increases hepatic fat content in a backcross of obese C57BL/6J New Zealand mice (115).

MTTP

The human microsomal triglyceride transfer protein (MTTP) works to lipidate and assemble the apoB-containing lipoproteins in the liver. It is critical to remove lipid from liver through the assembly and secretion of VLDL particles. Hsiao et al. reported the *MTTP* polymorphisms can modulate lipid homeostasis and determine the serum lipids and risk of MAFLD (116). A pediatric study also showed the association of *MTTP* rs2306986 variant and MAFLD in obese children (56).

LIPIN1

Lipin-1 encoded by the *LPIN1* gene, expressed mainly in adipose and the liver, has phosphatidic phosphatase activity (117). Valenti et al. reported children, but not adult, carrying the *LIPIN1* rs13412852 TT genotype had a lower prevalence of MAFLD, less severe liver damage and a lower liver fibrosis prevalence (57). The mutation in *LIPIN1* gene may result in decreasing the flux of free fatty acids (FFAs) to the liver.

INSULIN RESISTANCE

Insulin resistance is a feature of the MAFLD pathophysiology and occurs in its early phases (118, 119). It affects metabolic syndrome and MAFLD in obese children and adolescents (120), even in non-obese patients (121). Peripheral insulin resistance leads to excessive lipolysis in adipose tissue, releasing a lot of FFAs into the circulation (8). The liver then uptakes excessive FFAs and exceeds its capacity to transfer FFAs into neutral triglycerides, causing hepatic steatosis, lipotoxicity, and endoplasmic reticulum stress (122).

IRS1, ENPP1

The insulin receptor substrate 1 (*IRS1*) and ectonucleotide pyrophosphate phosphodiesterase (*ENPP1*) genes play crucial roles in controlling cell signaling in response to insulin. Once insulin binds to the insulin receptor, the IRS1 protein regulates hepatic gene expression that coordinates glucose homeostasis.

ENPP1 protein negatively modulates insulin receptors and induces insulin resistance if overexpressed.

Hepatic *IRS1* overexpression is associated with histological progression in patients with MAFLD (123). The *ENPP1* rs104449 and *IRS1* rs1801278 variants decrease hepatic insulin signaling and predispose adult patients with MAFLD to liver damage (58). In children, Hudert et al. reported *ENPP1* rs1044498, not *IRS1* rs1801278 variant was associated with pediatric MAFLD (59).

OXIDATIVE STRESS AND INFLAMMATION

The two-hit hypothesis is widely recognized as a model of MAFLD progression (124). The first hit causes hepatic fat accumulation and the second hit causes inflammation and fibrosis. The second hit usually results from excessive oxidative stress, such as mitochondrial stress and insulin resistance. In the other words, oxidative stress plays a critical role in the progression from simple steatosis to steatohepatitis (125, 126).

GPR120

G protein-coupled receptor 120 (GPR120) is a functional omega-3 fatty acid receptor that mediates anti-inflammatory and insulin sensitivity (127, 128). In 2014, Marzuillo et al. reported the association between the *GPR120* rs116454156 variant (R270H) and liver injury in obese children and adolescents (60). By regulating GPR120, docosahexaenoic acid (DHA) can reduce the inflammatory response of MAFLD in children (129).

UGT1A1

Genetic modifiers belonging to oxidative stress are involved in either the generation of reactive oxygen species (ROS) or the modulation of cellular antioxidant defense. Genetic variant in uridine-5'-diphosphoglucuronosyltransferase 1A1 (*UGT1A1*) increases serum bilirubin, which has anti-oxidative properties. Our previous study found that the variant *UGT1A1*6* genotype was associated with a lower risk of MAFLD in obese Taiwanese children (61). A subsequent study showed an inverse relation between serum bilirubin levels and the presence of MAFLD on Italian children, replicating our previous finding (130).

HO-1

Increasing heme oxygenase-1 (HO-1) activity can reverse complications related to obesity, metabolic syndrome, and MAFLD (131). HO-1 is a stress-responsive protein, defensing against the oxidative process. The (GT)n dinucleotide repeat within the *HO-1* gene promotor region is highly polymorphic. Our previous study found that obese children with the long repeat of *HO-1* (GT)n dinucleotide were more susceptible to MAFLD (63). In obese mice, liraglutide, a glucagon-like peptide 1 analog, ameliorated MAFLD severity through upregulating Sestrin2-mediated Nrf2/HO-1 pathway in obese mice (132), suggesting HO-1 could be a therapeutic target for MAFLD.

PPARGC1A

The peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*) gene encodes a PGC-1α protein that regulates mitochondrial functions, oxidative stress, and lipogenesis (133). Our previous study revealed the *PPARGC1A* rs8192678 risk A allele was associated with an increased risk of MAFLD in obese Taiwanese children (62). An animal study revealed loss of estrogen signaling contributes to oxidative damage caused by low levels of PPARGC1A in liver in mice (134).

CNR2

Innate immunity and inflammation are the hallmarks of progressive MAFLD (135). Inflammatory cytokines are involved in the progression from simple steatosis to steatohepatitis. The cannabinoid receptor type 2 (CB2), encoded by the *CNR2* gene, is a seven-transmembrane domain G protein-coupled receptor. Activation of CB2 receptor inhibits nuclear translocation of NF-kB, decreasing production of inflammatory cytokines. An animal experiment demonstrated CB2 activation can reduce hepatic injury and promote liver regeneration (136). Recent research revealed the role of the *CNR2* rs35761398 variant in modulating the hepatic inflammation state in obese children with MAFLD and in increasing susceptibility to liver damage (64).

KLB

β-Klotho gene (*KLB*) encodes a transmembrane protein mainly expressed in the liver. A recent study indicated *KLB* rs17618244 variant increased the risk of hepatocellular ballooning and lobular inflammation in children with MAFLD (65). The precise mechanism of KLB protein on MAFLD is not clear.

FIBROGENESIS

The liver fibrosis stage is the strongest predictor for disease-specific mortality in MAFLD (137). Accurate assessment of the risk of having advanced liver fibrosis in children with MAFLD is important in the clinical practice. However, because the fibrosis phenotype requires a period of cumulative damage, it is difficult to accurately measure the impact of genetic variation on the liver fibrosis in children with MAFLD. This is why there are fewer studies on the association between liver fibrosis and MAFLD in children. The limited available data on genetic variants associated with liver fibrosis in children with MAFLD are discussed below:

KLF6

Nobili et al. reported the Krueppel-like factor 6 (*KLF6*) rs3750861 variant reduced the risk of liver fibrosis in children with MAFLD (66). *KLF6* is up-regulated by activated HSCs following liver injury (138).

FDFT1

The farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*) gene encodes for squalene synthase and modulates the cholesterol biosynthesis. This *FDFT1* rs2645424 variant has been reported to be associated with the MAFLD activity score and moderate/severe fibrosis in a multiethnic cohort of obese youths (67). It is not currently clear whether the *FDFT1* rs2645424 variant affects the enzyme activity because it is an intronic variant.

INFLUENCES OF GENETIC VARIANTS ON MAFLD PATHOGENESIS

A schematic representation of the pathways in the pathogenesis of MAFLD development and progression affected by genetic factors is summarized in **Figure 1**. It currently remains difficult to accurately predict the development and progression of MAFLD because of the complicated interactions between genetic pathways and variable environmental influences.

TRANSLATIONAL IMPLICATIONS OF GENETIC VARIATIONS FOR MAFLD

Genetic variations in *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, and *HSD17B13*, etc. provide novel insights into the MAFLD pathophysiology and may be incorporated into predictive model for precision medicine in patients with MAFLD (139). Early recognition and treatment of MAFLD decreases long-term morbidity and mortality (140). Because MAFLD is a heterogeneous disease, the treatment option should be personal/individualized. Personalized prediction is required to guide risk stratification and treatment. Ma et al. reported improved diet quality is more effective in individuals at a high genetic risk of MAFLD (141). DHA supplementation may not be as effective as non-carriers in reducing liver fat levels in *PNPLA3* I148M carriers (142). Recently, Costanzo et al. reported a weighted-genetic risk score combining *PNPLA3*, *GCKR*, and *TM6SF2* risk alleles was associated with an 8-fold higher risk of MAFLD in obese children (143). In this regard, polygenic risk scores may be applied in risk stratification and guide the treatment.

Modulation of the human genetics associated with MAFLD presents the opportunity to develop a precision medicine. High-throughput technologies, such as gene array and next generation sequencing, can facilitate the translation of genetic testing in the care of children with MAFLD (144). Considering this complexity, computational models may help design personalized treatment strategies which account for genetic and environmental factors (145).

Despite its promise, the utility of genetic testing in patients with MAFLD remains controversial due to the lack of established evidence related to clinical benefits. Societal guidelines from ESPGHAN (European Society for Pediatric Gastroenterology Hepatology and Nutrition) in 2012 and NASPGHAN (North

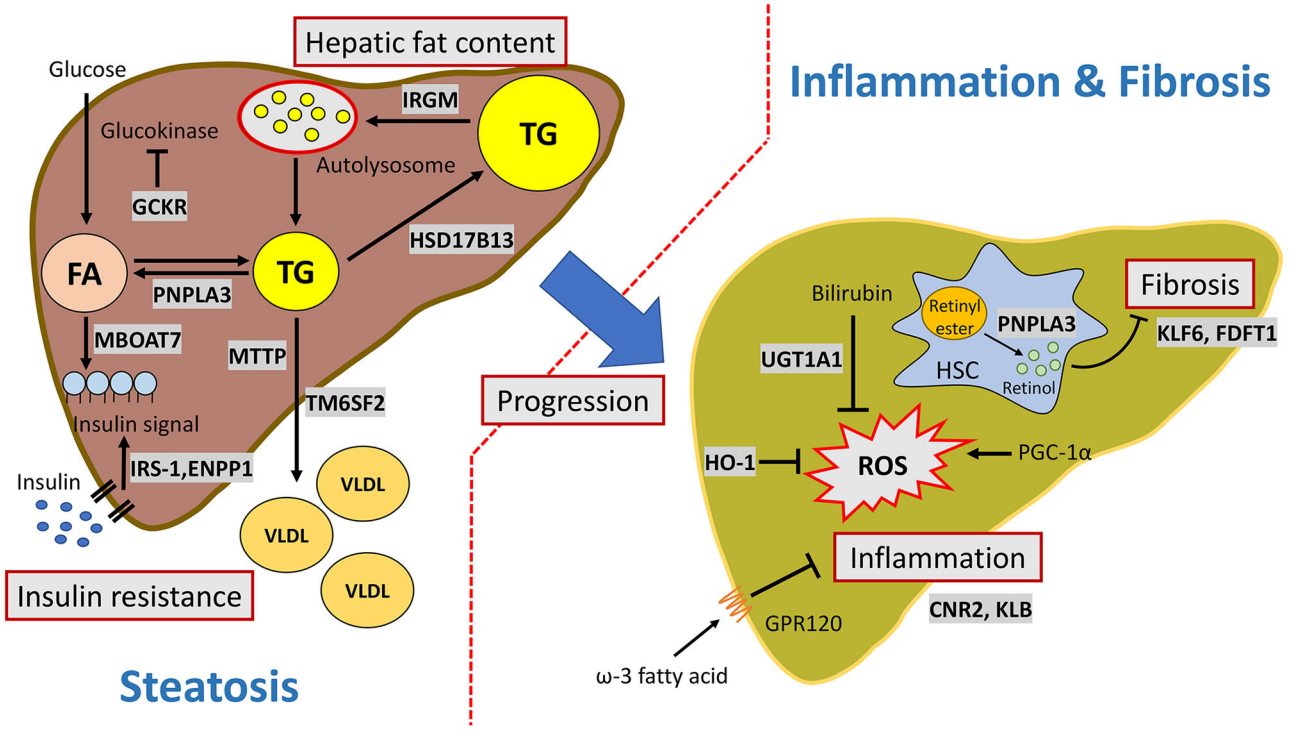

FIGURE 1 | Major pathways involved in the genetic susceptibility toward MAFLD. PNPLA3 facilitates triglyceride hydrolysis in hepatocytes and mediates retinol release from retinyl ester in hepatic stellate cells. IRGM activates autophagosome formation and increases hepatic lipophagy. TM6SF2 transfers triglyceride from cytoplasmic to VLDL particles. MBOAT7 is involved in phospholipid remodeling. HSD17B13 is a lipid droplet-related protein and has retinol dehydrogenase activity. IRS-1 and ENPP1control cell signaling in response to insulin. GCKR regulates glucokinase activity and facilitates *de novo* lipogenesis. GPR120 is a functional omega-3 fatty acid (such as DHA) receptor which mediates anti-inflammatory effects. PGC-1α (encoded by *PPARGC1A*) is a master regulator of reactive oxygen species. HO-1 and UGT1A1are involved in the bilirubin metabolism and antioxidant activity. CB2 (encoded by *CNR2*) and KLB are involved in the inflammation process, but the mechanism is still unknown. KLF6 and squalene synthase (encode by *FDFT1*) are associated with fibrosis. KLF6 activates hepatic stellate cells. Squalene synthase modulates cholesterol biosynthesis.

American Society for Pediatric Gastroenterology, Hepatology, and Nutrition) in 2017 recognize the genetic predisposition strongly affects the risk of MAFLD development in children, but they do not recommend routine genetic testing in children with NALFD (146, 147). In adults, 2016 EASL (European Association for the Study of the Liver) and 2018 AASLD (American Association for the Study of Liver Diseases) guidelines claimed that testing for genetic variants of MAFLD in routine clinical care is currently not advocated (148, 149). More trials are needed to be conducted to test the role of gene-based diagnosis and treatment for MAFLD before its clinical use.

CONCLUSION

This review article summarizes current knowledge and new advances related to the genetics of pediatric MAFLD. Overall, understanding human genetics and molecular mechanisms can give important information not only for prediction of risk but also on how to design drugs (150). To date, genetic studies have successfully advanced our understanding in the pathogenesis of MAFLD, but there are still gaps in translating these genetic studies into clinical applications in the real world. In the future, by analyzing more comprehensive personalized genetic and environmental factors, we will be able to accurately assess the risk of children with MAFLD and adopt personalized treatment.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol.* (2017) 23:8263–76. doi: 10.3748/wjg.v23.i47.8263
2. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* (2019) 4:389–98. doi: 10.1016/S2468-1253(19)30039-1
3. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. *PLoS ONE.* (2017) 12:e0173499. doi: 10.1371/journal.pone.0173499
4. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109
5. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol.* (2009) 7:234–8. doi: 10.1016/j.cgh.2008.11.005
6. Nobili V, Alisi A, Newton KP, Schwimmer JB. Comparison of the phenotype and approach to pediatric vs adult patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2016) 150:1798–810. doi: 10.1053/j.gastro.2016.03.009
7. Zhang X, Ji X, Wang Q, Li JZ. New insight into inter-organ crosstalk contributing to the pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Protein Cell.* (2018) 9:164–77. doi: 10.1007/s13238-017-0436-0
8. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology.* (2001) 120:1183–92. doi: 10.1053/gast.2001.23256
9. Di Sessa A, Umano GR, Miraglia Del Giudice E, Santoro N. From the liver to the heart: cardiac dysfunction in obese children with non-alcoholic fatty liver disease. *World J Hepatol.* (2017) 9:69–73. doi: 10.4254/wjh.v9.i2.69
10. Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. *J Hepatol.* (2020) 72:785–801. doi: 10.1016/j.jhep.2020.01.013
11. Di Costanzo A, Pacifico L, D'Erasmo L, Polito L, Martino MD, Perla FM, et al. Nonalcoholic Fatty Liver Disease (NAFLD), but not its susceptibility gene variants, influences the decrease of kidney function in overweight/obese children. *Int J Mol Sci.* (2019) 20:4444. doi: 10.3390/ijms20184444
12. Eslam M, Sanyal AJ, George J, International Consensus P. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology.* (2020) 158:1999–2014. doi: 10.1053/j.gastro.2019.11.312
13. Godoy-Matos AF, Silva Junior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr.* (2020) 12:60. doi: 10.1186/s13098-020-00570-y
14. Prokopowicz Z, Malecka-Tendera E, Matusik P. Predictive value of adiposity level, metabolic syndrome, and insulin resistance for the risk of nonalcoholic fatty liver disease diagnosis in obese children. *Can J Gastroenterol Hepatol.* (2018) 2018:9465784. doi: 10.1155/2018/9465784
15. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut.* (2008) 57:1441–7. doi: 10.1136/gut.2007.146019
16. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLoS ONE.* (2015) 10:e0140908. doi: 10.1371/journal.pone.0140908
17. Kleiner DE, Makhlouf HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. *Clin Liver Dis.* (2016) 20:293–312. doi: 10.1016/j.cld.2015.10.011
18. Brunt EM. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol.* (2010) 7:195–203. doi: 10.1038/nrgastro.2010.21
19. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2015) 149:389–97.e10. doi: 10.1053/j.gastro.2015.04.043
20. Crespo M, Lappe S, Feldstein AE, Alkhouri N. Similarities and differences between pediatric and adult nonalcoholic fatty liver disease. *Metabolism.* (2016) 65:1161–71. doi: 10.1016/j.metabol.2016.01.008
21. Fitzpatrick E, Dhawan A. Childhood and adolescent nonalcoholic fatty liver disease: is it different from adults? *J Clin Exp Hepatol.* (2019) 9:716–22. doi: 10.1016/j.jceh.2019.05.005
22. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology.* (2005) 42:641–9. doi: 10.1002/hep.20842

23. Goldner D, Lavine JE. Nonalcoholic fatty liver disease in children: unique considerations and challenges. *Gastroenterology.* (2020) 158:1967–83.e1. doi: 10.1053/j.gastro.2020.01.048
24. Sherif ZA, Saeed A, Ghavimi S, Nouraie SM, Laiyemo AO, Brim H, et al. Global epidemiology of nonalcoholic fatty liver disease and perspectives on US minority populations. *Dig Dis Sci.* (2016) 61:1214–25. doi: 10.1007/s10620-016-4143-0
25. Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology.* (2009) 50:1282–93. doi: 10.1002/hep.23119
26. Yu EL, Golshan S, Harlow KE, Angeles JE, Durelle J, Goyal NP, et al. Prevalence of nonalcoholic fatty liver disease in children with obesity. *J Pediatr.* (2019) 207:64–70. doi: 10.1016/j.jpeds.2018.11.021
27. Kim D, Kim WR. Nonobese fatty liver disease. *Clin Gastroenterol Hepatol.* (2017) 15:474–85. doi: 10.1016/j.cgh.2016.08.028
28. VanWagner LB, Armstrong MJ. Lean NAFLD: a not so benign condition? *Hepatol Commun.* (2018) 2:5–8. doi: 10.1002/hep4.1143
29. Weston SR, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology.* (2005) 41:372–9. doi: 10.1002/hep.20554
30. Marzuillo P, del Miraglia Giudice E, Santoro N. Pediatric fatty liver disease: role of ethnicity and genetics. *World J Gastroenterol.* (2014) 20:7347–55. doi: 10.3748/wjg.v20.i23.7347
31. Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics.* (2005) 115:e561–5. doi: 10.1542/peds.2004-1832
32. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol.* (2013) 10:686–90. doi: 10.1038/nrgastro.2013.171
33. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol.* (2017) 67:862–73. doi: 10.1016/j.jhep.2017.06.003
34. Szanto KB, Li J, Cordero P, Oben JA. Ethnic differences and heterogeneity in genetic and metabolic makeup contributing to nonalcoholic fatty liver disease. *Diabetes Metab Syndr Obes.* (2019) 12:357–67. doi: 10.2147/DMSO.S182331
35. Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology.* (2015) 149:1784–93. doi: 10.1053/j.gastro.2015.08.011
36. Dongiovanni. P, Anstee. QM, Valenti. L. Genetic predisposition in NAFLD and NASH impact on severity of liver disease and response to treatment. *Curr Pharm Design.* (2013) 19:5219–38. doi: 10.2174/1381612813199990381
37. Anstee QM, Daly AK, Day CP. Genetic modifiers of non-alcoholic fatty liver disease progression. *Biochim Biophys Acta.* (2011) 1812:1557–66. doi: 10.1016/j.bbadis.2011.07.017
38. Barbara M, Scott A, Alkhouri N. New insights into genetic predisposition and novel therapeutic targets for nonalcoholic fatty liver disease. *Hepatobiliary Surg Nutr.* (2018) 7:372–81. doi: 10.21037/hbsn.2018.08.05
39. Day CP. Genetic and environmental susceptibility to non-alcoholic fatty liver disease. *Dig Dis.* (2010) 28:255–60. doi: 10.1159/000282098
40. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology.* (2009) 137:865–72. doi: 10.1053/j.gastro.2009.06.005
41. Larrieta-Carrasco E, Leon-Mimila P, Villarreal-Molina T, Villamil-Ramirez H, Romero-Hidalgo S, Jacobo-Albavera L, et al. Association of the I148M/PNPLA3 variant with elevated alanine transaminase levels in normal-weight and overweight/obese Mexican children. *Gene.* (2013) 520:185–8. doi: 10.1016/j.gene.2013.03.038
42. Lin YC, Chang PF, Hu FC, Yang WS, Chang MH, Ni YH. A common variant in the PNPLA3 gene is a risk factor for non-alcoholic fatty liver disease in obese Taiwanese children. *J Pediatr.* (2011) 158:740–4. doi: 10.1016/j.jpeds.2010.11.016
43. Goran MI, Walker R, Le KA, Mahurkar S, Vikman S, Davis JN, et al. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. *Diabetes.* (2010) 59:3127–30. doi: 10.2337/db10-0554
44. Valenti L, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology.* (2010) 52:1274–80. doi: 10.1002/hep.23823
45. Grandone A, Cozzolino D, Marzuillo P, Cirillo G, Di Sessa A, Ruggiero L, et al. TM6SF2 Glu167Lys polymorphism is associated with low levels of LDL-cholesterol and increased liver injury in obese children. *Pediatr Obes.* (2016) 11:115–9. doi: 10.1111/ijpo.12032
46. Goffredo M, Caprio S, Feldstein AE, D'Adamo E, Shaw MM, Pierpont B, et al. Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: a multiethnic study. *Hepatology.* (2016) 63:117–25. doi: 10.1002/hep.28283
47. Lin YC, Chang PF, Chang MH, Ni YH. Genetic determinants of hepatic steatosis and serum cytokeratin-18 fragment levels in Taiwanese children. *Liver Int.* (2018) 38:1300–7. doi: 10.1111/liv.13689
48. Lin YC, Chang PF, Chang MH, Ni YH. Genetic variants in GCKR and PNPLA3 confer susceptibility to nonalcoholic fatty liver disease in obese individuals. *Am J Clin Nutr.* (2014) 99:869–74. doi: 10.3945/ajcn.113.079749
49. Santoro N, Zhang CK, Zhao H, Pakstis AJ, Kim G, Kursawe R, et al. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. *Hepatology.* (2012) 55:781–9. doi: 10.1002/hep.24806
50. Di Sessa A, Umano GR, Cirillo G, Del Prete A, Iacomino R, Marzuillo P, et al. The membrane-bound O-acyltransferase7 rs641738 variant in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr.* (2018) 67:69–74. doi: 10.1097/MPG.0000000000001979
51. Viitasalo A, Eloranta AM, Atalay M, Romeo S, Pihlajamaki J, Lakka TA. Association of MBOAT7 gene variant with plasma ALT levels in children: the PANIC study. *Pediatr Res.* (2016) 80:651–5. doi: 10.1038/pr.2016.139
52. Umano GR, Caprio S, Di Sessa A, Chalasani N, Dykas DJ, Pierpont B, et al. The rs626283 variant in the MBOAT7 gene is associated with insulin resistance and fatty liver in caucasian obese youth. *Am J Gastroenterol.* (2018) 113:376–83. doi: 10.1038/ajg.2018.1
53. Di Sessa A, Umano GR, Cirillo G, Marzuillo P, Arienzo MR, Pedulla M, et al. The rs72613567: TA variant in the hydroxysteroid 17-beta dehydrogenase 13 gene reduces liver damage in obese children. *J Pediatr Gastroenterol Nutr.* (2020) 70:371–4. doi: 10.1097/MPG.0000000000002573
54. Lin YC, Chang PF, Lin HF, Liu K, Chang MH, Ni YH. Variants in the autophagy-related gene IRGM confer susceptibility to non-alcoholic fatty liver disease by modulating lipophagy. *J Hepatol.* (2016) 65:1209–16. doi: 10.1016/j.jhep.2016.06.029
55. Bellini G, Miraglia Del Giudice E, Nobili V, Rossi F. The IRGM rs10065172 variant increases the risk for steatosis but not for liver damage progression in Italian obese children. *J Hepatol.* (2017) 67:653–5. doi: 10.1016/j.jhep.2017.02.037
56. Dai D, Wen F, Zhou S, Su Z, Liu G, Wang M, et al. Association of MTTP gene variants with pediatric NAFLD: a candidate-gene-based analysis of single nucleotide variations in obese children. *PLoS ONE.* (2017) 12:e0185396. doi: 10.1371/journal.pone.0185396
57. Valenti L, Motta BM, Alisi A, Sartorelli R, Buonaiuto G, Dongiovanni P, et al. LPIN1 rs13412852 polymorphism in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr.* (2012) 54:588–93. doi: 10.1097/MPG.0b013e3182442a55
58. Dongiovanni P, Valenti L, Rametta R, Daly AK, Nobili V, Mozzi E, et al. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut.* (2010) 59:267–73. doi: 10.1136/gut.2009.190801
59. Hudert CA, Selinski S, Rudolph B, Blaker H, Loddenkemper C, Thielhorn R, et al. Genetic determinants of steatosis and fibrosis progression in paediatric non-alcoholic fatty liver disease. *Liver Int.* (2019) 39:540–56. doi: 10.1111/liv.14006
60. Marzuillo P, Grandone A, Conte M, Capuano F, Cirillo G, Di Sessa A, et al. Novel association between a nonsynonymous variant (R270H) of the G-protein-coupled receptor 120 and liver injury in children and adolescents with obesity. *J Pediatr Gastroenterol Nutr.* (2014) 59:472–5. doi: 10.1097/MPG.0000000000000463
61. Lin YC, Chang PF, Hu FC, Chang MH, Ni YH. Variants in the UGT1A1 gene and the risk of pediatric nonalcoholic fatty liver disease. *Pediatrics.* (2009) 124:e1221–7. doi: 10.1542/peds.2008-3087

62. Lin Y-C, Chang P-F, Chang M-H, Ni Y-H. A common variant in the peroxisome proliferator–activated receptor-γ coactivator-1α gene is associated with nonalcoholic fatty liver disease in obese children. *Am J Clin Nutr.* (2013) 97:326–31. doi: 10.3945/ajcn.112.046417
63. Chang PF, Lin YC, Liu K, Yeh SJ, Ni YH. Heme oxygenase-1 gene promoter polymorphism and the risk of pediatric nonalcoholic fatty liver disease. *Int J Obes.* (2015) 39:1236–40. doi: 10.1038/ijo.2015.46
64. Rossi F, Bellini G, Alisi A, Alterio A, Maione S, Perrone L, et al. Cannabinoid receptor type 2 functional variant influences liver damage in children with non-alcoholic fatty liver disease. *PLoS ONE.* (2012) 7:e42259. doi: 10.1371/journal.pone.0042259
65. Dongiovanni P, Crudele A, Panera N, Romito I, Meroni M, De Stefanis C, et al. beta-Klotho gene variation is associated with liver damage in children with NAFLD. *J Hepatol.* (2020) 72:411–19. doi: 10.1016/j.jhep.2019.10.011
66. Nobili V, Donati B, Panera N, Vongsakulyanon A, Alisi A, Dallapiccola B, et al. A 4-polymorphism risk score predicts steatohepatitis in children with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr.* (2014) 58:632–6. doi: 10.1097/MPG.0000000000000279
67. Santoro N, Feldstein AE, Enoksson E, Pierpont B, Kursawe R, Kim G, et al. The association between hepatic fat content and liver injury in obese children and adolescents: effects of ethnicity, insulin resistance, and common gene variants. *Diabetes Care.* (2013) 36:1353–60. doi: 10.2337/dc12-1791
68. Huang Y, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci USA.* (2010) 107:7892–7. doi: 10.1073/pnas.1003585107
69. Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. *J Lipid Res.* (2006) 47:1940–9. doi: 10.1194/jlr.M600185-JLR200
70. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* (2008) 40:1461–5. doi: 10.1038/ng.257
71. Romeo S, Sentinelli F, Cambuli VM, Incani M, Congiu T, Matta V, et al. The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. *J Hepatol.* (2010) 53:335–8. doi: 10.1016/j.jhep.2010.02.034
72. Grimaudo S, Pipitone RM, Pennisi G, Celsa C, Camma C, Di Marco V, et al. Association between PNPLA3 rs738409 C>G variant and liver-related outcomes in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* (2020) 18:935–44.e3. doi: 10.1016/j.cgh.2019.08.011
73. Liu YM, Moldes M, Bastard JP, Bruckert E, Viguerie N, Hainque B, et al. Adiponutrin: a new gene regulated by energy balance in human adipose tissue. *J Clin Endocrinol Metab.* (2004) 89:2684–9. doi: 10.1210/jc.2003-031978
74. BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology.* (2017) 66:1111–24. doi: 10.1002/hep.29273
75. Pirazzi C, Adiels M, Burza MA, Mancina RM, Levin M, Stahlman M, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and *in vitro*. *J Hepatol.* (2012) 57:1276–82. doi: 10.1016/j.jhep.2012.07.030
76. Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res.* (2009) 50:2111–6. doi: 10.1194/jlr.P900013-JLR200
77. Pirazzi C, Valenti L, Motta BM, Pingitore P, Hedfalk K, Mancina RM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet.* (2014) 23:4077–85. doi: 10.1093/hmg/ddu121
78. Pingitore P, Dongiovanni P, Motta BM, Meroni M, Lepore SM, Mancina RM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. *Hum Mol Genet.* (2016) 25:5212–22. doi: 10.1093/hmg/ddw341
79. Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. *Hepatology.* (2017) 65:1875–90. doi: 10.1002/hep.29041
80. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology.* (2011) 53:1883–94. doi: 10.1002/hep.24283
81. Linden D, Ahnmark A, Pingitore P, Ciociola E, Ahlstedt I, Andreasson AC, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. *Mol Metab.* (2019) 22:49–61. doi: 10.1016/j.molmet.2019.01.013
82. Marzuillo P, Di Sessa A, Guarino S, Capalbo D, Umano GR, Pedulla M, et al. Nonalcoholic fatty liver disease and eGFR levels could be linked by the PNPLA3 I148M polymorphism in children with obesity. *Pediatr Obes.* (2019) 14:e12539. doi: 10.1111/ijpo.12539
83. Targher G, Mantovani A, Alisi A, Mosca A, Panera N, Byrne CD, et al. Relationship between PNPLA3 rs738409 polymorphism and decreased kidney function in children with NAFLD. *Hepatology.* (2019) 70:142–53. doi: 10.1002/hep.30625
84. Smagris E, Gilyard S, BasuRay S, Cohen JC, Hobbs HH. Inactivation of Tm6sf2, a gene defective in fatty liver disease, impairs lipidation but not secretion of very low density lipoproteins. *J Biol Chem.* (2016) 291:10659–76. doi: 10.1074/jbc.M116.719955
85. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* (2014) 46:352–6. doi: 10.1038/ng.2901
86. Luukkonen PK, Zhou Y, Nidhina Haridas PA, Dwivedi OP, Hyotylainen T, Ali A, et al. Impaired hepatic lipid synthesis from polyunsaturated fatty acids in TM6SF2 E167K variant carriers with NAFLD. *J Hepatol.* (2017) 67:128–36. doi: 10.1016/j.jhep.2017.02.014
87. Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun.* (2014) 5:4309. doi: 10.1038/ncomms5309
88. Ehrhardt N, Doche ME, Chen S, Mao HZ, Walsh MT, Bedoya C, et al. Hepatic Tm6sf2 overexpression affects cellular ApoB-trafficking, plasma lipid levels, hepatic steatosis and atherosclerosis. *Hum Mol Genet.* (2017) 26:2719–31. doi: 10.1093/hmg/ddx159
89. Viitasalo A, Pihlajamaki J, Paananen J, Atalay M, Lindi V, Lakka TA. Associations of TM6SF2 167K allele with liver enzymes and lipid profile in children: the PANIC study. *Pediatr Res.* (2016) 79:684–8. doi: 10.1038/pr.2016.3
90. Prill S, Caddeo A, Baselli G, Jamialahmadi O, Dongiovanni P, Rametta R, et al. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. *Sci Rep.* (2019) 9:11585. doi: 10.1038/s41598-019-47737-w
91. Marzuillo P, Di Sessa A, Cirillo G, Umano GR, Pedulla M, La Manna A, et al. Transmembrane 6 superfamily member 2 167K allele improves renal function in children with obesity. *Pediatr Res.* (2020) 88:300–4. doi: 10.1038/s41390-020-0753-5
92. Musso G, Cassader M, Gambino R. PNPLA3 rs738409 and TM6SF2 rs58542926 gene variants affect renal disease and function in nonalcoholic fatty liver disease. *Hepatology.* (2015) 62:658–9. doi: 10.1002/hep.27643
93. Rees MG, Wincovitch S, Schultz J, Waterstradt R, Beer NL, Baltrusch S, et al. Cellular characterisation of the GCKR P446L variant associated with type 2 diabetes risk. *Diabetologia.* (2012) 55:114–22. doi: 10.1007/s00125-011-2348-5
94. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet.* (2011) 7:e1001324. doi: 10.1371/journal.pgen.1001324
95. Zain SM, Mohamed Z, Mohamed R. Common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: a meta-analysis. *J Gastroenterol Hepatol.* (2015) 30:21–7. doi: 10.1111/jgh.12714
96. Fernandes Silva L, Vangipurapu J, Kuulasmaa T, Laakso M. An intronic variant in the GCKR gene is associated with multiple lipids. *Sci Rep.* (2019) 9:10240. doi: 10.1038/s41598-019-46750-3
97. Petta S, Miele L, Bugianesi E, Camma C, Rosso C, Boccia S, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS ONE.* (2014) 9:e87523. doi: 10.1371/journal.pone.0087523
98. Stender S, Kozlitina J, Nordestgaard BG, Tybjaerg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. *Nat Genet.* (2017) 49:842–7. doi: 10.1038/ng.3855

99. Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet.* (2009) 18:4081–8. doi: 10.1093/hmg/ddp357
100. Caddeo A, Jamialahmadi O, Solinas G, Pujia A, Mancina RM, Pingitore P, et al. MBOAT7 is anchored to endomembranes by six transmembrane domains. *J Struct Biol.* (2019) 206:349–60. doi: 10.1016/j.jsb.2019.04.006
101. Tanaka Y, Shimanaka Y, Caddeo A, Kubo T, Mao Y, Kubota T, et al. LPIAT1/MBOAT7 depletion increases triglyceride synthesis fueled by high phosphatidylinositol turnover. *Gut*. (2020). doi: 10.1136/gutjnl-2020-320646. [Epub ahead of print].
102. Meroni M, Longo M, Fracanzani AL, Dongiovanni P. MBOAT7 down-regulation by genetic and environmental factors predisposes to MAFLD. *EBioMedicine.* (2020) 57:102866. doi: 10.1016/j.ebiom.2020.102866
103. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology.* (2016) 150:1219–30.e6. doi: 10.1053/j.gastro.2016.01.032
104. Krawczyk M, Rau M, Schattenberg JM, Bantel H, Pathil A, Demir M, et al. Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738 variants on NAFLD severity: a multicenter biopsy-based study. *J Lipid Res.* (2017) 58:247–55. doi: 10.1194/jlr.P067454
105. Helsley RN, Varadharajan V, Brown AL, Gromovsky AD, Schugar RC, Ramachandiran I, et al. Obesity-linked suppression of membrane-bound O-acyltransferase 7 (MBOAT7) drives non-alcoholic fatty liver disease. *Elife.* (2019) 8:e49882. doi: 10.7554/eLife.49882
106. Su W, Mao Z, Liu Y, Zhang X, Zhang W, Gustafsson JA, et al. Role of HSD17B13 in the liver physiology and pathophysiology. *Mol Cell Endocrinol.* (2019) 489:119–25. doi: 10.1016/j.mce.2018.10.014
107. Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med.* (2018) 378:1096–106. doi: 10.1056/NEJMoa1712191
108. Sookoian S, Arrese M, Pirola CJ. Genetics meets therapy? Exome-wide association study reveals a loss-of-function variant in 17-beta-hydroxysteroid dehydrogenase 13 that protects patients from liver damage and nonalcoholic fatty liver disease progression. *Hepatology.* (2019) 69:907–10. doi: 10.1002/hep.30209
109. Di Sessa A, Umano GR, Cirillo G, Passaro AP, Verde V, Cozzolino D, et al. Pediatric non-alcoholic fatty liver disease and kidney function: effect of HSD17B13 variant. *World J Gastroenterol.* (2020) 26:5474–83. doi: 10.3748/wjg.v26.i36.5474
110. Su W, Wang Y, Jia X, Wu W, Li L, Tian X, et al. Comparative proteomic study reveals 17beta-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA.* (2014) 111:11437–42. doi: 10.1073/pnas.1410741111
111. Ma Y, Brown PM, Lin DD, Ma J, Feng D, Belyaeva OV, et al. Hsd17b13 deficiency does not protect mice from obesogenic diet injury. *Hepatology.* (2020). doi: 10.1002/hep.31517. [Epub ahead of print].
112. Ma Y, Karki S, Brown PM, Lin DD, Podszun MC, Zhou W, et al. Characterization of essential domains in HSD17B13 for cellular localization and enzymatic activity. *J Lipid Res.* (2020) 61:1400–9. doi: 10.1194/jlr.RA120000907
113. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. *Nature.* (2009) 458:1131–5. doi: 10.1038/nature07976
114. Chauhan S, Mandell MA, Deretic V. IRGM governs the core autophagy machinery to conduct antimicrobial defense. *Mol Cell.* (2015) 58:507–21. doi: 10.1016/j.molcel.2015.03.020
115. Schwerbel K, Kamitz A, Krahmer N, Hallahan N, Jahnert M, Gottmann P, et al. Immunity-related GTPase induces lipophagy to prevent excess hepatic lipid accumulation. *J Hepatol.* (2020) 73:771–82. doi: 10.1016/j.jhep.2020.04.031
116. Hsiao PJ, Lee MY, Wang YT, Jiang HJ, Lin PC, Yang YH, et al. MTTP-297H polymorphism reduced serum cholesterol but increased risk of non-alcoholic fatty liver disease-a cross-sectional study. *BMC Med Genet.* (2015) 16:93. doi: 10.1186/s12881-015-0242-6
117. Reue K, Zhang P. The lipin protein family: dual roles in lipid biosynthesis and gene expression. *FEBS Letters.* (2008) 582:90–6. doi: 10.1016/j.febslet.2007.11.014
118. Musso G, Cassader M, De Michieli F, Rosina F, Orlandi F, Gambino R. Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism. *Hepatology.* (2012) 56:933–42. doi: 10.1002/hep.25739
119. Honma M, Sawada S, Ueno Y, Murakami K, Yamada T, Gao J, et al. Selective insulin resistance with differential expressions of IRS-1 and IRS-2 in human NAFLD livers. *Int J Obes.* (2018) 42:1544–55. doi: 10.1038/s41366-018-0062-9
120. Yang HR, Chang EJ. Insulin resistance, body composition, and fat distribution in obese children with nonalcoholic fatty liver disease. *Asia Pac J Clin Nutr.* (2016) 25:126–33. doi: 10.6133/apjcn.2016.25.1.15
121. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia.* (2005) 48:634–42. doi: 10.1007/s00125-005-1682-x
122. Ibrahim SH, Kohli R, Gores GJ. Mechanisms of lipotoxicity in NAFLD and clinical implications. *J Pediatr Gastroenterol Nutr.* (2011) 53:131–40. doi: 10.1097/MPG.0b013e31822578db
123. Enooku K, Kondo M, Fujiwara N, Sasako T, Shibahara J, Kado A, et al. Hepatic IRS1 and ss-catenin expression is associated with histological progression and overt diabetes emergence in NAFLD patients. *J Gastroenterol.* (2018) 53:1261–75. doi: 10.1007/s00535-018-1472-0
124. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM.* (2010) 103:71–83. doi: 10.1093/qjmed/hcp158
125. Nobili V, Parola M, Alisi A, Marra F, Piemonte F, Mombello C, et al. Oxidative stress parameters in paediatric non-alcoholic fatty liver disease. *Int J Mol Med.* (2010) 26:471–6. doi: 10.3892/ijmm_00000487
126. Spahis S, Delvin E, Borys JM, Levy E. Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. *Antioxid Redox Signal.* (2017) 26:519–41. doi: 10.1089/ars.2016.6776
127. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* (2010) 142:687–98. doi: 10.1016/j.cell.2010.07.041
128. Im DS. Functions of omega-3 fatty acids and FFA4 (GPR120) in macrophages. *Eur J Pharmacol.* (2016) 785:36–43. doi: 10.1016/j.ejphar.2015.03.094
129. Nobili V, Carpino G, Alisi A, De Vito R, Franchitto A, Alpini G, et al. Role of docosahexaenoic acid treatment in improving liver histology in pediatric nonalcoholic fatty liver disease. *PLoS ONE.* (2014) 9:e88005. doi: 10.1371/journal.pone.0088005
130. Puri K, Nobili V, Melville K, Corte CD, Sartorelli MR, Lopez R, et al. Serum bilirubin level is inversely associated with nonalcoholic steatohepatitis in children. *J Pediatr Gastroenterol Nutr.* (2013) 57:114–8. doi: 10.1097/MPG.0b013e318291fefe
131. Drummond GS, Baum J, Greenberg M, Lewis D, Abraham NG. HO-1 overexpression and underexpression: clinical implications. *Arch Biochem Biophys.* (2019) 673:108073. doi: 10.1016/j.abb.2019.108073
132. Han X, Ding C, Zhang G, Pan R, Liu Y, Huang N, et al. Liraglutide ameliorates obesity-related nonalcoholic fatty liver disease by regulating Sestrin2-mediated Nrf2/HO-1 pathway. *Biochem Biophys Res Commun.* (2020) 525:895–901. doi: 10.1016/j.bbrc.2020.03.032
133. Soyal S, Krempler F, Oberkofler H, Patsch W. PGC-1alpha: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia.* (2006) 49:1477–88. doi: 10.1007/s00125-006-0268-6
134. Besse-Patin A, Leveille M, Oropeza D, Nguyen BN, Prat A, Estall JL. Estrogen signals through peroxisome proliferator-activated receptor-gamma coactivator 1alpha to reduce oxidative damage associated with diet-induced fatty liver disease. *Gastroenterology.* (2017) 152:243–56. doi: 10.1053/j.gastro.2016.09.017
135. Arrese M, Cabrera D, Kalergis AM, Feldstein AE. Innate immunity and inflammation in NAFLD/NASH. *Dig Dis Sci.* (2016) 61:1294–303. doi: 10.1007/s10620-016-4049-x

136. Teixeira-Clerc F, Belot MP, Manin S, Deveaux V, Cadoudal T, Chobert MN, et al. Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology*. (2010) 52:1046–59. doi: 10.1002/hep.23779
137. Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology*. (2015) 61:1547–54. doi: 10.1002/hep.27368
138. Miele L, Beale G, Patman G, Nobili V, Leathart J, Grieco A, et al. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. *Gastroenterology*. (2008) 135:282–91. doi: 10.1053/j.gastro.2008.04.004
139. Carlsson B, Linden D, Brolen G, Liljeblad M, Bjursell M, Romeo S, et al. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. (2020) 51:1305–20. doi: 10.1111/apt.15738
140. Giorgio V, Prono F, Graziano F, Nobili V. Pediatric non alcoholic fatty liver disease: old and new concepts on development, progression, metabolic insight and potential treatment targets. *BMC Pediatr*. (2013) 13:40. doi: 10.1186/1471-2431-13-40
141. Ma J, Hennein R, Liu C, Long MT, Hoffmann U, Jacques PF, et al. Improved diet quality associates with reduction in liver fat, particularly in individuals with high genetic risk scores for nonalcoholic fatty liver disease. *Gastroenterology*. (2018) 155:107–17. doi: 10.1053/j.gastro.2018.03.038
142. Nobili V, Bedogni G, Donati B, Alisi A, Valenti L. The I148M variant of PNPLA3 reduces the response to docosahexaenoic acid in children with non-alcoholic fatty liver disease. *J Med Food*. (2013) 16:957–60. doi: 10.1089/jmf.2013.0043
143. Di Costanzo A, Pacifico L, Chiesa C, Perla FM, Ceci F, Angeloni A, et al. Genetic and metabolic predictors of hepatic fat content in a cohort of Italian children with obesity. *Pediatr Res*. (2019) 85:671–7. doi: 10.1038/s41390-019-0303-1
144. Kovalic AJ, Banerjee P, Tran QT, Singal AK, Satapathy SK. Genetic and epigenetic culprits in the pathogenesis of nonalcoholic fatty liver disease. *J Clin Exp Hepatol*. (2018) 8:390–402. doi: 10.1016/j.jceh.2018.04.001
145. Skubic C, Drakulic Z, Rozman D. Personalized therapy when tackling nonalcoholic fatty liver disease: a focus on sex, genes, and drugs. *Expert Opin Drug Metab Toxicol*. (2018) 14:831–41. doi: 10.1080/17425255.2018.1492552
146. Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr*. (2012) 54:700–13. doi: 10.1097/MPG.0b013e318252a13f
147. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the expert committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr*. (2017) 64:319–34. doi: 10.1097/MPG.0000000000001482
148. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. (2016) 64:1388–1402.
149. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the study of liver diseases. *Hepatology*. (2018) 67:328–57. doi: 10.1002/hep.29367
150. Romeo S, Sanyal A, Valenti L. Leveraging human genetics to identify potential new treatments for fatty liver disease. *Cell Metab*. (2020) 31:35–45. doi: 10.1016/j.cmet.2019.12.002

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Socio-Demographic Factors Affect the Prevalence of Hematuria and Proteinuria Among School Children in Hualien, Taiwan: A Longitudinal Localization-Based Cohort Study

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Objective: Child hematuria/proteinuria is a risk factor for chronic kidney disease (CKD) in later life, and mass urinary screening could detect asymptomatic glomerulonephritis at an early stage. This study aimed to evaluate the longitudinal prevalence of hematuria/proteinuria and its association with socio-demographic factors among school children in Hualien, Taiwan.

Methods: The study cohort consisted of first and fourth graders enrolled from 2008 to 2015 in Hualien. We combined the data from two consecutive health examinations to ensure the validity of the body mass index (BMI), urbanization, proteinuria, and hematuria grouping. Prevalence and health status differences between sex, age, BMI, and urbanization level were examined.

Results: A total of 16,990 students within the same BMI and urbanization categories were included during the study interval. The prevalence of persistent hematuria was 1.0%. Fourth graders (odds ratio OR: 1.68, $p = 0.002$), girls (OR: 1.48, $p = 0.014$), and students from suburban/rural areas (OR: 1.99, and OR: 4.93, respectively; both $p < 0.001$) demonstrated higher hematuria risk. The prevalence of proteinuria was 0.2%. Fourth graders (OR: 4.44, $p < 0.001$) and students in suburban areas (OR: 0.27, $p = 0.031$) were associated with persistent proteinuria. After stratifying by age, the significant association remained. A higher risk of proteinuria was noted in underweight subjects (OR: 2.52, $p = 0.023$) among the fourth-grade students.

Conclusion: The prevalence of hematuria/proteinuria in Hualien was higher than the average reported for Taiwan. Hematuria/proteinuria was significantly associated with sex, age, BMI, and urbanization. Our longitudinal results can provide information for future pediatric CKD prevention in Taiwan.

Keywords: body mass index, children, chronic kidney disease, hematuria, mass urinary screening, proteinuria

INTRODUCTION

Chronic kidney disease (CKD) is currently a major global health problem. Not only can CKD progress to end-stage renal disease (ESRD), it is also a major risk factor for cardiovascular disease in later life (1). In Taiwan, the annual incidence of pediatric ESRD was reported to be 8.12 cases per million of age-matched population (2), with hematuria and proteinuria being the most common manifestations of pediatric CKD at diagnosis (3). Early aggressive treatment of patients with nephrotic-range proteinuria and combined hematuria-proteinuria could delay CKD progression (4). Asymptomatic glomerulonephritis (GN), manifesting as urinary abnormalities, can be detected via the school mass urinary screening (MUS) system, and MUS programs have been established for early detection of GN in Asian countries such as Japan, Korea, Singapore, and Taiwan, where GN is frequently the primary cause of ESRD (4–7). Dipstick screening has been widely used as a simple and inexpensive method for detecting urinary abnormalities and has subsequently promoted public awareness of CKD (5, 7–9).

Socio-demographic factors have been shown to have a significant impact on CKD progression (10, 11). Adult studies have revealed that the prevalence of CKD is highest among low to middle-income countries (10), and Fraser et al. have also reported that CKD and proteinuria are associated with patient socioeconomic status (11). Hualien County, which is located in the East of Taiwan, has the largest aboriginal population (25% of all residents) and the second least urbanized county in Taiwan. From 1991 to 1998 in Taiwan, 0.3% of screened students had an abnormal urinalysis. Among them, 7.4% of these students had heavy proteinuria, with increased risks of developing hypertension and nephritis (5). Notably, students in Hualien had the third-highest 4-year prevalence of heavy proteinuria in Taiwan at that time (12). Furthermore, the prevalence of metabolic syndrome in junior high school students in East Taiwan was 5.4%, which was the second-highest percentage in Taiwan in 2010. The prevalence of increased low-density lipoprotein (8.0%) and decreased high-density lipoprotein (8.6%) in the blood is highest among children in East Taiwan as compared with Taiwan in general (13). These epidemiology studies, therefore, imply that children in Hualien may suffer from renal damage and an increased incidence of abnormal urinalysis.

Since 2007, health examinations, including urinalysis, have been provided for all grades 1, 4, and 7 school children in Hualien by the same tertiary center. Our prior studies have reported that the overall prevalence of hematuria and proteinuria among Hualien school children increased gradually between 2010 and 2013 (3.8 vs. 4.1% with hematuria and 2.4 vs. 5.7% with proteinuria, respectively) (13, 14). However, little information is available regarding the influence of socio-demographic factors on the same participant after long-term follow-up, or on the possible risk stratification of these students in Taiwan. Furthermore, a recent study also revealed that childhood socioeconomic status is associated with adult CKD progression (15). Therefore, the objective of the present study was to evaluate the longitudinal prevalence of hematuria/proteinuria and its association with different socio-demographic factors, including sex, age, body mass index (BMI), and level of urbanization, in school children within Hualien, Taiwan.

MATERIALS AND METHODS

Subjects

Health examination and MUS data were routinely collected for the same students in his or her first (aged 7–8 years), fourth (aged 10–11 years), and seventh graders (aged 13–14 years) in Hualien County, Taiwan. Thus, we enrolled first and fourth graders in 2008, 2009, 2011, and 2012 to form the cohort. Consecutive health examination and MUS data after 3 years were also included to assure BMI, urbanization, proteinuria, and hematuria status. We compared the same students within the 3-year intervals in grades 1–4 or grades 4–7 to evaluate the risk factors associated with the longitudinal progression of proteinuria and hematuria. The study period was divided into four 3-year epochs: epoch 1 (2008–2011), epoch 2 (2009–2012), epoch 3 (2011–2014), and epoch 4 (2012–2015). A flow chart detailing our study design is depicted in **Figure 1**. A total of 24,788 students from 25 junior high schools and 105 elementary schools, with complete follow-up data, were enrolled in this longitudinal study. Students with the same BMI and urbanization category within these 3-year intervals ($n = 16{,}990$, 68.5% of the total population) were included in the analysis. Informed consent was obtained from the students' parents or guardians. The study was approved by the Hualien County Government Education Bureau and the Protection of Human Subjects Institutional Review Board of Tzu Chi University and Hospital (REC No.: IRB101-125 and IRB105-52-B).

Measurements and Procedures

This study evaluated the longitudinal prevalence of hematuria and proteinuria and its association with different socio-demographic factors among school children in Hualien, Taiwan in two study periods. Risk factors believed to be associated with these abnormal urinalyses in school children, including sex, age, BMI, and urbanization levels, were also recorded. Follow-up measurements were performed 3 years after baseline testing at the elementary or junior high schools for all available children. The children were instructed to empty the bladder completely the night before sample collection. A midstream first-morning urine specimen was collected the following day and was examined with the dipstick method to detect hematuria and proteinuria as previously described (13, 16). Leukocyte and nitrite levels were also recorded for later adjustment for abnormalities indicating urinary tract infection. For both isolated hematuria and proteinuria, positive reactions were defined as persistent 1+ (25 red blood cells/L and protein 30 mg/dL for hematuria and proteinuria 1+, respectively) or higher in both baseline testing and follow-up measurements during the 3-year interval (8). Children were categorized as underweight, normal, or overweight/obese according to the age-gender-specific Taiwanese national BMI reference values for children and adolescents, as reported by the Ministry of Health and Welfare in Taiwan (17). The population density (people per square kilometer, km^2) was used to determine the urbanization

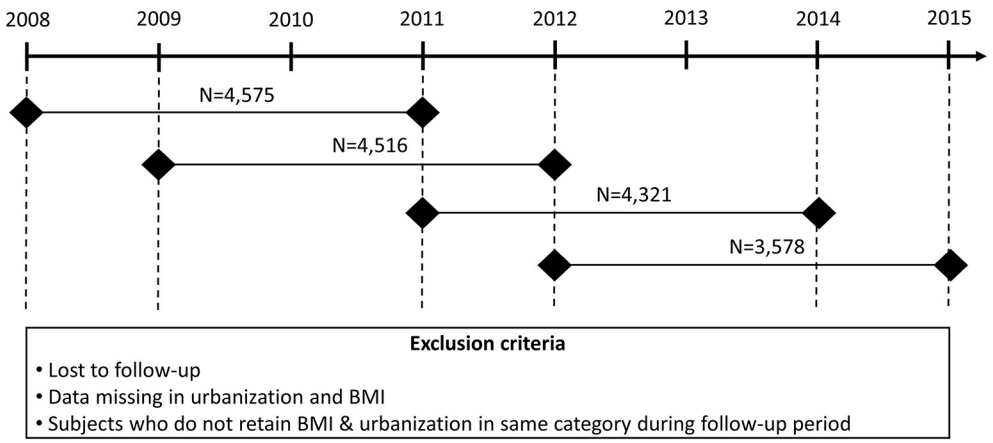

FIGURE 1 | The study cohort consisted of first and fourth graders in 2008, 2009, 2011, and 2012. We combined the data from two consecutive health examinations and urine analyses to ensure the validity of the BMI group, level of urbanization, proteinuria, and hematuria.

level. According to the population densities of residential areas, urbanization levels were divided into the following three groups: urban (>500 people/km^2), suburban (15–500 people/km^2), and rural (<15 people/km^2) (18). Based on this categorization, three (23%) of the included areas were defined as urban, seven (54%) as suburban, and three (23%) as rural.

Statistical Analysis

Data were analyzed using SPSS for Windows (version 21; SPSS Inc., Chicago, IL, USA). Descriptive statistics for hematuria and proteinuria are presented as absolute numbers and percentages. A Chi-square test was performed to identify parameters that were significantly associated with BMI. Logistic regression models were used to analyze associations between the prevalence of hematuria/proteinuria and socio-demographic factors. Crude and adjusted odds ratios, and 95% confidence intervals, were calculated. A $p < 0.05$ was defined as statistically significant.

RESULTS

Demographic information on the 16,990 students accepting examination during the four study epochs (2008–2011, 2009–2012, 2011–2014, and 2012–2015) is presented in **Table 1**. The overall prevalence of persistent hematuria and proteinuria was 1% (164/16,990), and 0.2% (42/16,990), respectively. Of the enrolled students, 47.0% were first-graders and 52.2% were male. Of the students with an unaltered urbanization level throughout the study period, 72.4, 22.9, and 4.8% of children lived in urban, suburban, or rural areas, respectively. We analyzed students that remained in the same BMI group throughout the study period; 64.1% had a normal BMI, 10.9% were underweight, and 25.0% were overweight/obese.

To clarify whether abnormal urinalysis was associated with a higher risk of these socio-demographic factors, we performed multiple logistic regression analysis. The results are summarized in **Table 2**. Female sex, older age, and habitation in suburban/rural areas were identified as risk factors for persistent hematuria. Girls had a significantly higher odds ratio (1.48) of hematuria as compared to boys ($P = 0.014$). Students enrolled in the fourth grade had a significantly higher odds ratio (1.68) of hematuria as compared to those enrolled in the first grade ($P = 0.002$). Students living in suburban and rural areas had significantly higher odds ratios (1.99 and 4.93, respectively) of hematuria as compared to those living in urban areas (both $P < 0.001$).

In proteinuria risk factor studies, older age and living in suburban areas were associated with persistent proteinuria. The older students (fourth graders) had a significantly higher odds ratio (4.44) of proteinuria as compared to the younger ones (first graders) ($p < 0.001$). Students living in suburban areas had significantly lower odds ratios (0.27) of proteinuria than those living in the urban area ($P = 0.031$).

A positive correlation between BMI and hematuria was observed, although underweight and overweight/obese students did not have a significantly lower and higher odds ratio of hematuria as compared to that in the normal group. Interestingly, a negative correlation between BMI and proteinuria was noted throughout the study. Students who were overweight/obese had a lower odds ratio (0.92), and underweight children tended to have a higher odds ratio (2.13) of proteinuria as compared to those who remained at a normal weight, although this was not statistically significant ($P = 0.839$ and 0.055, respectively).

To further clarify the influence of age among these socio-demographic risk factors for hematuria or proteinuria, subgroup analysis was performed after stratifying by age (the results are shown in **Table 3**). Among first grade students, differences between sex and urbanization levels were observed. Female students had a significantly higher risk of persistent hematuria than male students, whereas students living in suburban/rural areas had a significantly higher risk of persistent hematuria than students living in urban areas. The difference in urbanization remained only in fourth-grade students. When evaluating risk factors related to proteinuria, there was no statistically significant difference between sexes, urbanization levels, or BMI among

TABLE 1 | Demographic information among students accepting physical examination ($n = 16,990$).

Variable	Period				Total
	2008–2011	2009–2012	2011–2014	2012–2015	
N	4,575	4,516	4,321	3,578	16,990
Initial school grade					
1	2,231 (48.8%)	2,068 (45.8%)	2,025 (46.9%)	1,654 (46.2%)	7,978 (47.0%)
4	2,344 (51.2%)	2,448 (54.2%)	2,296 (53.1%)	1,924 (53.8%)	9,012 (53.0%)
Sex					
Male	2,417 (52.8%)	2,368 (52.4%)	2,175 (50.3%)	1,866 (52.2%)	8,826 (51.9%)
Female	2,158 (47.2%)	2,148 (47.6%)	2,146 (49.7%)	1,712 (47.8%)	8,164 (48.1%)
BMI patterns					
BMI remaining normal	3,191 (69.7%)	2,712 (60.1%)	2,992 (69.2%)	1,988 (55.6%)	10,883 (64.1%)
BMI remaining underweight	170 (3.7%)	781 (17.3%)	178 (4.1%)	727 (20.3%)	1,856 (10.9%)
BMI remaining overweight/obese	1,214 (26.5%)	1,023 (22.7%)	1,151 (26.6%)	863 (24.1%)	4,251 (25.0%)
Urbanization					
Urban	3,300 (72.1%)	3,231 (71.5%)	3,108 (71.9%)	2,660 (74.3%)	12,299 (72.4%)
Suburban	1,052 (23.0%)	1,106 (24.5%)	997 (23.1%)	728 (20.3%)	3,883 (22.9%)
Rural	223 (4.9%)	179 (4.0%)	216 (5.0%)	190 (5.3%)	808 (4.8%)
Hematuria					
No	4,519 (98.8%)	4,462 (98.8%)	4,295 (99.4%)	3,550 (99.2%)	16,826 (99.0%)
Yes	56 (1.2%)	54 (1.2%)	26 (0.6%)	28 (0.8%)	164 (1.0%)
Proteinuria					
No	4,570 (99.9%)	4,509 (99.8%)	4,311 (99.8%)	3,558 (99.4%)	16,948 (99.8%)
Yes	5 (0.1%)	7 (0.2%)	10 (0.2%)	20 (0.6%)	42 (0.2%)

Data are presented as absolute numbers and percentages.
**$p < 0.05$ was considered statistically significant.*
BMI, body mass index.

first graders. However, among the fourth graders, underweight students demonstrated a significantly higher odds ratio (2.52) of proteinuria compared with students who remained at a normal weight ($P = 0.023$).

DISCUSSION

The major findings from our longitudinal school children's urinary screening analysis are summarized as follows: female sex, older age, and habitation in suburban/rural areas were identified as significant risk factors for persistent hematuria. Older age was also identified as a risk factor for persistent proteinuria. After stratifying by age, similar socio-demographic risk factors were identified for persistent hematuria and proteinuria in first-grade students. However, a significantly higher risk of persistent hematuria was still noted among students living in suburban/rural areas, and a higher risk of persistent proteinuria was noted in underweight students who were older (fourth graders).

The measured prevalence of pediatric hematuria or proteinuria varies depending on different study methods, ethnic background, or geographic region (4, 5, 16, 19). Using the dipstick method in the urine specimen from the first screen, the prevalence of hematuria and proteinuria was 6.8 and 1.2%, respectively, in a screening program of 12-year-old school children in Singapore (19). In Taiwan, an epidemiological study revealed that the most common manifestations at diagnosis in children with CKD were proteinuria (41%) and hematuria (28%), with even higher rates of proteinuria (68.2%) and hematuria (44.1%) when GN is the underlying etiology of CKD (3). Our present study has revealed that the overall prevalence of persistent hematuria and proteinuria in Hualien County were 1 and 0.2%, respectively, which were higher than those of a previous study analyzing school students in Taiwan from August 1999 to January 2000 (20), suggesting that the incidence of hematuria and proteinuria have been gradually increasing in recent years.

A previous MUS study of school children in Taiwan revealed that girls were affected by proteinuria more often than boys (12). This contrasts with the present study in which we found no sex differences in students with proteinuria. However, we found that female students had a significantly higher risk of hematuria than male students. A study in Saudi Arabia also revealed that hematuria is more frequently observed in healthy adolescent girls, with a female to male ratio of 1.5:1, and without a significant correlation with ethnicity or nationality (21). Also, older female students had a higher prevalence of hematuria in the present study, which is consistent with previous reports (13, 16). Although this phenomenon may correlate with the possibility of menstrual blood contamination, persistent false positives due to menstrual blood contamination are less likely within our 3-year interval follow-up. Furthermore, the

TABLE 2 | Factors associated with a higher risk of abnormal screening items ($n = 16,990$).

Variable	Hematuria				Proteinuria			
	Crude		Adjusted[a]		Crude		Adjusted[a]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
School grade								
1	1		1		1		1	
4	1.42 (1.04, 1.95)	0.028*	1.68 (1.21, 2.34)	0.002*	4.44 (1.97, 10.00)	<0.001*	4.62 (2.02, 10.56)	<0.001*
Sex								
Male	1		1		1		1	
Female	1.46 (1.07, 1.99)	0.018*	1.48 (1.08, 2.03)	0.014*	1.19 (0.65, 2.18)	0.574	1.16 (0.63, 2.14)	0.628
Urbanization								
Urban	1		1		1		1	
Suburban	1.98 (1.41, 2.79)	<0.001*	1.99 (1.41, 2.80)	<0.001*	0.26 (0.08, 0.83)	0.023*	0.27 (0.08, 0.89)	0.031*
Rural	4.11 (2.58, 6.55)	<0.001*	4.93 (3.04, 8.02)	<0.001*	0.82 (0.20, 3.42)	0.788	1.58 (0.37, 6.78)	0.539
BMI pattern								
BMI remaining normal	1		1		1		1	
BMI remaining underweight	0.70 (0.38, 1.27)	0.234	0.79 (0.43, 1.45)	0.450	2.21 (1.02, 4.75)	0.044*	2.13 (0.98, 4.60)	0.055
BMI remaining Overweight/obese	1.30(0.92, 1.82)	0.133	1.36 (0.97, 1.91)	0.077	0.96 (0.45, 2.07)	0.917	0.92 (0.43, 1.99)	0.839

[a]*Adjusted for school grade, sex, urbanization, and BMI pattern. BMI, body mass index; CI, confidence interval; OR, odds ratio.*

*$p < 0.05$ *was considered statistically significant after logistic regression analysis.*

prevalence of GN is higher in Asian countries than in other geographic regions and is frequently the primary cause of ESRD as mentioned above (22). A recent study by Chiou et al. also confirmed that IgA nephritis and lupus nephritis are the most important primary and secondary GN, and the Taiwan Pediatric Renal Collaborative Study identified it as the most common disease in patients with CKD due to nephritis (37.2%) (3, 5). In Taiwan, pediatric CKD patients with nephritis were older (average age at diagnosis: 10.2 years), were more likely to be female (54.6%), had a higher prevalence of hematuria (44.1%) at diagnosis, and had a higher prevalence of autoimmune diseases (3). Furthermore, after further age-sex specific analyses, our previous cross-sectional study of students in Hualien also showed that seventh-grade female adolescents had the highest prevalence of hematuria and proteinuria compared with other students (13). Results from these studies indicate that females may be more prone to abnormal urinalysis due to autoimmune-mediated GN, and this may have contributed to the difference in hematuria distribution between sexes in our study population.

Proteinuria has been shown to be a significant risk factor for pediatric CKD progression (23). Our present study reported a significantly high prevalence of proteinuria in older students. Not surprisingly, the prevalence of renal disease increased gradually with age, and many studies have reported similar findings (12, 13, 16). An epidemiological study in Taiwanese pediatric patients with CKD due to nephritis also revealed an even higher prevalence of proteinuria than hematuria at diagnosis. Furthermore, the median age of nephritis-induced CKD patients was 10.2 years, which is similar to our findings in the fourth graders (10–11 years) (3). Although many studies have demonstrated that an increased BMI is significantly associated with proteinuria risk (21, 24–27), our present study revealed that the prevalence of proteinuria was not elevated in persistently overweight or obese students. This result may be affected by our study population, since prior studies stated that the correlation between BMI and CKD was mainly attributed to the subcategory of morbid obesity, with a BMI >35 kg/m^2 (28). Most students classified as overweight/obese in our study did not fit the criteria for morbid obesity (only 63 (0.4%) students were morbidly obese); thus, no associations were identified between proteinuria prevalence and BMI in our 3-year interval study period. Moreover, the progression of renal damage increased with age, but most of the study population were younger elementary school students with less comorbidity-induced renal damage. Therefore, future long-term follow-up of these overweight/obese students, especially morbidly obese individuals, is necessary.

Conversely, our study revealed that persistently underweight students had an increased prevalence of proteinuria and this became statistically significant with increasing age, as confirmed by further subgroup analysis stratified for age. There is increasing evidence that the association between proteinuria and BMI shows a clear U-shape trend, as well as remarkable differences between the sexes (29). Previous studies have shown that low BMI is associated with proteinuria (30), and that proteinuria and decreased BMI are independent risk factors for developing CKD and ESRD (31, 32). Our findings are consistent with a recent study by Lin et al., which found that students with CKD with albuminuria in a Taiwanese elementary school had significantly lower body weights than students without CKD (33). Although the relationship between low BMI and pediatric proteinuria is still uncertain, decreasing BMI could be due to muscle wasting, malnutrition, or volume depletion, which can aggravate CKD progression and subsequently increase the severity of proteinuria (32). In addition, a larger odds ratio of

TABLE 3 | Factors associated with a higher risk of abnormal screening items, stratified for grade (n = 16,990).

Stratification	Variable	Hematuria				Proteinuria			
		Crude		Adjusted[a]		Crude		Adjusted[a]	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
First Graders (N = 7,978)	**Sex**								
	Male	1		1		1		1	
	Female	1.67 (1.01, 2.78)	0.046*	1.72 (1.03, 2.86)	0.038*	2.74 (0.53, 14.15)	0.228	3.01 (0.58, 15.68)	0.190
	Urbanization								
	Urban	1		1		1		1	
	Suburban	2.64 (1.47, 4.76)	0.001*	2.65 (1.47, 4.77)	0.001*	0.00 (NA)	0.988	0.00 (NA)	0.987
	Rural	6.48 (3.50, 12.01)	<0.001*	6.38 (3.43, 11.87)	<0.001*	1.41 (0.17, 11.71)	0.752	1.26 (0.15, 10.51)	0.830
	BMI pattern								
	BMI remaining normal	1		1		1		1	
	BMI remaining underweight	0.75 (0.30, 1.91)	0.548	0.93 (0.36, 2.38)	0.876	0.00 (NA)	0.992	0.00 (NA)	0.991
	BMI remaining overweight/obese	1.30 (0.75, 2.28)	0.352	1.39 (0.79, 2.44)	0.254	2.17 (0.49, 9.71)	0.311	2.39 (0.53, 10.79)	0.256
Fourth graders (N = 9,012)	**Sex**								
	Male	1		1		1		1	
	Female	1.33 (0.90, 1.98)	0.154	1.36 (0.91, 2.02)	0.132	1.01 (0.52, 1.96)	0.981	0.97 (0.50, 1.90)	0.936
	Urbanization								
	Urban	1		1		1		1	
	Suburban	1.75 (1.15, 2.68)	0.010*	1.73 (1.13, 2.64)	0.012*	0.32 (0.10, 1.05)	0.060	0.34 (0.10, 1.10)	0.072
	Rural	3.52 (1.40, 8.88)	0.008*	3.48 (1.37, 8.80)	0.009*	1.40 (0.19, 10.33)	0.741	1.56 (0.21, 11.57)	0.666
	BMI pattern								
	BMI remaining normal	1		1		1		1	
	BMI remaining underweight	0.65(0.30, 1.43)	0.289	0.71 (0.32, 1.56)	0.390	2.59 (1.18, 5.70)	0.018*	2.52 (1.14, 5.57)	0.023*
	BMI remaining overweight/obese	1.25 (0.82, 1.92)	0.299	1.33 (0.86, 2.04)	0.197	0.69 (0.28, 1.73)	0.430	0.70 (0.28, 1.75)	0.443

[a]*Adjusted for school grade, sex, urbanization, and BMI pattern. OR, odds ratio; CI, confidence interval.*

**p < 0.05 was considered statistically significant after logistic regression analysis.*

proteinuria was particularly prominent in the younger subjects with the lowest BMI, and this relationship was not found in the higher BMI group, suggesting the presence of more GN than postural proteinuria or nephrosclerosis in the younger subjects with the lowest BMIs (29).

Our present study showed that persistent hematuria was more prevalent in children living in suburban and rural areas than in those living in urban areas. After performing age-specific analyses, this phenomenon remained. In Taiwan, low levels of urbanization often accompany poor sanitary conditions, increased toxins exposure, and reduced medical resources. Although herbal remedies, such as aristolochic acid, have been prohibited since November 2003 in Taiwan, these herbs may be inappropriately used in the less urbanized area and could increase the risk of renal damage (34). Furthermore, Hualien water contains a high amount of calcium, and the drinking water is classified as moderately hard to hard according to the reference data from Taiwan Water Supply Corporation (13). Consuming unsanitized water with elevated calcium levels may increase the risk of hypercalciuria-induced hematuria in the students living in more rural areas (12, 35).

The strength of this study is that, to the best of our knowledge, it is the first location-based, longitudinal cohort study in Taiwan evaluating the risk factors of hematuria and proteinuria in school children with adjustment for socio-demographic factors. In our study, we analyzed the same children using a 3-year interval follow-up, and most of the school-aged children of Hualien County live in the same urbanization area. The distribution of urbanization, after comparing four study epochs, showed no statistical significance; hence, this

follow-up study represents a true longitudinal evaluation of CKD risk in these students without bias resulting from loss to follow-up of specific groups. Secondly, students within the same BMI classification throughout the study period were included for analysis, and using age-specific BMI cutoffs allowed the variability in age at measurement to be taken into account. Thirdly, health examination data were collected from 2008 to 2015 for longitudinal analysis, and thus, the directionality cause-effect relationship between hematuria/proteinuria and different socio-demographic factors was more evidenced than in the previous cross-sectional study (13). There is little information on socio-demographic factors influencing CKD progression in the pediatric population. A previous study in Australia revealed that socioeconomic and geographical disadvantages had no significant influence on hematuria and proteinuria among 2,266 Australian indigenous and non-indigenous children (36). Our large cohort study consisting of 16,990 students after a 6-year follow-up revealed that socio-demographic factors had a significant association with hematuria and proteinuria, although the results are not in line with those of the Australian's study. Our results could be helpful for providing preventive measures in school children, before they may develop kidney disease not only in Taiwan but also in other countries.

Our current study had the following limitations. First, several risk factors, such as local toxins, use of herbal remedies, and genetic effects which may influence the occurrence of hematuria and proteinuria are not available in our database. This information may pose some bias in our study and further longitudinal researches are needed to validate these results. Secondly, there may have been a high rate of false-positive results for hematuria or proteinuria as a result of the students' physical activity, presence of fever, diet, supine or standing position, and hydration status (37). The specificity of urine tests for abnormal urine examination can be increased with repeat dipstick and/or microscope examinations (38). In the present study, although our urine examination was performed on a single occasion, students with persistent 1+ or higher grade hematuria or proteinuria, in both initial and follow-up testing within the 3-year interval, were selected for analysis. This strategy can elevate the accuracy of our urinalysis. Thirdly, although increasing evidence has revealed that obesity-related glomerulopathy can develop even in childhood (27), our results revealed a non-statistically significant association between persistently overweight students and hematuria or proteinuria. Due to the retrospective nature of the study, cardiometabolic risk factors, such as hypertension, dysglycemia, and dyslipidemia, could not be included in the analysis, which may have created some bias in our study. Further prospective studies to determine associations between these metabolic syndrome components and abnormal urinalysis in obese students should be considered.

CONCLUSION

Routine health examination with urinalysis screening for hematuria and proteinuria can detect and predict early-stage chronic renal disease in school children. Our study suggests that children with abnormal BMI should be offered a temporal follow-up from early elementary school age (7–8 years). Our study also highlights the need for long-term prospective observational studies on the risk factors of CKD in adolescents and young adults in Taiwan. In summary, our current study provides useful data for policymakers, primary health care practitioners, and pediatricians to establish necessary future protocols on pediatric CKD prevention in Taiwan.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Protection of Human Subjects Institutional Review Board of Tzu Chi University and Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

M-CC, Y-HC, and C-FC conceived and designed the experiments and wrote the paper. M-CC and J-HW analyzed the data. All authors contributed to the article and approved the submitted manuscript.

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REFERENCES

1. Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt KU, et al. Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from kidney disease improving global outcomes. *Kidney Int.* (2007) 72:247–59. doi: 10.1038/sj.ki.5002343
2. Lin HH, Tsai CW, Lin PH, Cheng KF, Wu HD, Wang IK, et al. Survival analysis of pediatric dialysis patients in Taiwan. *Nephrology.* (2012) 17:621–7. doi: 10.1111/j.1440-1797.2012.01613.x
3. Chiou YY, Lin CY, Chen MJ, Chiou YH, Wang YF, Wang HH, et al. Etiology and pediatric chronic kidney disease progression: Taiwan Pediatric Renal Collaborative study. *J Formos Med Assoc.* (2016) 115:752–63. doi: 10.1016/j.jfma.2015.07.019

4. Cho BS, Hahn WH, Cheong HI, Lim I, Ko CW, Kim SY, et al. A nationwide study of mass urine screening tests on Korean school children and implications for chronic kidney disease management. *Clin Exp Nephrol.* (2013) 17:205–10. doi: 10.1007/s10157-012-0672-9
5. Lin CY, Hsieh CC, Chen WP, Yang LY, Wang HH. The underlying diseases and follow-up in Taiwanese children screened by urinalysis. *Pediatr Nephrol.* (2001) 16:232–7. doi: 10.1007/s004670000529
6. Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1,850 biopsied cases. Research Group on Progressive Chronic Renal Disease. *Nephron*. (1999) 82:205–13. doi: 10.1159/000045404
7. Cho BS, Kim SD. School urinalysis screening in Korea. *Nephrology.* (2007) 12(Suppl. 3):S3–7. doi: 10.1111/j.1440-1797.2007.00873.x
8. Shajari A, Shajari MF-ZH. Screening of renal diseases in the first primary school children in Shiraz. *Acta Medica Iranica.* (2007) 45:215–8.
9. Yamagata K, Iseki K, Nitta K, Imai H, Iino Y, Matsuo S, et al. Chronic kidney disease perspectives in Japan and the importance of urinalysis screening. *Clin Exp Nephrol.* (2008) 12:1–8. doi: 10.1007/s10157-007-0010-9
10. Chartier MJ, Tangri N, Komenda P, Walld R, Koseva I, Burchill C, et al. Prevalence, socio-demographic characteristics, and comorbid health conditions in pre-dialysis chronic kidney disease: results from the Manitoba chronic kidney disease cohort. *BMC Nephrol.* (2018) 19:255. doi: 10.1186/s12882-018-1058-3
11. Fraser SD, Roderick PJ, Aitken G, Roth M, Mindell JS, Moon G, et al. Chronic kidney disease, albuminuria and socioeconomic status in the Health Surveys for England 2009 and 2010. *J Public Health.* (2014) 36:577–86. doi: 10.1093/pubmed/fdt117
12. Lin CY, Sheng CC, Lin CC, Chen CH, Chou P. Mass urinary screening and follow-up for school children in Taiwan Province. *Acta Paediatr Taiwan.* (2001) 42:134–40. doi: 10.7097/APT.200106.0134
13. Chen MC, Wang JH, Chu CH, Cheng CF. Differential prevalence of hematuria and proteinuria with socio-demographic factors among school children in Hualien, Taiwan. *Pediatr Neonatol.* (2018) 59:360–7. doi: 10.1016/j.pedneo.2017.11.011
14. Chu CH, Wang JH, Jan RH, Huang CH, Cheng CF. Association between health examination items and body mass index among school children in Hualien, Taiwan. *BMC Public Health.* (2013) 13:975. doi: 10.1186/1471-2458-13-975
15. Surachman A, Daw J, Bray BC, Alexander LM, Coe CL, Almeida DM. Childhood socioeconomic status, comorbidity of chronic kidney disease risk factors, and kidney function among adults in the midlife in the United States (MIDUS) study. *BMC Nephrol.* (2020) 21:188. doi: 10.1186/s12882-020-01846-1
16. Murakami M, Yamamoto H, Ueda Y, Murakami K, Yamauchi K. Urinary screening of elementary and junior high-school children over a 13-year period in Tokyo. *Pediatr Nephrol.* (1991) 5:50–3. doi: 10.1007/BF00852844
17. Chen W, Tsai C-Y, Chen A-C, Wu S-F, Lin T-W, Lin H-C. Growth charts of Taiwanese youth: norms based on health-related physical fitness. *Mid Taiwan J Med.* (2003) 8:S85–93.
18. Liu C-Y, Hung Y, Chuang Y, Chen Y, Weng W, Liu J, et al. Incorporating development stratification of Taiwan townships into sampling design of large scale health interview survey. *J Health Manag.* (2006) 4:1–22.
19. Yap HK, Quek CM, Shen Q, Joshi V, Chia KS. Role of urinary screening programmes in children in the prevention of chronic kidney disease. *Ann Acad Med Singapore.* (2005) 34:3–7.
20. Lin CC, Chiang CC, Jen JJ, Kao SM. The developments of mass urinary screening in Taiwan. *Acta Paediatr Taiwan*. (2001) 42:130–3. doi: 10.7097/APT.200106.0130
21. Hothan KA, Alasmari BA, Alkhelaiwi OK, Althagafi KM, Alkhaldi AA, Alfityani AK, et al. Prevalence of hypertension, obesity, hematuria and proteinuria amongst healthy adolescents living in Western Saudi Arabia. *Saudi Med J.* (2016) 37:1120–6. doi: 10.15537/smj.2016.10.14784
22. Woo KT, Chan CM, Chin YM, Choong HL, Tan HK, Foo M, et al. Global evolutionary trend of the prevalence of primary glomerulonephritis over the past three decades. *Nephron Clin Pract.* (2010) 116:c337–46. doi: 10.1159/000319594
23. van den Belt SM, Heerspink HJL, Gracchi V, de Zeeuw D, Wühl E, Schaefer F. Early proteinuria lowering by angiotensin-converting enzyme inhibition predicts renal survival in children with CKD. *J Am Soc Nephrol.* (2018) 29:2225–33. doi: 10.1681/ASN.2018010036
24. Wang Y, Chen X, Song Y, Caballero B, Cheskin LJ. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int.* (2008) 73:19–33. doi: 10.1038/sj.ki.5002586
25. Wuhl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, Zurowska A, et al. Strict blood-pressure control and progression of renal failure in children. *N Engl J Med.* (2009) 361:1639–50. doi: 10.1056/NEJMoa0902066
26. Wu Y, Liu Z, Xiang Z, Zeng C, Chen Z, Ma X, et al. Obesity-related glomerulopathy: insights from gene expression profiles of the glomeruli 1derived from renal biopsy samples. *Endocrinology.* (2006) 147:44–50. doi: 10.1210/en.2005-0641
27. Correia-Costa L, Azevedo A, Caldas Afonso A. Childhood obesity and impact on the kidney. *Nephron.* (2019) 143:8–11. doi: 10.1159/000492826
28. Cohen E, Fraser A, Goldberg E, Milo G, Garty M, Krause I. Association between the body mass index and chronic kidney disease in men and women. a population-based study from Israel. *Nephrol Dial Transplant.* (2013) 28(Suppl. 4):iv130–5. doi: 10.1093/ndt/gft072
29. Sato Y, Fujimoto S, Konta T, Iseki K, Moriyama T, Yamagata K, et al. U-shaped association between body mass index and proteinuria in a large Japanese general population sample. *Clin Exp Nephrol.* (2014) 18:75–86. doi: 10.1007/s10157-013-0809-5
30. Ramirez SP, McClellan W, Port FK, Hsu SI. Risk factors for proteinuria in a large, multiracial, southeast Asian population. *J Am Soc Nephrol.* (2002) 13:1907–17. doi: 10.1097/01.ASN.0000018406.20282.C8
31. Iseki K, Tokashiki K, Iseki C, Kohagura K, Kinjo K, Takishita S. Proteinuria and decreased body mass index as a significant risk factor in developing end-stage renal disease. *Clin Exp Nephrol.* (2008) 12:363–9. doi: 10.1007/s10157-008-0061-6
32. Tokashiki K, Tozawa M, Iseki C, Kohagura K, Kinjo K, Takishita S, et al. Decreased body mass index as an independent risk factor for developing chronic kidney disease. *Clin Exp Nephrol.* (2009) 13:55–60. doi: 10.1007/s10157-008-0085-y
33. Lin CY, Huang SM. Childhood albuminuria and chronic kidney disease is associated with mortality and end-stage renal disease. *Pediatr Neonatol.* (2016) 57:280–7. doi: 10.1016/j.pedneo.2015.09.013
34. Lai MN, Lai JN, Chen PC, Tseng WL, Chen YY, Hwang JS, et al. Increased risks of chronic kidney disease associated with prescribed Chinese herbal products suspected to contain aristolochic acid. *Nephrology.* (2009) 14:227–34. doi: 10.1111/j.1440-1797.2008.01061.x
35. Fallahzadeh MK, Fallahzadeh MH, Mowla A, Derakhshan A. Hypercalciuria in children with urinary tract symptoms. *Saudi J Kidney Dis Transpl.* (2010) 21:673–7.
36. Haysom L, Williams R, Hodson EM, Lopez-Vargas PA, Roy LP, Lyle DM, et al. Natural history of chronic kidney disease in Australian Indigenous and non-Indigenous children: a 4-year population-based follow-up study. *Med J Aust.* (2009) 190:303–6. doi: 10.5694/j.1326-5377.2009.tb02417.x
37. Pugia MJ, Lott JA, Kajima J, Saambe T, Sasaki M, Kuromoto K, et al. Screening school children for albuminuria, proteinuria and occult blood with dipsticks. *Clin Chem Lab Med.* (1999) 37:149–57. doi: 10.1515/CCLM.1999.027
38. Koshy SM, Garcia-Garcia G, Pamplona JS, Renoirte-Lopez K, Perez-Cortes G, Gutierrez ML, et al. Screening for kidney disease in children on World Kidney Day in Jalisco, Mexico. *Pediatr Nephrol.* (2009) 24:1219–25. doi: 10.1007/s00467-009-1136-7

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REVIEW
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Childhood and Adolescent Obesity: A Review

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Obesity is a complex condition that interweaves biological, developmental, environmental, behavioral, and genetic factors; it is a significant public health problem. The most common cause of obesity throughout childhood and adolescence is an inequity in energy balance; that is, excess caloric intake without appropriate caloric expenditure. Adiposity rebound (AR) in early childhood is a risk factor for obesity in adolescence and adulthood. The increasing prevalence of childhood and adolescent obesity is associated with a rise in comorbidities previously identified in the adult population, such as Type 2 Diabetes Mellitus, Hypertension, Non-alcoholic Fatty Liver disease (NAFLD), Obstructive Sleep Apnea (OSA), and Dyslipidemia. Due to the lack of a single treatment option to address obesity, clinicians have generally relied on counseling dietary changes and exercise. Due to psychosocial issues that may accompany adolescence regarding body habitus, this approach can have negative results. Teens can develop unhealthy eating habits that result in Bulimia Nervosa (BN), Binge- Eating Disorder (BED), or Night eating syndrome (NES). Others can develop Anorexia Nervosa (AN) as they attempt to restrict their diet and overshoot their goal of "being healthy." To date, lifestyle interventions have shown only modest effects on weight loss. Emerging findings from basic science as well as interventional drug trials utilizing GLP-1 agonists have demonstrated success in effective weight loss in obese adults, adolescents, and pediatric patients. However, there is limited data on the efficacy and safety of other weight-loss medications in children and adolescents. Nearly 6% of adolescents in the United States are severely obese and bariatric surgery as a treatment consideration will be discussed. In summary, this paper will overview the pathophysiology, clinical, and psychological implications, and treatment options available for obese pediatric and adolescent patients.

Keywords: obesity, childhood, review (article), behavior, adolescent

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INTRODUCTION

Obesity is a complex issue that affects children across all age groups (1–3). One-third of children and adolescents in the United States are classified as either overweight or obese. There is no single element causing this epidemic, but obesity is due to complex interactions between biological, developmental, behavioral, genetic, and environmental factors (4). The role of epigenetics and the gut microbiome, as well as intrauterine and intergenerational effects, have recently emerged

as contributing factors to the obesity epidemic (5, 6). Other factors including small for gestational age (SGA) status at birth, formula rather than breast feeding in infancy, and early introduction of protein in infant's dietary intake have been reportedly associated with weight gain that can persist later in life (6–8). The rising prevalence of childhood obesity poses a significant public health challenge by increasing the burden of chronic non-communicable diseases (1, 9).

Obesity increases the risk of developing early puberty in children (10), menstrual irregularities in adolescent girls (1, 11), sleep disorders such as obstructive sleep apnea (OSA) (1, 12), cardiovascular risk factors that include Prediabetes, Type 2 Diabetes, High Cholesterol levels, Hypertension, NAFLD, and Metabolic syndrome (1, 2). Additionally, obese children and adolescents can suffer from psychological issues such as depression, anxiety, poor self-esteem, body image and peer relationships, and eating disorders (13, 14).

So far, interventions for overweight/obesity prevention have mainly focused on behavioral changes in an individual such as increasing daily physical exercise or improving quality of diet with restricting excess calorie intake (1, 15, 16). However, these efforts have had limited results. In addition to behavioral and dietary recommendations, changes in the community-based environment such as promotion of healthy food choices by taxing unhealthy foods (17), improving lunch food quality and increasing daily physical activity at school and childcare centers, are extra measures that are needed (16). These interventions may include a ban on unhealthy food advertisements aimed at children as well as access to playgrounds and green spaces where families can feel their children can safely recreate. Also, this will limit screen time for adolescents as well as younger children.

However, even with the above changes, pharmacotherapy and/or bariatric surgery will likely remain a necessary option for those youth with morbid obesity (1). This review summarizes our current understanding of the factors associated with obesity, the physiological and psychological effects of obesity on children and adolescents, and intervention strategies that may prevent future concomitant issues.

DEFINITION OF CHILDHOOD OBESITY

Body mass index (BMI) is an inexpensive method to assess body fat and is derived from a formula derived from height and weight in children over 2 years of age (1, 18, 19). Although more sophisticated methods exist that can determine body fat directly, they are costly and not readily available. These methods include measuring skinfold thickness with a caliper, Bioelectrical impedance, Hydro densitometry, Dual-energy X-ray Absorptiometry (DEXA), and Air Displacement Plethysmography (2).

BMI provides a reasonable estimate of body fat indirectly in the healthy pediatric population and studies have shown that BMI correlates with body fat and future health risks (18). Unlike in adults, Z-scores or percentiles are used to represent BMI in children and vary with the age and sex of the child. BMI Z-score cut off points of >1.0, >2.0, and >3.0 are recommended by the World Health Organization (WHO) to define at risk of overweight, overweight and obesity, respectively (19). However, in terms of percentiles, overweight is applied when BMI is >85th percentile <95th percentile, whereas obesity is BMI > 95th percentile (20–22). Although BMI Z-scores can be converted to BMI percentiles, the percentiles need to be rounded and can misclassify some normal-weight children in the under or overweight category (19). Therefore, to prevent these inaccuracies and for easier understanding, it is recommended that the BMI Z-scores in children should be used in research whereas BMI percentiles are best used in the clinical settings (20).

As BMI does not directly measure body fat, it is an excellent screening method, but should not be used solely for diagnostic purposes (23). Using 85th percentile as a cut off point for healthy weight may miss an opportunity to obtain crucial information on diet, physical activity, and family history. Once this information is obtained, it may allow the provider an opportunity to offer appropriate anticipatory guidance to the families.

PATHOPHYSIOLOGY OF OBESITY

The pathophysiology of obesity is complex that results from a combination of individual and societal factors. At the individual level, biological, and physiological factors in the presence of ones' own genetic risk influence eating behaviors and tendency to gain weight (1). Societal factors include influence of the family, community and socio-economic resources that further shape these behaviors (**Figure 1**) (3, 24).

Biological Factors

There is a complex architecture of neural and hormonal regulatory control, the Gut-Brain axis, which plays a significant role in hunger and satiety (**Figure 2**). Sensory stimulation (smell, sight, and taste), gastrointestinal signals (peptides, neural signals), and circulating hormones further contribute to food intake (25–27).

The hypothalamus is the crucial region in the brain that regulates appetite and is controlled by key hormones. Ghrelin, a hunger-stimulating (orexigenic) hormone, is mainly released from the stomach. On the other hand, leptin is primarily secreted from adipose tissue and serves as a signal for the brain regarding the body's energy stores and functions as an appetite -suppressing (anorexigenic) hormone. Several other appetite-suppressing (anorexigenic) hormones are released from the pancreas and gut in response to food intake and reach the hypothalamus through the brain-blood barrier (BBB) (28–32). These anorexigenic and orexigenic hormones regulate energy balance by stimulating hunger and satiety by expression of various signaling pathways in the arcuate nucleus (ARC) of the hypothalamus (**Figure 2**) (28, 33). Dysregulation of appetite due to blunted suppression or loss of caloric sensing signals can result in obesity and its morbidities (34).

Emotional dysfunction due to psychiatric disorders can cause stress and an abnormal sleep-wake cycles. These modifications in biological rhythms can result in increased appetite, mainly due to ghrelin, and can contribute to emotional eating (35).

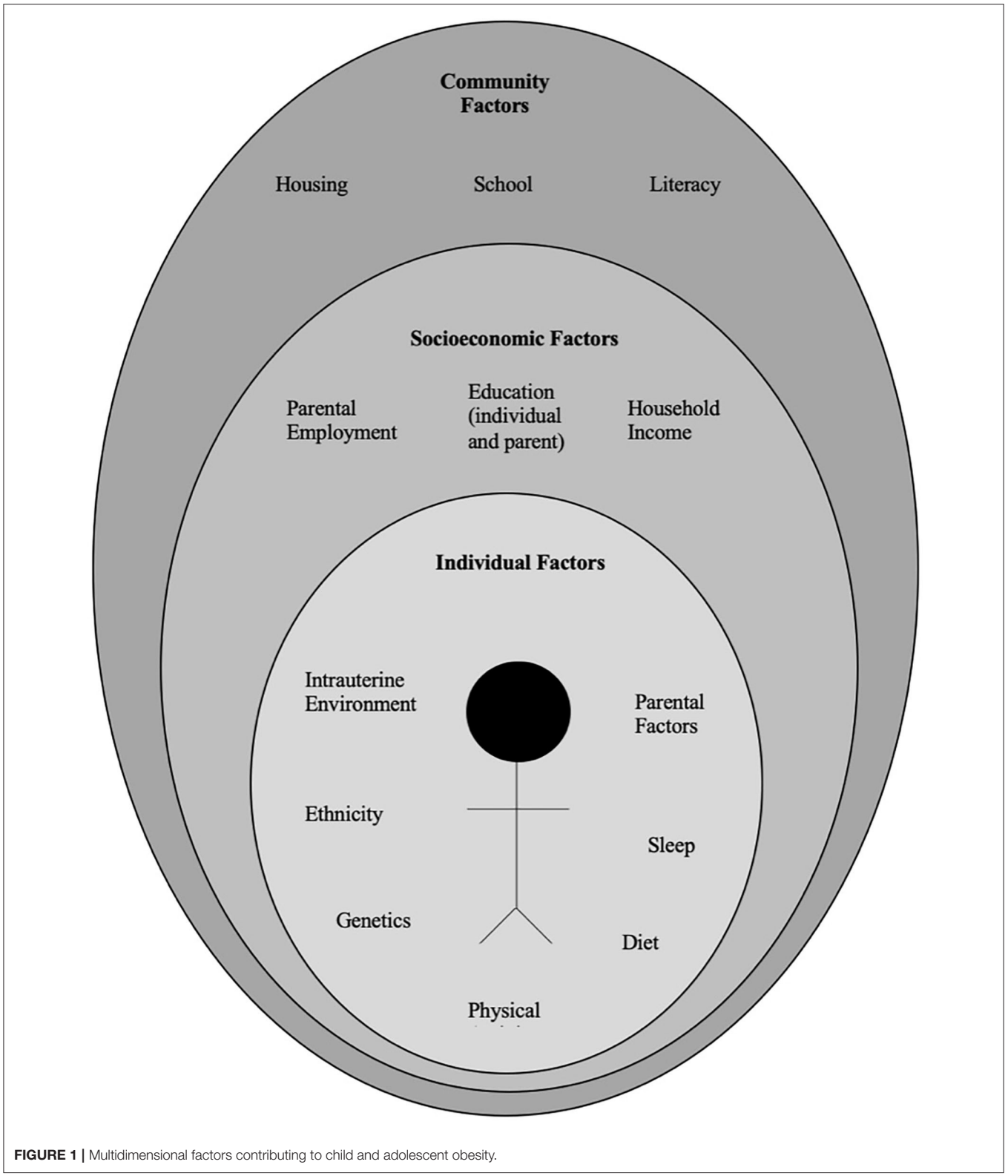

FIGURE 1 | Multidimensional factors contributing to child and adolescent obesity.

Recently, the role of changes in the gut microbiome with increased weight gain through several pathways has been described in literature (36, 37). The human gut serves as a host to trillions of microorganisms, referred to as gut microbiota. The dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with Firmicutes and Bacteroidetes representing 90% of human gut microbiota (5, 38). The microbes

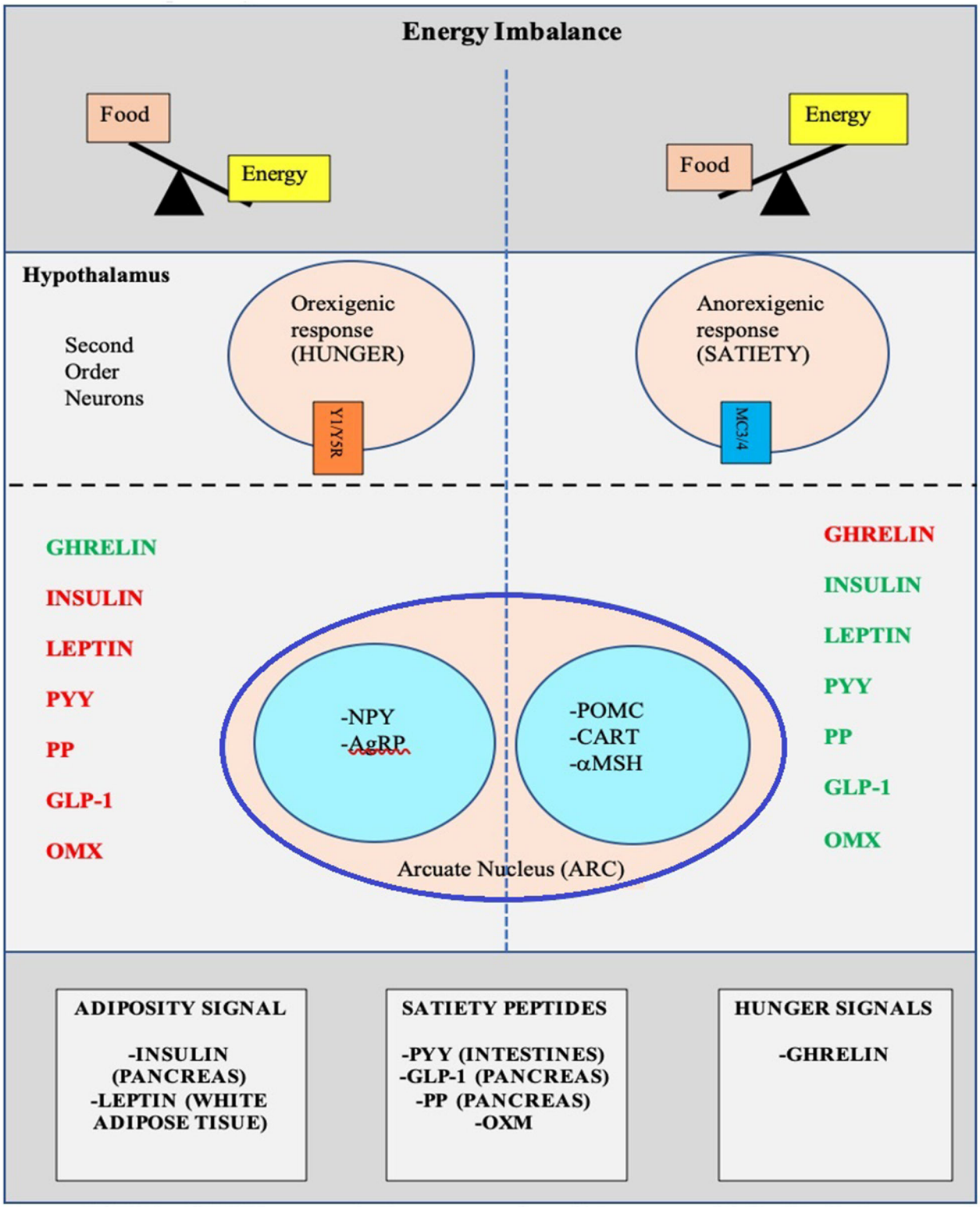

FIGURE 2 | Pictorial representation of the Hunger-Satiety pathway[a] and the various hormones[b] involved in the pathway. a, Y1/Y5R and MC3/4 are second order neuro receptors which are responsible in either the hunger or satiety pathway. Neurons in the ARC include: NPY, Neuropeptide Y; AgRP, Agouti-Related Peptide; POMC, Pro-Opiomelanocortin; CART, Cocaine-and Amphetamine-regulated Transcript; α-MSH, α-Melanocyte Stimulating Hormone. b, PYY, Peptide YY; PP, Pancreatic Polypeptide; GLP-1, Glucagon-Like Peptide- I; OMX, Oxyntomodulin.

in the gut have a symbiotic relationship within their human host and provide a nutrient-rich environment. Gut microbiota can be affected by various factors that include gestational age at birth, mode of infant delivery, type of neonatal and infant feeding, introduction of solid food, feeding practices and external factors like antibiotic use (5, 38). Also, the maturation of the bacterial phyla that occurs from birth to adulthood (39), is influenced by genetics, environment, diet, lifestyle, and gut physiology and stabilizes in adulthood (5, 39, 40). Gut microbiota is unique to each individual and plays a specific role in maintaining structural integrity, and the mucosal barrier of the gut, nutrient metabolism, immune response, and protection against pathogens (5, 37, 38). In addition, the microbiota ferments the indigestible food and synthesizes other essential micronutrients as well as short chain fatty acids (SCFAs') (40, 41). Dysbiosis or imbalance of the gut microbiota, in particularly the role of SCFA has been linked with the patho-physiology of obesity (36, 38, 41, 42). SCFAs' are produced by anaerobic fermentation of dietary fiber and indigestible starch and play a role in mammalian energy metabolism by influencing gut-brain communication axis. Emerging evidence has shown that increased ratio of Firmicutes to Bacteroidetes causes increased energy extraction of calories from diets and is evidenced by increased production of short chain fatty acids (SCFAs') (43–45). However, this relationship is not affirmed yet, as a negative relationship between SCFA levels and obesity has also been reported (46). Due to the conflicting data, additional randomized control trials are needed to clarify the role of SCFA's in obese and non-obese individuals.

The gut microbiota also has a bidirectional interaction with the liver, and various additional factors such as diet, genetics, and the environment play a key role in this relationship. The Gut- Liver Axis is interconnected at various levels that include the mucus barrier, epithelial barrier, and gut microbiome and are essential to maintain normal homeostasis (47). Increased intestinal mucosal permeability can disrupt the gut-liver axis, which releases various inflammatory markers, activates an innate immune response in the liver, and results in a spectrum of liver diseases that include hepatic steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) (48, 49).

Other medical conditions, including type 2 Diabetes Mellitus, Metabolic Syndrome, eating disorders as well as psychological conditions such as anxiety and depression are associated with the gut microbiome (50–53).

Genetic Factors

Genetic causes of obesity can either be monogenic or polygenic types. Monogenic obesity is rare, mainly due to mutations in genes within the leptin/melanocortin pathway in the hypothalamus that is essential for the regulation of food intake/satiety, body weight, and energy metabolism (54). Leptin regulates eating behaviors, the onset of puberty, and T-cell immunity (55). About 3% of obese children have mutations in the leptin (*LEP*) gene and the leptin receptor (LEPR) and can also present with delayed puberty and immune dysfunction (55, 56). Obesity caused by other genetic mutations in the leptin-melanocortin pathway include proopiomelanocortin (POMC) and melanocortin receptor 4 (MC4R), brain-derived neurotrophic factor (BDNF), and the tyrosine kinase receptor B (NTRK2) genes (57, 58). Patients with monogenic forms generally present during early childhood (by 2 years old) with severe obesity and abnormal feeding behaviors (59). Other genetic causes of severe obesity are Prader Willi Syndrome (PWS), Alström syndrome, Bardet Biedl syndrome. Patients with these syndromes present with additional characteristics, including cognitive impairment, dysmorphic features, and organ-specific developmental abnormalities (60). Individuals who present with obesity, developmental delay, dysmorphic features, and organ dysfunction should receive a genetics referral for further evaluation.

Polygenic obesity is the more common form of obesity, caused by the combined effect of multiple genetic variants. It is the result of the interplay between genetic susceptibility and the environment, also known as the Gene-Environment Interaction (GEI) (61–64). Genome-wide association studies (GWAS) have identified gene variants [single nucleotide polymorphism (SNPs)] for body mass index (BMI) that likely act synergistically to affect body weight (65). Studies have identified genetic variants in several genes that may contribute to excessive weight gain by increasing hunger and food intake (66–68). When the genotype of an individual confers risk for obesity, exposure to an obesogenic environment may promote a state of energy imbalance due to behaviors that contribute to conserving rather than expending energy (69, 70). Research studies have shown that obese individuals have a genetic variation that can influence their actions, such as increased food intake, lack of physical activity, a decreased metabolism, as well as an increased tendency to store body fat (63, 66, 67, 69, 70).

Recently the role of epigenetic factors in the development of obesity has emerged (71). The epigenetic phenomenon may alter gene expression without changing the underlying DNA sequence. In effect, epigenetic changes may result in the addition of chemical tags known as methyl groups, to the individual's chromosomes. This alteration can result in a phenomenon where critical genes are primed to on and off regulate. Complex physiological and psychological adjustment occur during infancy and can thereafter set the stage for health vs. disease. Developmental origins of health and disease (DOHaD) shows that early life environment can impact the risk of chronic diseases later in life due to fetal programming secondary to epigenetic changes (72). Maternal nutrition during the prenatal or early postnatal period may trigger these epigenetic changes and increase the risk for chronic conditions such as obesity, metabolic and cardiovascular disease due to epigenetic modifications that may persist and cause intergenerational effect on the health children and adults (58, 73, 74). Similarly, adverse childhood experiences (ACE) have been linked to a broad range of negative outcomes through epigenetic mechanisms (75) and promote unhealthy eating behaviors (76, 77). Other factors such as diet, physical activity, environmental and psychosocial stressors can cause epigenetic changes and place an individual at risk for weight gain (78).

Developmental Factors

Eating behaviors evolve over the first few years of life. Young children learn to eat through their direct experience with food and observing others eating around them (79). During infancy, feeding defines the relationship of security and trust between a child and the parent. Early childhood eating behaviors shift to more self-directed control due to rapid physical, cognitive, communicative, and social development (80). Parents or caregivers determine the type of food that is made available to the infant and young child. However, due to economic limitations and parents having decreased time to prepare nutritious meals, consumption of processed and cheaper energy-dense foods have occurred in Western countries. Additionally, feeding practices often include providing large or super-sized portions of palatable foods and encouraging children to finish the complete meal (clean their plate even if they do not choose to), as seen across many cultures (81, 82). Also, a segment of parents are overly concerned with dietary intake and may pressurize their child to eat what they perceive as a healthy diet, which can lead to unintended consequences (83). Parents' excessive restriction of food choices may result in poor self-regulation of energy intake by their child or adolescent. This action may inadvertently promote overconsumption of highly palatable restricted foods when available to the child or adolescent outside of parental control with resultant excessive weight gain (84, 85).

During middle childhood, children start achieving greater independence, experience broader social networks, and expand their ability to develop more control over their food choices. Changes that occur in the setting of a new environment such as daycare or school allow exposure to different food options, limited physical activity, and often increased sedentary behaviors associated with school schedules (24). As the transition to adolescence occurs, physical and psychosocial development significantly affect food choices and eating patterns (25). During the teenage years, more independence and interaction with peers can impact the selection of fast foods that are calorically dense. Moreover, during the adolescent years, more sedentary behaviors such as video and computer use can limit physical exercise. Adolescence is also a period in development with an enhanced focus on appearance, body weight, and other psychological concerns (86, 87).

Environmental Factors

Environmental changes within the past few decades, particularly easy access to high-calorie fast foods, increased consumption of sugary beverages, and sedentary lifestyles, are linked with rising obesity (88). The easy availability of high caloric fast foods, and super-sized portions, are increasingly common choices as individuals prefer these highly palatable and often less expensive foods over fruits and vegetables (89). The quality of lunches and snacks served in schools and childcare centers has been an area of debate and concern. Children and adolescents consume one-third to one-half of meals in the above settings. Despite policies in place at schools, encouraging foods, beverages, and snacks that are deemed healthier options, the effectiveness of these policies in improving children's dietary habits or change in obesity rate has not yet been seen (90). This is likely due to the fact that such policies primarily focus on improving dietary quality but not quantity which can impact the overweight or obese youth (91). Policies to implement taxes on sugary beverages are in effect in a few states in the US (92) as sugar and sugary beverages are associated with increased weight gain (2, 3). This has resulted in reduction in sales of sugary drinks in these states, but the sales of these types of drinks has risen in neighboring states that did not implement the tax (93). Due to advancements in technology, children are spending increased time on electronic devices, limiting exercise options. Technology advancement is also disrupting the sleep-wake cycle, causing poor sleeping habits, and altered eating patterns (94). A study published on Canadian children showed that the access to and night-time use of electronic devices causes decreased sleep duration, resulting in excess body weight, inferior diet quality, and lower physical activity levels (95).

Infant nutrition has gained significant popularity in relation to causing overweight/obesity and other diseases later in life. Breast feeding is frequently discussed as providing protection against developing overweight/obesity in children (8). Considerable heterogeneity has been observed in studies and conducting randomized clinical trials between breast feeding vs. formula feeding is not feasible (8). Children fed with a low protein formula like breast milk are shown to have normal weight gain in early childhood as compared to those that are fed formulas with a high protein load (96). A recent Canadian childbirth cohort study showed that breast feeding within first year of life was inversely associated with weight gain and increased BMI (97). The effect was stronger if the child was exclusively breast fed directly vs. expressed breast milk or addition of formula or solid food (97). Also, due to the concern of poor growth in preterm or SGA infants, additional calories are often given for nutritional support in the form of macronutrient supplements. Most of these infants demonstrate "catch up growth." In fact, there have been reports that in some children the extra nutritional support can increase the risk for overweight/obesity later in life. The association, however, is inconsistent. Recently a systemic review done on randomized controlled trials comparing the studies done in preterm and SGA infants with feeds with and without macronutrient supplements showed that macronutrient supplements may increase weight and length in toddlers but did not show a significant increase in the BMI during childhood (98). Increased growth velocity due to early introduction of formula milk and protein in infants' diet, may influence the obesity pathways, and can impact fetal programming for metabolic disease later in life (99).

General pediatricians caring for children with overweight/obesity, generally recommend endocrine testing as parents often believe that there may be an underlying cause for this condition and urge their primary providers to check for conditions such as thyroid abnormalities. Endocrine etiologies for obesity are rarely identified and patients with underlying endocrine disorders causing excessive weight gain usually are accompanied by attenuated growth patterns, such that a patient continues to gain weight with a decline in linear height (100). Various endocrine etiologies that one could consider in a patient with excessive weight gain in the setting of slow linear

growth: severe hypothyroidism, growth hormone deficiency, and Cushing's disease/syndrome (58, 100).

CLINICAL-PHYSIOLOGY OF PEDIATRIC OBESITY

It is a well-known fact that early AR(increased BMI) before the age of 5 years is a risk factor for adult obesity, obesity-related comorbidities, and metabolic syndrome (101–103). Typically, body mass index (BMI) declines to a minimum in children before it starts increasing again into adulthood, also known as AR. Usually, AR happens between 5 and 7 years of age, but if it occurs before the age of 5 years is considered early AR. Early AR is a marker for higher risk for obesity-related comorbidities. These obesity-related health comorbidities include cardiovascular risk factors (hypertension, dyslipidemia, prediabetes, and type 2 diabetes), hormonal issues, orthopedic problems, sleep apnea, asthma, and fatty liver disease (**Figure 3**) (9).

CLINICAL COMORBIDITIES OF OBESITY IN CHILDREN

Growth and Puberty

Excess weight gain in children can influence growth and pubertal development (10). Childhood obesity can cause prepubertal acceleration of linear growth velocity and advanced bone age in boys and girls (104). Hyperinsulinemia is a normal physiological state during puberty, but children with obesity can have abnormally high insulin levels (105). Leptin resistance also occurs in obese individuals who have higher leptin levels produced by their adipose tissue (55, 106). The insulin and leptin levels can act on receptors that impact the growth plates with a resultant bone age advancement (55).

Adequate nutrition is essential for the typical timing and tempo of pubertal onset. Excessive weight gain can initiate early puberty, due to altered hormonal parameters (10). Obese children may present with premature adrenarche, thelarche, or precocious puberty (PP) (107). The association of early pubertal changes with obesity is consistent in girls, and is well-reported; however, data is sparse in boys (108). One US study conducted in racially diverse boys showed obese boys had delayed puberty, whereas overweight boys had early puberty as compared to normal-weight boys (109). Obese girls with PP have high leptin levels (110, 111). Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) is a cross-sectional study and suggested an indirect relationship between elevated leptin levels, early puberty, and cardiometabolic and inflammatory markers in obese girls (112). Additionally, obese girls with premature adrenarche carry a higher risk for developing polycystic ovary syndrome (PCOS) in the future (113, 114).

Sleep Disorders

Obesity is an independent risk factor for obstructive sleep apnea (OSA) in children and adolescents (12, 115). Children with OSA have less deleterious consequences in terms of cardiovascular stress of metabolic syndrome when compared to adolescents and adults (116, 117). In children, abnormal behaviors and neurocognitive dysfunction are the most critical and frequent end-organ morbidities associated with OSA (12). However, in adolescents, obesity and OSA can independently cause oxidative systemic stress and inflammation (118, 119), and when this occurs concurrently, it can result in more severe metabolic dysfunction and cardiovascular outcomes later in life (120).

Other Comorbidities

Obesity is related to a clinical spectrum of liver abnormalities such as NAFLD (121); the most important cause of liver disease in children (122–124). NAFLD includes steatosis (increased liver fat without inflammation) and NASH (increased liver fat with inflammation and hepatic injury). While in some adults NAFLD can progress to an end-stage liver disease requiring liver transplant (125, 126), the risk of progression during childhood is less well-defined (127). NAFLD is closely associated with metabolic syndrome including central obesity, insulin resistance, type 2 diabetes, dyslipidemia, and hypertension (128).

Obese children are also at risk for slipped capital femoral epiphysis (SCFE) (129), and sedentary lifestyle behaviors may have a negative influence on the brain structure and executive functioning, although the direction of causality is not clear (130, 131).

CLINICAL COMORBIDITIES OF OBESITY IN ADOLESCENTS

Menstrual Irregularities and PCOS

At the onset of puberty, physiologically, sex steroids can cause appropriate weight gain and body composition changes that should not affect normal menstruation (132, 133). However, excessive weight gain in adolescent girls can result in irregular menstrual cycles and puts them at risk for PCOS due to increased androgen levels. Additionally, they can have excessive body hair (hirsutism), polycystic ovaries, and can suffer from distorted body images (134, 135). Adolescent girls with PCOS also have an inherent risk for insulin resistance irrespective of their weight. However, weight gain further exacerbates their existing state of insulin resistance and increases the risk for obesity-related comorbidities such as metabolic syndrome, and type 2 diabetes. Although the diagnosis of PCOS can be challenging at this age due to an overlap with predictable pubertal changes, early intervention (appropriate weight loss and use of hormonal methods) can help restore menstrual cyclicity and future concerns related to childbearing (11).

Metabolic Syndrome and Sleep Disorders

Metabolic syndrome (MS) is a group of cardiovascular risk factors characterized by acanthosis nigricans, prediabetes, hypertension, dyslipidemia, and non-alcoholic steatohepatitis (NASH), that occurs from insulin resistance caused by obesity (136). Diagnosis of MS in adults requires at least three out of the five risk factors: increased central adiposity, hypertension, hyperglycemia, hypertriglyceridemia, or low HDL level. Definitions to diagnose MS are controversial in younger age groups, and many definitions have been

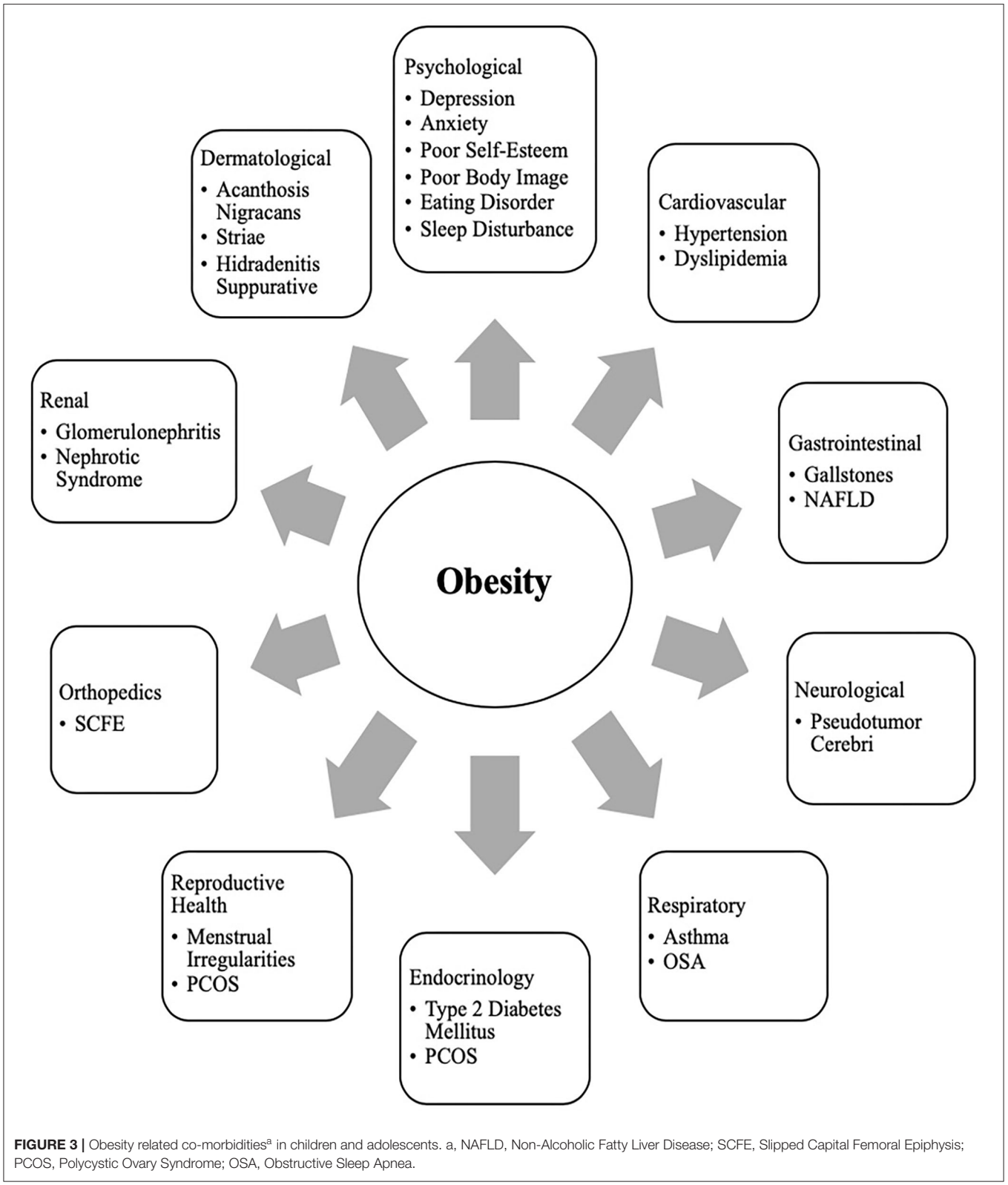

FIGURE 3 | Obesity related co-morbidities[a] in children and adolescents. a, NAFLD, Non-Alcoholic Fatty Liver Disease; SCFE, Slipped Capital Femoral Epiphysis; PCOS, Polycystic Ovary Syndrome; OSA, Obstructive Sleep Apnea.

proposed (136). This is due to the complex physiology of growth and development during puberty, which causes significant overlap between MS and features of normal growth. However, childhood obesity is associated with an inflammatory state even before puberty (137). In obese children and adolescents, hyperinsulinemia during puberty (138, 139)

and unhealthy sleep behaviors increase MS's risk and severity (140). Even though there is no consensus on diagnosis regarding MS in this age group, when dealing with obese children and adolescents, clinicians should screen them for MS risk factors and sleep behaviors and provide recommendations for weight management.

Social Psychology of Pediatric Obesity in Children and Adolescents

Obese children and adolescents may experience psychosocial sequelae, including depression, bullying, social isolation, diminished self-esteem, behavioral problems, dissatisfaction with body image, and reduced quality of life (13, 141). Compared with normal-weight counterparts, overweight/obesity is one of the most common reasons children and adolescents are bullied at school (142). The consequence of stigma, bullying, and teasing related to childhood obesity are pervasive and can have severe implications for emotional and physical health and performance that can persist later in life (13).

In adolescents, psychological outcomes associated with obesity are multifactorial and have a bidirectional relationship (**Figure 4**). Obese adolescents due to their physique may have a higher likelihood of psychosocial health issues, including depression, body image/dissatisfaction, lower self-esteem, peer victimization/bullying, and interpersonal relationship difficulties. They may also demonstrate reduced resilience to challenging situations compared to their non-obese/overweight counterparts (9, 143–146). Body image dissatisfaction has been associated with further weight gain but can also be related to the development of a mental health disorder or an eating disorder (ED) or disorder eating habits (DEH). Mental health disorders such as depression are associated with poor eating habits, a sedentary lifestyle, and altered sleep patterns. ED or DEH that include anorexia nervosa (AN), bulimia nervosa (BN), binge-eating disorder (BED) or night eating syndrome (NES) may be related to an individual's overvaluation of their body shape and weight or can result during the treatment for obesity (147–150). The management of obesity can place a patient at risk of AN if there is a rigid focus on caloric intake or if a patient overcorrects and initiates obsessive self-directed dieting. Healthcare providers who primarily care for obese patients, usually give the advice to diet to lose weight and then maintain it. However, strict dieting (hypocaloric diet), which some patients may later engage in can lead to an eating disorder such as anorexia nervosa (151). This behavior leads to a poor relationship with food, and therefore, adolescents perseverate on their weight and numbers (152).

Providers may not recognize DEHs when a morbidly obese patient loses the same weight as a healthy weight individual (149). It may appear as a positive result with families and others praising the individual without realizing that this youth may be engaging in destructive behaviors related to weight control. Therefore, it is essential to screen regarding the process of how weight loss was achieved (144, 150).

Support and attention to underlying psychological concerns can positively affect treatment, overall well-being, and reduce the risk of adult obesity (150). The diagram above represents the complexity of the different psychological issues which can impact the clinical care of the obese adolescent.

Eating family meals together can improve overall dietary intake due to enhanced food choices mirrored by parents. It has also may serve as a support to individuals with DEHs if there is less attention to weight and a greater focus on appropriate, sustainable eating habits (148).

TREATMENT

Prevention and Anticipatory Guidance

It is essential to recognize and provide preventive measures for obesity during early childhood and adolescence (100, 153, 154). It is well-established that early AR is a risk factor for adult obesity (66–68). Therefore, health care providers caring for the pediatric population need to focus on measures such as BMI but provide anticipatory guidance regarding nutritional counseling without stigmatizing or judging parents for their children's overweight/obesity (155). Although health care providers continue to pursue effective strategies to address the obesity epidemic; ironically, they frequently exhibit weight bias and stigmatizing behaviors. Research has demonstrated that the language that health care providers use when discussing a patient's body weight can reinforce stigma, reduce motivation for weight loss, and potentially cause avoidance of routine preventive care (155). In adolescents, rather than motivating positive changes, stigmatizing language regarding weight may negatively impact a teen and result in binge eating, decreased physical activity, social isolation, avoidance of health care services, and increased weight gain (156, 157). Effective provider-patient communication using motivational interviewing techniques are useful to encourage positive behavior changes (155, 158).

Anticipatory guidance includes educating the families on healthy eating habits and identifying unhealthy eating practices, encouraging increased activity, limiting sedentary activities such as screen time. Lifestyle behaviors in children and adolescents are influenced by many sectors of our society, including the family (**Figure 1**) (3, 24). Therefore, rather than treating obesity in isolation as an individual problem, it is crucial to approach this problem by focusing on the family unit. Family-based multi-component weight loss behavioral treatment is the gold standard for treating childhood obesity, and it is having been found useful in those between 2 and 6 years old (150, 159). Additionally, empowering the parents to play an equal role in developing and implementing an intervention for weight management has shown promising results in improving the rate of obesity by decreasing screen time, promoting healthy eating, and increasing support for children's physical activity (160, 161).

When dietary/lifestyle modifications have failed, the next option is a structured weight -management program with a multidisciplinary approach (15). The best outcomes are associated with an interdisciplinary team comprising a physician,

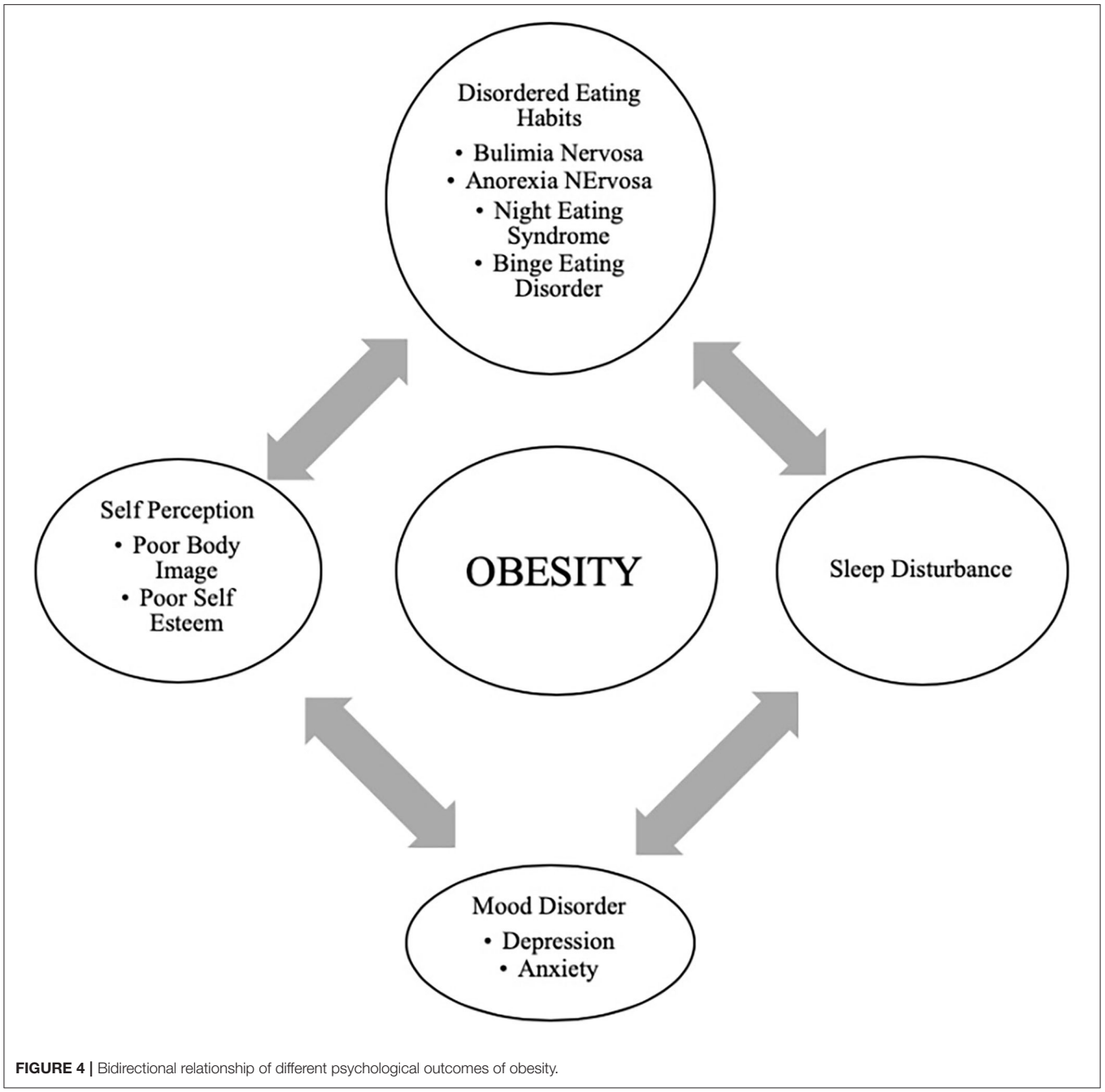

FIGURE 4 | Bidirectional relationship of different psychological outcomes of obesity.

dietician, and psychologist generally 1–2 times a week (15, 162). However, this treatment approach is not effective in patients with severe obesity (122). Although healthier lifestyle recommendations for weight loss are the current cornerstone for obesity management, they often fail. As clinicians can attest, these behavioral and dietary changes are hard to achieve, and all too often is not effective in patients with severe obesity. Failure to maintain substantial weight loss over the long term is due to poor adherence to the prescribed lifestyle changes as well as physiological responses that resist weight loss (163). American TV hosts a reality show called "The Biggest Loser" that centers on overweight and obese contestants attempting to lose weight for a cash prize. Contestants from "The Biggest Loser" competition, had metabolic adaptation (MA) after drastic weight loss, regained more than they lost weight after 6 years due to a significant slow resting metabolic rate (164). MA is a physiological response which is a reduced basal metabolic rate seen in individuals who are losing or have lost weight. In MA, the body alters how efficient it is at turning the food eaten into energy; it is a natural defense mechanism against starvation and is a response to caloric restriction. Plasma leptin levels decrease substantially during caloric restriction,

suggesting a role of this hormone in the drop of energy expenditure (165).

Pharmacological Management

The role of pharmacological therapy in the treatment of obesity in children and adolescents is limited.

Orlistat is the only FDA approved medication for weight loss in 12-18-year-olds but has unpleasant side effects (166). Another medicine, Metformin, has been used in children with signs of insulin resistance, may have some impact on weight, but is not FDA approved (167). The combination of phentermine/topiramate (Qsymia) has been FDA approved for weight loss in obese individuals 18 years and older. In studies, there has been about 9–10% weight loss over 2 years. However, caution must be taken in females as it can lead to congenital disabilities, especially with use in the first trimester of pregnancy (167).

GLP-1 agonists have demonstrated great success in effective weight loss and are approved by the FDA for adult obesity (168–170). A randomized control clinical trial recently published showed a significant weight loss in those using liraglutide (3.0 mg)/day plus lifestyle therapy group compared to placebo plus lifestyle therapy in children between the ages of 12–18 years (171).

Recently during the EASL conference, academic researchers and industry partners presented novel interventions targeting different gut- liver axis levels that include intestinal content, intestinal microbiome, intestinal mucosa, and peritoneal cavity (47). The focus for these therapeutic interventions within the gut-liver axis was broad and ranged anywhere from newer drugs protecting the intestinal mucus lining, restoring the intestinal barriers and improvement in the gut microbiome. One of the treatment options was Hydrogel technology which was shown to be effective toward weight loss in patients with metabolic syndrome. Hydrogel technology include fibers and high viscosity polysaccharides that absorb water in the stomach and increasing the volume, thereby improving satiety (47). Also, a clinical trial done in obese pregnant mothers using Docosahexaenoic acid (DHA) showed that the mothers' who got DHA had children with lower adiposity at 2 and 4 years of age (172). Recently the role of probiotics in combating obesity has emerged. Probiotics are shown to alter the gut microbiome that improves intestinal digestive and absorptive functions of the nutrients. Intervention including probiotics may be a possible solution to manage pediatric obesity (173, 174). Additionally, the role of Vitamin E for treating the comorbidities of obesity such as diabetes, hyperlipidemia, NASH, and cardiovascular risk, has been recently described (175, 176). Vitamin E is a lipid- soluble compound and contains both tocopherols and tocotrienols. Tocopherols have lipid-soluble antioxidants properties that interact with cellular lipids and protects them from oxidation damage (177). In metabolic disease, certain crucial pathways are influenced by Vitamin E and some studies have summarized the role of Vitamin E regarding the treatment of obesity, metabolic, and cardiovascular disease (178). Hence, adequate supplementation of Vitamin E as an appropriate strategy to help in the treatment of the prevention of obesity and its associated comorbidities has been suggested. Nonetheless, some clinical trials have shown contradictory results with Vitamin E supplementation (177). Although Vitamin E has been recognized as an antioxidant that protects from oxidative damage, however, a full understanding of its mechanism of action is still lacking.

Bariatric Surgery

Bariatric surgery has gained popularity since the early 2000s in the management of severe obesity. If performed earlier, there are better outcomes for reducing weight and resolving obesity-related comorbidities in adults (179–182). Currently, the indication for bariatric in adolescents; those who have a BMI >35 with at least one severe comorbidity (Type 2 Diabetes, severe OSA, pseudotumor cerebri or severe steatohepatitis); or BMI of 40 or more with other comorbidities (hypertension, hyperlipidemia, mild OSA, insulin resistance or glucose intolerance or impaired quality of life due to weight). Before considering bariatric surgery, these patients must have completed most of their linear growth and participated in a structured weight-loss program for 6 months (159, 181, 183). The American Society for Metabolic and Bariatric Surgery (AMBS) outlines the multidisciplinary approach that must be taken before a patient undergoing bariatric surgery. In addition to a qualified bariatric surgeon, the patient must have a pediatrician or provider specialized in adolescent medicine, endocrinology, gastroenterology and nutrition, registered dietician, mental health provider, and exercise specialist (181). A mental health provider is essential as those with depression due to obesity or vice versa may have persistent mental health needs even after weight loss surgery (184).

Roux-en-Y Gastric Bypass (RYGB), laparoscopic Sleeve Gastrectomy (LSG), and Gastric Banding are the options available. RYGB and LSG currently approved for children under 18 years of age (166, 181, 185). At present, gastric banding is not an FDA recommended procedure in the US for those under 18y/o. One study showed some improvements in BMI and severity of comorbidities but had multiple repeat surgeries and did not believe a suitable option for obese adolescents (186).

Compared to LSG, RYGB has better outcomes for excess weight loss and resolution of obesity-related comorbidities as shown in studies and clinical trials (183, 184, 187). Overall, LSG is a safer choice and may be advocated for more often (179–181). The effect on the Gut-Brain axis after Bariatric surgery is still inconclusive, especially in adolescents, as the number of procedures performed is lower than in adults. Those who underwent RYGB had increased fasting and post-prandial PYY and GLP-1, which could have contributed to the rapid weight loss (185); this effect was seen less often in patients with gastric banding (185). Another study in adult patients showed higher bile acid (BA) subtype levels and suggested a possible BA's role in the surgical weight loss response after LSG (188). Adolescents have lower surgical complication rates than their adult counterparts, hence considering bariatric surgery earlier rather than waiting until adulthood has been entertained (180). Complications after surgery include nutritional imbalance in iron, calcium, Vitamin D, and B12 and should be monitored closely (180, 181, 185). Although 5-year data for gastric

bypass in very obese teens is promising, lifetime outcome is still unknown, and the psychosocial factors associated with adolescent adherence post-surgery are also challenging and uncertain.

CONCLUSION

Obesity in childhood and adolescence is not amenable to a single easily modified factor. Biological, cultural, and environmental factors such as readily available high-density food choices impact youth eating behaviors. Media devices and associated screen time make physical activity a less optimal choice for children and adolescents. This review serves as a reminder that the time for action is now. The need for interventions to change the obesogenic environment by instituting policies around the food industry and in the schools needs to be clarified. In clinical trials GLP-1 agonists are shown to be effective in weight loss in children but are not yet FDA approved. Discovery of therapies to modify the gut microbiota as treatment for overweigh/obesity through use of probiotics or fecal transplantation would be revolutionary. For the present, ongoing clinical research efforts in concert with pharmacotherapeutic and multidisciplinary lifestyle programs hold promise.

AUTHOR CONTRIBUTIONS

AK, SL, and MJ contributed to the conception and design of the study. All authors contributed to the manuscript revision, read, and approved the submitted version.

REFERENCES

1. Gurnani M, Birken C, Hamilton. J. Childhood obesity: causes, consequences, and management. *Pediatr Clin North Am.* (2015) 62:821–40. doi: 10.1016/j.pcl.2015.04.001
2. Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, and Bhadoria. AS. Childhood obesity: causes and consequences. *J Family Med Prim Care.* (2015) 4:187–92. doi: 10.4103/2249-4863.154628
3. Brown CL, Halvorson EE, Cohen GM, Lazorick S, Skelton JA. Addressing childhood obesity: opportunities for prevention. *Pediatr Clin North Am.* (2015) 62:1241–61. doi: 10.1016/j.pcl.2015.05.013
4. Qasim A, Turcotte M, de Souza RJ, Samaan MC, Champredon D, Dushoff J, et al. On the origin of obesity: identifying the biological, environmental, and cultural drivers of genetic risk among human populations. *Obes Rev. (*2018*)* 19:121–49. doi: 10.1111/obr.12625
5. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. *What is the healthy gut microbiota composition?* a changing ecosystem across age, environment, diet, and diseases. *Microorganisms.* (2019) 7:14. doi: 10.3390/microorganisms7010014
6. Indrio F, Martini S, Francavilla R, Corvaglia L, Cristofori F, Mastrolia SA, et al. Epigenetic matters: the link between early nutrition, microbiome, and long-term health development. *Front Pediatr.* (2017) 5:178. doi: 10.3389/fped.2017.00178
7. Marcovecchio ML, Gorman S, Watson LPE, Dunger DB, Beardsall K. Catch-up growth in children born small for gestational age related to body composition and metabolic risk at six years of age in the UK. *Horm Res Paediatr.* (2020) 93:119–27. doi: 10.1159/000508974
8. Koletzko B, Fishbein M, Lee WS, Moreno L, Mouane N, Mouzaki M, et al. Prevention of childhood obesity: a position paper of the global federation of international societies of paediatric gastroenterology, hepatology nutrition (FISPGHAN). *J Pediatr Gastroenterol Nutr.* (2020) 70:702–10. doi: 10.1097/MPG.0000000000002708
9. Pulgarón ER. Childhood obesity: a review of increased risk for physical and psychological comorbidities. *Clin Ther.* (2013) 35:A18–32. doi: 10.1016/j.clinthera.2012.12.014
10. De Leonibus C, Marcovecchio ML, Chiarelli F. Update on statural growth and pubertal development in obese children. *Pediatr Rep.* (2012) 4:e35. doi: 10.4081/pr.2012.e35
11. Witchel SF, Burghard AC, Tao RH, Oberfield SE. *The diagnosis and treatment of PCOS in adolescents. Curr Opin Pediatr.* (2019) 31:562–9. doi: 10.1097/MOP.0000000000000778
12. Marcus CL, Brooks LJ, Draper KA, Gozal D, Halbower AC, Jones J, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics.* (2012) 130:e714–55. doi: 10.1542/peds.2012-1672
13. Rankin J, Matthews L, Cobley S, Han A, Sanders R, Wiltshire HD, et al. Psychological consequences of childhood obesity: psychiatric comorbidity and prevention. *Adolesc Health Med Ther.* (2016) 7:125–46. doi: 10.2147/AHMT.S101631
14. Topçu S, Orhon FS, Tayfun M, Uçaktürk SA, Demirel F. Anxiety, depression, and self-esteem levels in obese children: a case-control study. *J Pediatr Endocrinol Metabol.* (2016) 29:357–61. doi: 10.1515/jpem-2015-0254
15. Katzmarzyk PT, Barlow S, Bouchard C, Catalano PM, Hsia DS, Inge TH, et al. An evolving scientific basis for the prevention and treatment of pediatric obesity. *Int J Obes.* (2014) 38:887–905. doi: 10.1038/ijo.2014.49
16. Brown T, Moore TH, Hooper L, Gao Y, Zayegh A, Ijaz S, et al. Interventions for preventing obesity in children. *Cochrane Database Syst Rev.* (2019) 7:CD001871. doi: 10.1002/14651858.CD001871.pub4
17. Smith E, Scarborough P, Rayner M, Briggs ADM. Should we tax unhealthy food and drink? *Proc Nutr Soc.* (2019) 77:314–20. doi: 10.1017/S0029665117004165
18. Adab P, Pallan M, Whincup PH. Is BMI the best measure of obesity? *BMJ.* (2018) 360:k 1274. doi: 10.1136/bmj.k1274
19. Anderson LN, Carsley S, Lebovic G, Borkhoff CM, Maguire JL, Parkin PC, et al. Misclassification of child body mass index from cut-points defined by rounded percentiles instead of Z-scores. *BMC Res Notes.* (2017) 10:639. doi: 10.1186/s13104-017-2983-0
20. Must A, Anderson SE. Body mass index in children and adolescents: consideration for population-based applications. *Int J Obes.* (2006) 30:590–4. doi: 10.1038/sj.ijo.0803300
21. Flegal KM, Wei R, Ogden C. Weight-for-stature compared with body mass index-for-age growth charts for the United States from the centers for disease control and prevention. *Am J Clin Nutr.* (2002) 75:761–6.22. doi: 10.1093/ajcn/75.4.761
22. Himes JH, Dietz WH. Guidelines for overweight in adolescent preventive services: recommendations from an expert committee. *The expert committee on clinical guidelines for overweight in adolescent preventive services. Am J Clin Nutr.* (1994) 59:307–16. doi: 10.1093/ajcn/59.2.307
23. Lazarus R, Baur L, Webb K, Blyth F. Body mass index in screening for adiposity in children and adolescents: systematic evaluation using receiver operating characteristic curves. *Am J Clin Nutr.* (1996) 63:500–6. doi: 10.1093/ajcn/63.4.500
24. McGinnis JM, Gootman JA. *Food Marketing to Children and Youth: Threat or Opportunity? Institute of Medicine of the National Academies.* Washington, DC: The National Academies Press. (2006).
25. Chaudhri OB, Salem V, Murphy KG, Bloom SR. Gastrointestinal satiety signals. *Annu Rev Physiol.* (2008) 70:239–55. doi: 10.1146/annurev.physiol.70.113006.100506
26. Scaglioni S, De Cosmi V, Ciappolino V, Parazzini F, Brambilla P, Agostoni C. Factors influencing children's eating behaviours. *Nutrients.* (2018) 10:706. doi: 10.3390/nu10060706
27. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. *Endocrinol Metab Clin North Am.* (2008) 37:811–23. doi: 10.1016/j.ecl.2008.08.005

28. Niswender KD, Baskin DG, Schwartz MW. Review insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends Endocrinol Metab.* (2004) 15:362–9. doi: 10.1016/j.tem.2004.07.009
29. Niswender KD, Schwartz MW. Review insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol.* (2003) 24:1–10. doi: 10.1016/S0091-3022(02)00105-X
30. Amitani M, Asakawa A, Amitani H, Inui. A. The role of leptin in the control of insulin-glucose axis. *Front Neurosci.* (2013) 7:51. doi: 10.3389/fnins.2013.00051
31. Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron.* (2003) 37:649–61. doi: 10.1016/S0896-6273(03)00063-1
32. Buhmann, H., le Roux CW, Bueter M. The gut–brain axis in obesity. *Best Prac Res Clin Gastroenterol.* (2014) 28:559–71. doi: 10.1016/j.bpg.2014.07.003
33. Cone RD. Review anatomy and regulation of the central melanocortin system. *Nat Neurosci.* (2005) 8:571–8. doi: 10.1038/nn1455
34. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech.* (2017) 10:679–89. doi: 10.1242/dmm.026609
35. Labarthe A, Fiquet O, Hassouna R, Zizzari P, Lanfumey L, Ramoz N, et al. Ghrelin-derived peptides: a link between appetite/reward, gh axis, and psychiatric disorders? *Front Endocrinol.* (2014) 5:163. doi: 10.3389/fendo.2014.00163
36. Hills, R. D. Jr., Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut microbiome: profound implications for diet and disease. *Nutrients.* (2019) 11:1613. doi: 10.3390/nu11071613
37. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol Hepatol.* (2017) 2:747–56. doi: 10.1016/S2468-1253(17)30147-4
38. Gérard P. Gut microbiota and obesity. *Cell Mol Life Sci.* (2016) 73:147–62. doi: 10.1007/s00018-015-2061-5
39. Derrien M, Alvarez AS, de Vos WM. The gut microbiota in the first decade of life. *Trends Microbiol.* (2019) 27:997–1010.40. doi: 10.1016/j.tim.2019.08.001
40. Dao, MC., and Clément K. Gut microbiota and obesity: concepts relevant to clinical care. *Eur J Intern Med.* (2018) 48:18–24.41. doi: 10.1016/j.ejim.2017.10.005
41. Kim KN, Yao,Y., Ju SY. Short chain fatty acids and fecal microbiota abundance in humans with obesity: a systematic review and meta-analysis. *Nutrients.* (2019) 11:2512. doi: 10.3390/nu11102512
42. Castaner O, Goday A, Park YM, Lee SH, Magkos F, Shiow STE, et al. *The gut microbiome profile in* obesity: a systematic review. *Int J Endocrinol. (2*018) 2018:4095789. doi: 10.1155/2018/4095789
43. Riva A, Borgo F, Lassandro C, Verduci E, Morace G, Borghi E, et al. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in firmicutes populations. *Enviroin Microbiol.* (2017) 19:95–105. doi: 10.1111/1462-2920.13463
44. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TMS, Comelli EM. *Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. Nutr Diabetes.* (2014) 4:e121. doi: 10.1038/nutd.2014.23
45. Rahat-Rozenbloom S, Fernandes J, Gloor GB, Wolever TMS. *Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. Int J Obes.* (2014) 38:1525–31. doi: 10.1038/ijo.2014.46
46. Barczyńska R, Litwin M, Slizewska K, Szalecki M, Berdowska A, Bandurska K, et al. Bacterial microbiota fatty acids in the faeces of overweight obese children. *Pol. J. Microbiol.* (2018) 67:339–45. doi: 10.21307/pjm-2018-041
47. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol.* (2020) 72:558–77. doi: 10.1016/j.jhep.2019.10.003
48. Yu EL, Golshan S, Harlow KE, Angeles JE, Durelle J, Goyal NP, et al. Prevalence of nonalcoholic fatty liver disease in children with obesity. *J Pediatr. (*2019*)* 207:64–70. doi: 10.1016/j.jpeds.2018.11.021
49. Ranucci G, Spagnuolo MI, Iorio R. Obese children with fatty liver: Between reality and disease mongering. *World J Gastroenterol.* (2017) 23:8277–82. doi: 10.3748/wjg.v23.i47.8277
50. Cox AJ, West NP, Cripps. A. W. Obesity, inflammation, and the gut microbiota. *Lancet Diabet Endocrinol.* (2015) 3:207–15. doi: 10.1016/S2213-8587(14)70134-2
51. Seitz J, Trinh S, Herpertz-Dahlmann B. The microbiome and eating disorders. *Psychiatr Clin North Am.* . (2019) 42:93–103. doi: 10.1016/j.psc.2018.10.004
52. Deans E. Microbiome and mental health in the modern environment. *J Physiol Anthropol.* (2016) 36:1. doi: 10.1186/s40101-016-0101-y
53. Peirce JM, Alviña K. *The role of inflammation and the gut microbiome in depression and anxiety. J Neurosci Res.* (2019) 97:1223–41. doi: 10.1002/jnr.24476
54. Ranadive SA, Vaisse C. Lessons from extreme human obesity: monogenic disorders. *Endocrinol Metab Clin North Am.* (2008) 37:733–51. doi: 10.1016/j.ecl.2008.07.003
55. Soliman AT, Yasin M, Kassem A. Leptin in pediatrics: a hormone from adipocyte that wheels several functions in children. *Indian J Endocrinol Metab*. (2012) 16(Suppl. 3):*S*577–87. doi: 10.4103/2230-8210.105575
56. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM. et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med.* (2007) 356:237–47. doi: 10.1056/NEJMoa063988
57. Mutch DM, Clément K. Unraveling the genetics of human obesity. *PLoS Genet.* (2006) 2:e188. doi: 10.1371/journal.pgen.0020188
58. Crocker MK, Yanovski JA. Pediatric obesity: etiology and treatment. *Endocrinol Metab Clin North Am.* (2009) 38:525–48. doi: 10.1016/j.ecl.2009.06.007
59. Huvenne H, Dubern B, Clément K, Poitou C. Rare genetic forms of obesity: clinical approach and current treatments in 2016. *Obes Facts.* (2016) 9:158–73. doi: 10.1159/000445061
60. Stefan M, Nicholls RD. What have rare genetic syndromes taught us about the pathophysiology of the common forms of obesity? *Curr Diab Rep.* (2004) 4:143–50. doi: 10.1007/s11892-004-0070-0
61. Hetherington MM, Cecil JE. Gene-Environment interactions in obesity. *Forum Nutr.* (2009) 63:195–203. doi: 10.1159/000264407
62. Reddon H, Guéant JL, Meyre D. The importance of gene-environment interactions in human obesity. *Clin Sci.* (2016) 130:1571–97. doi: 10.1042/CS20160221
63. Castillo JJ, Orlando RA, Garver WS. Gene-nutrient interactions and susceptibility to human obesity. *Genes Nutr.* (2017) 12:29. doi: 10.1186/s12263-017-0581-3
64. Heianza Y, Qi L. Gene-Diet interaction and precision nutrition in obesity. *Int J Mol Sci.* (2017) 18:787. doi: 10.3390/ijms18040787
65. Goodarzi MO. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol.* (2018) 6:223–36. . doi: 10.1016/S2213-8587(17)30200-0
66. Bouchard L, Drapeau V, Provencher V, Lemieux S, Chagnon Y, Rice T, et al. Neuromedin beta: a strong candidate gene linking eating behaviors and susceptibility to obesity. *Am J Clin Nutr.* (2004) 80:1478–86. . doi: 10.1093/ajcn/80.6.1478
67. Grimm ER, Steinle NI. Genetics of eating behavior: established and emerging concepts. *Nutr Rev.* (2011) 69:52–60. . doi: 10.1111/j.1753-4887.2010.00361.x
68. van der Klaauw AA, Farooqi IS. The hunger genes: pathways to obesity. *Cell.* (2015) 161:119–32. . doi: 10.1016/j.cell.2015.03.008
69. Martinez JA. Bodyweight regulation causes of obesity. *Proc Nutr Soc.* (2000) 59:337–45. Review. doi: 10.1017/S0029665100000380
70. Rask-Andersen M, Karlsson T, Ek WE, Johansson Å. Gene-environment interaction study for BMI reveals interactions between genetic factors and physical activity, alcohol consumption and socioeconomic status. *PLoS Genet.* (2017) 5:1. doi: 10.1371/journal.pgen.1006977
71. Xulong S, Pengzhou, L. , Xiangwu Y, Weizheng L, Xianjie, Q. , Shaihong Z, et al. From genetics and epigenetics to the future of precision treatment for obesity. *Gastroenterol Rep.* (2017) 5:266–70. doi: 10.1093/gastro/gox033
72. Bianco-Miotto T, Craig JM, Gasser YP, van dijk SJ, Ozanne SE. Epigenetics and DOHaD: from basics to birth and beyond. *J Dev Orig Health Dis.* (2017) 8:513–9. doi: 10.1017/S2040174417000733
73. van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS, Members of EpiSCOPE. Epigenetics and human obesity. *Int J Obes.* (2015) 39:85–97. doi: 10.1038/ijo.2014.34

74. Li Y. Epigenetic mechanisms link maternal diets and gut microbiome to obesity in the offspring. *Front Genet*. (2018) 9:342. doi: 10.3389/fgene.2018.00342
75. Kaufman J, Montalvo-Ortiz JL, Holbrook H, O'Loughlin K, Orr C, Kearney C, et al. Adverse childhood experiences, epigenetic measures, and obesity in youth. *J Pediatr.* (2018) 202:150–6.76. doi: 10.1016/j.jpeds.2018.06.051
76. May Gardner R, Feely A, Layte R, Williams J, McGavock J. Adverse childhood experiences are associated with an increased risk of obesity in early adolescence: a population-based prospective cohort study. *Pediatr Res.* (2019) 86:522–28. doi: 10.1038/s41390-019-0414-8
77. Cheon BK,, Hong YY. *Mere experience of low subjective socioeconomic status stimulates appetite food intake. Proc Natl Acad Sci USA*. (2017) 114:72–7. doi: 10.1073/pnas.1607330114
78. Alegría-Torres JA, Baccarelli A, Bollati V. Epigenetics lifestyle. *Epigenomics*. (2011) 3:267-77. doi: 10.2217/epi.11.22
79. Birch LL, Fisher JO. Development of eating behaviors among children and adolescents. *Pediatrics*. (2011) 101:539–49.
80. Birch L, Savage JS, Ventura A. Influences on the development of children's eating behaviours: from infancy to adolescence. *Can J Diet Pract Res.* (2007) 68:s1–s56.
81. Nielsen SJ, Popkin BM. Patterns and trends in food portion sizes, 1977- 1998. *JAMA*. (2003) 289:450–53. . doi: 10.1001/jama.289.4.450
82. Munoz KA, Krebs-Smith SM, Ballard-Barbash R, Cleveland LE. Food intakes of US children and adolescents compared with recommendations. *Pediatrics.* (1997) 100:323–29. doi: 10.1542/peds.100.3.323
83. Fisher JO, Birch LL. Restricting access to palatable foods affects children's behavioral response, food selection, and intake. *Am J Clin Nutr.* (1999) 69:1264–72. doi: 10.1093/ajcn/69.6.1264
84. Faith MS, Scanlon KS, Birch LL, Francis LA, Sherry B. Parent-child feeding strategies and their relationships to child eating and weight status. *Obes Res.* (2004) 12:1711–22. . doi: 10.1038/oby.2004.212
85. Smith AD, Sanchez N, Reynolds C, Casamassima M, Verros M, Annameier SK, et al. Associations of parental feeding practices and food reward responsiveness with adolescent stress-eating. *Appetite.* (2020) 152:104715. doi: 10.1016/j.appet.2020.104715
86. Lowe CJ, Morton JB, Reichelt AC. Adolescent obesity and dietary decision making-a brain-health perspective. *Lancet Child Adolesc Health.* (2020) 4:388–96. doi: 10.1016/S2352-4642(19)30404-3
87. Goran MI, Treuth MS. Energy expenditure, physical activity, and obesity in children. *Pediatr Clin North Am.* (2001) 48:931–53. doi: 10.1016/S0031-3955(05)70349-7
88. Romieu I, Dossus L, Barquera S, Blottière HM, Franks PW, Gunter M, et al. Energy balance and obesity: what are the main drivers? *Cancer Causes Control.* (2017) 28:247–58. doi: 10.1007/s10552-017-0869-z
89. Mattes R, Foster GD. Food environment and obesity. *Obesity.* (2014) 22:2459–61. doi: 10.1002/oby.20922
90. Ickovics JR, O'Connor Duffany K, Shebl FM, Peters SM, Read MS, Gilstad-Hayden KR, et al. Implementing school-based policies to prevent obesity: cluster randomized trial. *Am J Prev Med.* (2019) 56:e1–11. doi: 10.1016/j.amepre.2018.08.026
91. Micha R, Karageorgou D, Bakogianni I, Trichia E, Whitsel LP, Story M, et al. Effectiveness of school food environment policies on children's dietary behaviors: A systematic review and meta-analysis. *PLoS ONE. (*2018*)* 13:e0194555. doi: 10.1371/journal.pone.0194555
92. Cawley J, Frisvold D, Hill A, Jones DJ. The impact of the philadelphia beverage tax on purchases and consumption by adults and children. *Health Econ.* (2019) 67:102225. doi: 10.1016/j.jhealeco.2019.102225
93. John Cawley J, Thow AM, Wen K, Frisvold D. The economics of taxes on sugar-sweetened beverages: a review of the effects on prices, sales, cross-border shopping, and consumption. *Annu Rev Nutr.* (2019) 39:317–38. doi: 10.1146/annurev-nutr-082018-124603
94. Fuller C, Lehman E, Hicks S, Novick MB. Bedtime use of technology and associated sleep problems in children. *Glob Pediatr Health.* (2017) 4:2333794X17736972. doi: 10.1177/2333794X17736972
95. Chahal H, Fung C, Kuhle S, Veugelers PJ. Availability and night-time use of electronic entertainment and communication devices are associated with short sleep duration and obesity among Canadian children. *Pediatr Obes.* (2012) 8:42–51. doi: 10.1111/j.2047-6310.2012.00085.x
96. Minghua T. Protein intake during the first two years of life and its association with growth and risk of overweight. *Int J Environ Res Public Health. (*2018*)* 15:1742. doi: 10.3390/ijerph15081742
97. Azad MB, Vehling L, Chan D, Klopp A, Nickel NC, McGavock JM, et al. Infant feeding and weight gain: separating breast milk from breastfeeding and formula from food. *Pediatrics.* (2018) 142:e20181092. doi: 10.1542/peds.2018-1092
98. Lin L, Amissah E, Gamble GD, Crowther CA, Harding, JE. Impact of macronutrient supplements on later growth of children born preterm or small for gestational age: a systematic review and meta-analysis of randomised and quasirandomised controlled trials. *PLoS Med.* (2020) 17:e1003122. . doi: 10.1371/journal.pmed.1003122
99. Rzehak P, Oddy WH, Mearin ML, Grote V, Mori TA, Szajewska H, et al. Infant feeding and growth trajectory patterns in childhood and body composition in young adulthood. *Am J Clin Nutr. (*2017*)* 106:568–80. doi: 10.3945/ajcn.116.140962
100. Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric obesity-assessment, treatment, and prevention: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* (2017) 102:709–57. doi: 10.1210/jc.2016-2573
101. Whitaker RC, Pepe MS, Wright JA, Seidel KD, Dietz WH. Early adiposity rebound and the risk of adult obesity. *Pediatrics*. (1998) 101:E5. doi: 10.1542/peds.101.3.e5
102. Geserick M, Vogel M, Gausche R, Lipek T, Spielau U, Keller E, et al. Acceleration of BMI in early childhood and risk of sustained obesity. *N Engl J Med.* (2018) 379:1303–12. doi: 10.1056/NEJMoa1803527
103. Jabakhanji SB, Boland F, Ward M, Biesma RJ. Body mass index changes in early childhood. *Pediatrics.* (2018) 202:106–14. doi: 10.1016/j.jpeds.2018.06.049
104. Chung S. Growth and puberty in obese children and implications of body composition. *J Obes Metab Syndr.* (2017) 26:243–50. doi: 10.7570/jomes.2017.26.4.243
105. Tagi VM, Giannini C, Chiarelli F. Insulin resistance in children. *Front Endocrinol.* (2019) 10:342. doi: 10.3389/fendo.2019.00342
106. Kelesidis I, Mantzoros CS. Leptin and its emerging role in children and adolescents. *Clin Pediatr Endocrinol*. (2006) 15:1–14. doi: 10.1297/cpe.15.1
107. Burt Solorzano CM, McCartney CR, Obesity and the pubertal transition in girls and boys. *Reproduction*. (2010) 140:399–410. doi: 10.1530/REP-10-0119
108. Li W, Liu Q, Deng X, Chen Y, Liu S, Story M. Association between obesity and puberty timing: a systematic review and meta-analysis. *Int J Environ Res Public Health.* (2017) 14:1266. doi: 10.3390/ijerph14101266
109. Lee JM, Wasserman R, Kaciroti N, Gebremariam A, Steffes J, Dowshen S. et al. Timing of puberty in overweight vs. obese boys. *Pediatrics.* (2016) 137:e20150164. doi: 10.1542/peds.2015-0164
110. He J, Kang Y, Zheng L. *Serum levels of LH, IGF-1 and leptin in girls with idiopathic central precocious puberty (ICPP)* and the correlations with the development of ICPP. *Minerva Pediatr*. (2018). doi: 10.23736/S0026-4946.18.05069-7
111. Kang MJ, Oh YJ, Shim YS, Baek JW, Yang S, Hwang IT. The usefulness of circulating levels of leptin, kisspeptin, and neurokinin B in obese girls with precocious puberty. *Gynecol Endocrinol.* (2018) 34:627–30. doi: 10.1080/09513590.2017.1423467
112. Rendo-Urteaga T, Ferreira de Moraes AC, Torres-Leal FL, Manios Y, Gottand F, Sjöström M, et al. Leptin and adiposity as mediators on the association between early puberty and several biomarkers in European adolescents: the helena study. *J Pediatr Endocrinol Metab.* (2018) 31:1221–29. doi: 10.1515/jpem-2018-0120
113. Franks S. Adult polycystic ovary syndrome begins in childhood. *Best Pract Res Clin Endocrinol Metab.* (2002) 16:263–72. doi: 10.1053/beem.2002.0203
114. Franks S. Polycystic ovary syndrome in adolescents. *Int J Obes.* (2008) 32:1035–41. doi: 10.1038/ijo.2008.61
115. Jehan S, Zizi F, Pandi-Perumal SR, Wall S, Auguste E, Myers K, et al. Obstructive sleep apnea and obesity: implications for public health. *Sleep Med Disord.* (2017) 1:00019.
116. Patinkin ZW, Feinn R, Santos M. Metabolic consequences of obstructive sleep apnea in adolescents with obesity: a systematic literature review and meta-analysis. *Childhood Obes.* (2017) 13:102–10. doi: 10.1089/chi.2016.0248

117. Kaditis A. From obstructive *sleep* apnea in childhood to cardiovascular disease in adulthood: what is the evidence? *Sleep*. (2010) 33:1279–80. doi: 10.1093/sleep/33.10.1279
118. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rose G, et al. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci*. (2014) 16:378–400. doi: 10.3390/ijms16010378
119. Eisele HJ, Markart P, Schulz R. *Obstructive sleep apnea, oxidative stress, and cardiovascular* disease: evidence from human studies. *Oxid Med Cell Longev*. (2015) 2015:608438. doi: 10.1155/2015/608438
120. Hui W, Slorach C, Guerra V, Parekh RS, Hamilton J, Messiha S, et al. Effect of obstructive sleep apnea on cardiovascular function in obese youth. *Am J Cardiol*. (2019) 123:341–7. doi: 10.1016/j.amjcard.2018.09.038
121. Matteoni CA, Younossi, Z.m., Gramlich T, Boparai N, Liu YC, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology*. (1999) 1999:116:1413. doi: 10.1016/S0016-5085(99)70506-8
122. Lavine JE, Schwimmer JB. Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis. (*2004*)* 8:549. doi: 10.1016/j.cld.2004.04.010
123. Huang JS, Barlow SE, Quiros-Tejeira RE, Scheimann A, Skelton J, Suskind D, et al. Childhood obesity for pediatric gastroenterologists. *J Pediatr Gastroenterol Nutr*. (2013) 2013:56:99. doi: 10.1097/MPG.0b013e31826d3c62
124. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLoS ONE. (*2015*)* 10:e0140908. doi: 10.1371/journal.pone.0140908
125. Nobili V, Alisi A, Newton KP, Schwimmer JB. Comparison of the phenotype and approach to pediatric vs adult patients with nonalcoholic fatty liver disease. *Gastroenterology. (*2016*)* 150:1798–810. doi: 10.1053/j.gastro.2016.03.009
126. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol. (*2009*)* 7:234–38. doi: 10.1016/j.cgh.2008.11.005
127. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angula P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut. (*2009*)* 58:1538. doi: 10.1136/gut.2008.171280
128. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook, S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. Circulation. (2008) 118:277. doi: 10.1161/CIRCULATIONAHA.107.739920
129. Perry DC, Metcalfe D, Lane S, Turner S. Childhood obesity and slipped capital femoral epiphysis. *Pediatrics*. (2018) 142:e20181067. doi: 10.1542/peds.2018-1067
130. Zavala-Crichton JP, Esteban-Cornejo I, Solis-Urra P, Mora-Gonzalez J, Cadenas-Sanchez C, Rodriguez-Ayllon M, et al. Association of sedentary behavior with brain structure and intelligence in children with overweight or obesity: *Active Brains Project*. (2020) 9:1101. doi: 10.3390/jcm9041101
131. Ronan L, Alexander-Bloch A, Fletcher PC. Childhood obesity, cortical structure, and executive function in healthy children. *Cereb Cortex*. (2019) 30:2519–28. doi: 10.1093/cercor/bhz257
132. Baker ER. Body weight and the initiation of puberty. *Clin Obstetr Gynecol*. (1985) 28:573–9. doi: 10.1097/00003081-198528030-00013
133. Siervogel RM, Demerath EW, Schubert C, Remsberg KE, Chumlea WM, Sun S, et al. Puberty and body composition. *Horm Res*. (2003) 60:36–45. doi: 10.1159/000071224
134. Sadeeqa S, Mustafa T, Latif S. Polycystic ovarian syndrome- related depression in adolescent girls. *J Pharm Bioallied Sci*. (2018) 10:55–9. doi: 10.4103/JPBS.JPBS_1_18
135. Himelein MJ, Thatcher SS. Depression and body image among women with polycystic ovary syndrome. *J Health Psychol*. (2006) 11:613–25. doi: 10.1177/1359105306065021
136. Magge SN, Goodman E, Armstrong, SC. The metabolic syndrome in children and adolescents: shifting the focus to cardiometabolic risk factor clustering. *Pediatrics*. (2017) 140:e20171603. doi: 10.1542/peds.2017-1603
137. Mauras N, Delgiorno C, Kollman C, Bird K, Morgan M, Sweeten S, et al. Obesity without established comorbidities of the metabolic syndrome is associated with a proinflammatory and prothrombotic state, even before the onset of puberty in children. *J Clin Endocrinol Metab*. (2010) 95:1060–8. doi: 10.1210/jc.2009-1887
138. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*. (2004) 350:2362–74. doi: 10.1056/NEJMoa031049
139. Erdmann J, Kallabis B, Oppel U, Sypchenko O, Wagenpfeil S, Schusdziarra V. Development of hyperinsulinemia and insulin resistance during the early stage of weight gain. *Am J Physiol Endocrinol Metabol*. (2008) 294:e568–75. . doi: 10.1152/ajpendo.00560.2007
140. Pulido-Arjona L, Correa-Bautista JE, Agostinis-Sobrinho C, Mota J, Santos R, Correa-Rodrigues M, et al. Role of sleep duration and sleep- related problems in the metabolic syndrome among children and adolescents. *Ital J Pediatr*. (2018) 44:9. doi: 10.1186/s13052-018-0451-7
141. Harriger JA, Thompson JK. Psychological consequences of obesity: weight bias and body image in overweight and obese youth. *Int Rev Psychiatry*. (2012) 24:247–53. . doi: 10.3109/09540261.2012.678817
142. Bacchini D, Licenziati MR, Garrasi A, Corciulo N, Driul D, Tanas R, et al. Bullying and victimization in overweight and obese outpatient children and adolescents: an italian multicentric study. *PLoS ONE*. (2015) 10:e0142715. doi: 10.1371/journal.pone.0142715
143. Loth KA, Watts AW, Berg PVD, Neumark-Sztainer D. Does body satisfaction help or harm overweight teens? A 10-year longitudinal study of the relationship between body satisfaction and body mass index. *J Adolesc Health*. (2015) 57:559–61. doi: 10.1016/j.jadohealth.2015.07.008
144. Gowey MA, Lim CS, Clifford LM, Janicke DM. Disordered eating and health-related quality of life in overweight and obese children. *J Pediatr Psychol*. (2014) 39:552–61. doi: 10.1093/jpepsy/jsu012
145. Mannan M, Mamun A, Doi S, Clavarino A. Prospective associations between depression and obesity for adolescent males and females- a systematic review and meta-analysis of longitudinal studies. *PLoS ONE*. (2016) 11:e0157240. doi: 10.1371/journal.pone.0157240
146. Ruiz LD, Zuelch ML, Dimitratos SM, Scherr RE. Adolescent obesity: diet quality, psychosocial health, and cardiometabolic risk factors. *Nutrients*. (2019) 12:43. doi: 10.3390/nu12010043
147. Goldschmidt AB, Aspen VP, Sinton MM, Tanofsky-Kraff M, Wilfley DE. Disordered eating attitudes and behaviors in overweight youth. *Obesity*. (2008) 16:257–64. doi: 10.1038/oby.2007.48
148. Golden NH, Schneider M, Wood C. Preventing obesity and eating disorders in adolescents. *Pediatrics*. (2016) 138:e1–e12. doi: 10.1542/peds.2016-1649
149. Rastogi R, Rome. ES. Restrictive eating disorders in previously overweight adolescents and young adults. *Cleve Clin J Med*. (2020) 87:165–71. doi: 10.3949/ccjm.87a.19034
150. Hayes JF, Fitzsimmons-Craft EE, Karam AM, Jakubiak JL, Brown ME, Wilfley D. Disordered eating attitudes and behaviors in youth with overweight and obesity: implications for treatment. *Curr Obes Rep*. (2018) 7:235. doi: 10.1007/s13679-018-0316-9
151. Goldschmidt AB, Wall MM, Loth KA, Neumark-Sztainer D. Risk factors for disordered eating in overweight adolescents and young adults: Table I. *J Pediatr Psychol*. (2015) 40:1048–55. doi: 10.1093/jpepsy/jsv053
152. Follansbee-Junger K, Janicke DM, Sallinen BJ. The influence of a behavioral weight management program on disordered eating attitudes and behaviors in children with overweight. *J Am Diet Assoc*. (2010) 110:653–9. doi: 10.1016/j.jada.2010.08.005
153. Blake-Lamb TL, Locks LM, Perkins ME, Woo Baidal JA, Cheng ER, Taveras EM. Interventions for childhood obesity in the first 1,000 days a systematic review. *Am J Prev Med*. (2016) 50:780–9. doi: 10.1016/j.amepre.2015.11.010
154. McGuire S. Institute of Medicine (IOM). Early childhood obesity prevention policies. Washington, DC: The National Academies Press. *Adv Nutr*. (2011) 3:56–7. doi: 10.3945/an.111.001347
155. Pont SJ, Puhl R, Cook SR, Slusser W. Stigma experienced by children and adolescents with obesity. *Pediatrics*. (2017) 140:e20173034. doi: 10.1542/peds.2017-3034
156. Puhl R, Suh Y. Health consequences of weight stigma: implications for obesity prevention and treatment. *Curr Obes Rep*. (2015) 4:182–90. doi: 10.1007/s13679-015-0153-z
157. Schwimmer JB, Burwinkle TM, Varni JW. Health-related quality of life of severely obese children and adolescents. *JAMA*. (2003) 289:1813–9. doi: 10.1001/jama.289.14.1813

158. Carcone AI, Jacques-Tiura AJ, Brogan Hartlieb KE, Albrecht T, Martin T. Effective patient-provider communication in pediatric obesity. *Pediatr Clin North Am.* (2016) 63:525–38. doi: 10.1016/j.pcl.2016.02.002
159. Coppock JH, Ridolfi DR, Hayes JF, Paul MS, Wilfley DE. Current approaches to the management of pediatric overweight and obesity. *Curr Treat Options Cardiovasc Med.* (2014) 16:343. doi: 10.1007/s11936-014-0343-0
160. Davison KK, Jurkowski JM, Li K, Kranz S, Lawson HA. A childhood obesity intervention developed by families for families: results from a pilot study. *Int J Behav Nutr Phys Act.* (2013) 10:3. doi: 10.1186/1479-5868-10-3
161. Krystia O, Ambrose T, Darlington G, Ma DWL, Buchholz AC, Haines J. A randomized home- based childhood obesity prevention pilot intervention has favourable effects on parental body composition: preliminary evidence from the guelph family health study. *BMC Obes.* (2019) 6:10. doi: 10.1186/s40608-019-0231-y
162. Skjåkødegård HF, Danielsen YS, Morken M, Linde SRF, Kolko RP, Balantekin KN, et al. Study protocol: a randomized controlled trial evaluating the effect of family-based behavioral treatment of childhood and adolescent obesity–The FABO-study. *BMC Public Health.* (2016) 16:1106. doi: 10.1186/s12889-016-3755-9
163. Hall KD, Kahan S. Maintenance of lost weight and long-term management of obesity. *Med Clin North Am.* (2018) 102:183–97. doi: 10.1016/j.mcna.2017.08.012
164. Hall KD. Diet vs. exercise in "the biggest loser" weight loss competition. *Obesity.* (2013) 21:957–9. doi: 10.1002/oby.20065
165. Lecoultre V, Ravussin E, Redman LM. The fall in leptin concentration is a major determinant of the metabolic adaptation induced by caloric restriction independently of the changes in leptin circadian rhythms. *J Clin Endocrinol Metabol.* (2011) 96:E1512–E516. doi: 10.1210/jc.2011-1286
166. Kaur KK, Allahbadia G, Singh M. Childhood obesity: a comprehensive review of epidemiology, aetiopathogenesis and management of this global threat of the 21st century. *Acta Sci Paediatr.* (2019) 2:56–66. doi: 10.31080/ASPE.2019.02.0132
167. Crimmins NA, Xanthakos SA. Obesity. In *Neinstein's Adolescent and Young Adult Health*, Guide. Philadelphia, PA: Wolters Kluwer (2016). p. 295–300.
168. Astrup A, Rossner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, et al. Effects of liraglutide in the treatment of obesity: a randomized, double-blind, placebo-controlled study. *Lancet.* (2009) 374:1606–16. doi: 10.1016/S0140-6736(09)61375-1
169. Monami M, Dicembrini I, Marchionni N, Rotella CM, Mannucci E. Effects of glucagon-like peptide-1 receptor agonists on body weight: a meta-analysis. *Exp Diabetes Res.* (2012) 2012:672658. doi: 10.1155/2012/672658
170. Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf., et al. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N Engl J Med.* (2015) 373:11–22 . doi: 10.1056/NEJMoa1411892
171. Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, et al. A randomized, controlled trial of liraglutide for adolescents with obesity. *N Engl J Med.* (2020) 382:2117–28. doi: 10.1056/NEJMoa1916038
172. Foster BA, Escaname E, Powell T, Larsen B, Siddiqui SK, Menchaca J, et al. Randomized controlled trial of DHA supplementation during pregnancy: child adiposity outcomes. *Nutrients. (*2017*)* 9:566. doi: 10.3390/nu9060566
173. Abenavoli L, Scarpellini E, Colica C, Boccuto L, Salehi B, Sharifi-Rad J, et al. Gut microbiota and obesity: a role for probiotics. *Nutrients.* (2019) 11:2690. doi: 10.3390/nu11112690
174. Vajro P, Mandato C, Veropalumbo C, De Micco I. Probiotics: a possible role in treatment of adult and pediatric nonalcoholic fatty liver disease. *Ann Hepatol. (*2013*)* 12:161–63. doi: 10.1016/S1665-2681(19)31401-2
175. Zhao L, Fang X, Marshall M, Chung S. Regulation of obesity and metabolic complications by gamma and delta tocotrienols. *Molecules.* (2016) 21:344. doi: 10.3390/molecules21030344
176. Wong SK, Chin K-Y, Suhaimi FH, Ahmad F, Ima-Nirwana S. Vitamin E as a potential interventional treatment for metabolic syndrome: evidence from animal and human studies. *Front Pharmacol.* (2017) 8:444. doi: 10.3389/fphar.2017.00444
177. Galli F, Azzi A, Birringer A, Cook-Mills JM, Eggersdorfer M, Frank J, et al. Vitamin E: Emerging aspects and new directions. *Free Radic Biol Med.* (2017) 102:16–36. doi: 10.1016/j.freeradbiomed.2016.09.017
178. Galmés S, Serra,F., Palou A. Vitamin E metabolic effects and genetic variants: a challenge for precision nutrition in obesity and associated disturbances. *Nutrients. (*2018*)* 10:1919. doi: 10.3390/nu10121919
179. Ahn SM. Current issues in bariatric surgery for adolescents with severe obesity: durability, complications, and timing of intervention. *J. Obes Metabol Syndrome.* (2020) 29:4–11. doi: 10.7570/jomes19073
180. Lamoshi A, Chernoguz A, Harmon CM, Helmrath M. Complications of bariatric surgery in adolescents. *Semin Pediatr Surg.* (2020) 29:150888. doi: 10.1016/j.sempedsurg.2020.150888
181. Weiss AL, Mooney A, Gonzalvo JP. Bariatric surgery. *Adv Pediatr.* (2017) 6:269–83. doi: 10.1016/j.yapd.2017.03.005
182. Stanford FC, Mushannen T, Cortez P, Reyes KJC, Lee H, Gee DW, et al. Comparison of short and long-term outcomes of metabolic and bariatric surgery in adolescents and adults. *Front Endocrinol.* (2020) 11:157. doi: 10.3389/fendo.2020.00157
183. Inge TH, Zeller MH, Jenkins TM, Helmrath M, Brandt ML, Michalsky MP, et al. Perioperative outcomes of adolescents undergoing bariatric surgery: the teen-longitudinal assessment of bariatric surgery (Teen-LABS) *study. JAMA Pediatr.* (2014) 168:47–53. doi: 10.1001/jamapediatrics.2013.4296
184. Järvholm K, Bruze G, Peltonen M, Marcus C, Flodmark CE, Henfridsson P, et al. 5-year mental health and eating pattern outcomes following bariatric surgery in adolescents: a prospective cohort study. *Lancet Child AdolescHealth*. (2020) 4:210–9. doi: 10.1016/S2352-4642(20)30024-9
185. Xanthakos SA. Bariatric surgery for extreme adolescent obesity: indications, outcomes, and physiologic effects on the gut–brain axis. *Pathophysiology.* (2008) 15:135–46. doi: 10.1016/j.pathophys.2008.04.005
186. Zitsman JL, Digiorgi MF, Kopchinski JS, Sysko R, Lynch L, Devlin M, et al. Adolescent Gastric Banding: a five-year longitudinal study in 137 individuals. *Surg Obes Relat Dis.* (2018) 14. doi: 10.1016/j.soard.2018.09.030
187. Inge TH, Jenkins TM, Xanthakos SA, Dixon JB, Daniels SR, Zeller MH, et al. Long-term outcomes of bariatric surgery in adolescents with severe obesity (FABS-5+). *A prospective follow-up analysis. Lancet Diabet Endocrinol.* (2017) 5:165–73. doi: 10.1016/S2213-8587(16)30315-1
188. Kindel TL, Krause C, Helm MC, Mcbride CL, Oleynikov D, Thakare R, et al. Increased glycine-amidated hyocholic acid correlates to improved early weight loss after sleeve gastrectomy. *Surg Endosc.* (2017) 32:805–12. doi: 10.1007/s00464-017-5747-y

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Alternative Polyadenylation and Differential Regulation of *Ucp1*: Implications for Brown Adipose Tissue Thermogenesis Across Species

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Brown adipose tissue (BAT) is a thermogenic organ owing to its unique expression of uncoupling protein 1 (UCP1), which is a proton channel in the inner mitochondrial membrane used to dissipate the proton gradient and uncouple the electron transport chain to generate heat instead of adenosine triphosphate. The discovery of metabolically active BAT in human adults, especially in lean people after cold exposure, has provoked the "thermogenic anti-obesity" idea to battle weight gain. Because BAT can expend energy through UCP1-mediated thermogenesis, the molecular mechanisms regulating UCP1 expression have been extensively investigated at both transcriptional and posttranscriptional levels. Of note, the 3′-untranslated region (3′-UTR) of *Ucp1* mRNA is differentially processed between mice and humans that quantitatively affects UCP1 synthesis and thermogenesis. Here, we summarize the regulatory mechanisms underlying UCP1 expression, report the number of poly(A) signals identified or predicted in *Ucp1* genes across species, and discuss the potential and caution in targeting UCP1 for enhancing thermogenesis and metabolic fitness.

Keywords: alternative polyadenylation, brown adipose tissue, thermogenesis, transcriptional control, translational control, uncoupling protein 1

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INTRODUCTION

Brown and white adipocytes possess unique functions in thermogenesis and energy storage, respectively; however, to some extent, one could cover the function of the other. A high fat diet (HFD) can induce brown-to-white adipocyte conversion when brown adipocytes store excess lipids to appear unilocular. Some white adipocytes are known as beige adipocytes, which can be browned in response to adrenergic signaling to become thermogenic (1). Cold-induced metabolic activity of brown adipose tissue (BAT) is positively correlated with resting metabolic rate and negatively with body mass index and body fat percentage in humans (2, 3). Therefore, a "thermogenic anti-obesity" approach has been proposed to combat obesity if the metabolic furnace, BAT, can be pharmacologically activated to burn excess fat (4). Because BAT defends against fat accumulation *via* uncoupling protein 1 (UCP1)-mediated thermogenesis, extensive efforts have uncovered how UCP1 activity is controlled by allosteric conformation changes and gene expression to affect thermogenesis. Several polymorphisms in the promoter, non-coding, and coding regions of *UCP1*

gene were found associated with obesity and type 2 diabetes (5–8). Of note, genomic variations in *Ucp1* across species indicate that non-shivering thermogenic function and regulation are not necessarily conserved through evolution.

Discovery of *Ucp1*, Whose 3′-UTR Is Processed Differently Among Species

UCP1 was first isolated from BAT of rats and hamsters in 1980 and named for its heat-producing function by uncoupling the oxidative phosphorylation process in mitochondria (**Figure 1A**) (9). At the basal status, the binding of purine nucleotide di- and tri-phosphates on the outer facing cavity of UCP1 blocks its proton-translocating activity (10, 11). In response to nutrients or cold environment, thermogenesis is activated *via* the release of norepinephrine from sympathetic circuits onto β3 adrenergic receptors (β3ARs) in brown adipocytes (4, 12, 13). β3AR signaling elevates cyclic adenosine monophosphate (cAMP) level to activate protein kinase A and lipolysis and acutely enhances UCP1 activity *via* free fatty acid-induced allosteric changes (14). Chronic activation of β3ARs promotes UCP1 synthesis by multiple transcriptional and posttranscriptional mechanisms to be discussed later.

Rat and mouse *Ucp1* cDNAs were cloned in 1985 (15, 16) and later sequenced to reveal their coding sequences and untranslated regions (UTRs) (17–19). Soon after, the rabbit *Ucp1* cDNA sequence was reported (20). The 3′-UTR of *Ucp1* in all three species contains two poly(A) signals (i.e., AAUAAA). Northern blot analysis revealed two *Ucp1* transcripts of ∼1.5 and 1.9 kb (hereafter called *Ucp1S* and *Ucp1L*) in mouse and rat BAT (15, 16). The transcripts result from an alternative use of the poly(A) signal to yield 3′-UTRs of 78 and 485 bp in mice, respectively (21) (**Figure 1B**). By contrast, only the distal poly(A) signal is used to generate *Ucp1L* in rabbit BAT (20). The human *UCP1* coding sequence was identified by screening a genomic library with a rat *Ucp1* cDNA probe, and a 1.9-kb *UCP1* mRNA was detected in human perirenal BAT (22). More recently, the sequence of human *UCP1* cDNA (ENST00000262999) revealed only one poly(A) site in the 3′-UTR, and RT-PCR analysis confirmed that human *UCP1* encodes only the long form carrying a 465-bp 3′-UTR (21).

Thermogenic UCP1 Is Not Preserved in All Endothermic Placental Mammals

The functional *Ucp1* gene consists of six exons and spans a region of ∼8–10 kb among species (an example of mouse *Ucp1* is in **Figure 1B**). The phylogenetic relationship of *Ucp1* across species has revealed that the non-thermogenic UCP1 in fish and frogs (23) was derived from a hypothetical proto-UCP hundreds of millions of years ago. Non-thermogenic UCP1 was first lost in Sauropsida (reptiles and birds) around 300 million years ago, then thermogenic UCP1 emerged in endothermic placental mammals about 100 million years ago (24, 25). Although UCP1-mediated non-shivering thermogenesis is believed to provide a survival advantage in the cold and retain the body temperature of eutherian neonates after birth, eight mammalian clades, namely Xenarthra, Pholidota, Cetacea, Sirenia, Proboscidea, Hyracoidea, Equidae, and the most well-known Suidae (pigs), showed inactivation of *Ucp1* (26). The loss of exons 3 to 5 in pig *Ucp1* occurred ∼20 million years ago, so pigs produce no functional UCP1 and depend on shivering for thermoregulation (27).

The previous studies aligned the genomic or amino acid sequences of *Ucp1* across species to build up the phylogenetic tree and derive an evolutionary timeline for *Ucp1* (24–26). We followed these evolutionary patterns by aligning the nucleotide sequences of the *Ucp1* coding regions in selected mammals and analyzed whether alternative polyadenylation could follow any evolutionary path (**Figure 1C**). The phylogenetic tree of *Ucp1* grouped most species following the canonical taxonomy, classifying animals of the same order in the same branch. The three exceptions are *Ucp1* in the small Madagascar hedgehog in *Afrosoricida* more related to rodents, in the gray mouse lemur in *Primates* more related to rodents and rabbits, and in the dog quite different from other animals in *Carnivora* (24).

The 3′-UTR information for *Ucp1* cDNAs in various species is often lacking or incomplete. We predicted "alternative polyadenylation" by searching the poly(A) signal (i.e., AATAAA) within 1 kb downstream of the stop codon from *Ucp1* genomic sequences because the 3′-UTR, including in mouse and human *Ucp1* mRNAs, is limited in the last coding exon of most transcripts. Our analyses revealed many representative animals carrying two poly(A) sites, such as mice, rats, and squirrels in *Rodentia*; rabbits in *Lagomorpha*; and cats, dogs, cows, leopards, and bears in *Laurasiatheria*. However, microbats and greater horseshoe bats in *Chiroptera* are the exceptions, with only one poly(A) motif in the 3′-flanking region. Notably, almost all primates, such as humans, chimpanzees, orangutans, and macaques, have only one poly(A) site in *Ucp1*, with the exception of mouse lemurs, which carry two poly(A) signals at the position similar to those of rodent *Ucp1*. The nucleotide sequence of the *Ucp1* coding region in mouse lemurs was unexpectedly more similar to those in rodents than primates (**Figure 1C**). Although the platypus, a unique placental mammal, has two adjacent poly(A) signals in *Ucp1*, alternative polyadenylation, even if it occurs, would generate two 3′-UTRs of only 32 bp difference. The poly(A) site of *Ucp1* in koala and gray short-tailed opossum in *Marsupialia* is located at 1,389 and 2,074 bp downstream of the stop codon, respectively, implying *Ucp1* in both species may be subjected to more complex posttranscriptional regulation. Other than the 3′-UTRs of rat *Ucp1S*, mouse *Ucp1S* and *Ucp1L*, and human *UCP1* having been confirmed by cDNA sequencing (17, 18, 21), the actual usage and preference of the two putative polyadenylation sites in other species require further investigation.

Transcriptional Regulation of *Ucp1* Gene

Cold exposure markedly increases both *Ucp1L* and *Ucp1S* mRNA levels in mouse and rat BAT (15, 16), so elaborative efforts have identified the molecular mechanisms controlling *Ucp1* transcription (28–30). DNA footprinting and promoter assays were performed to identify a 212-bp distal enhancer (−2,494 to −2,283 bp from the transcription start site) and a 555-bp proximal promoter (−611 to −57 bp) in the 5′ non-coding region

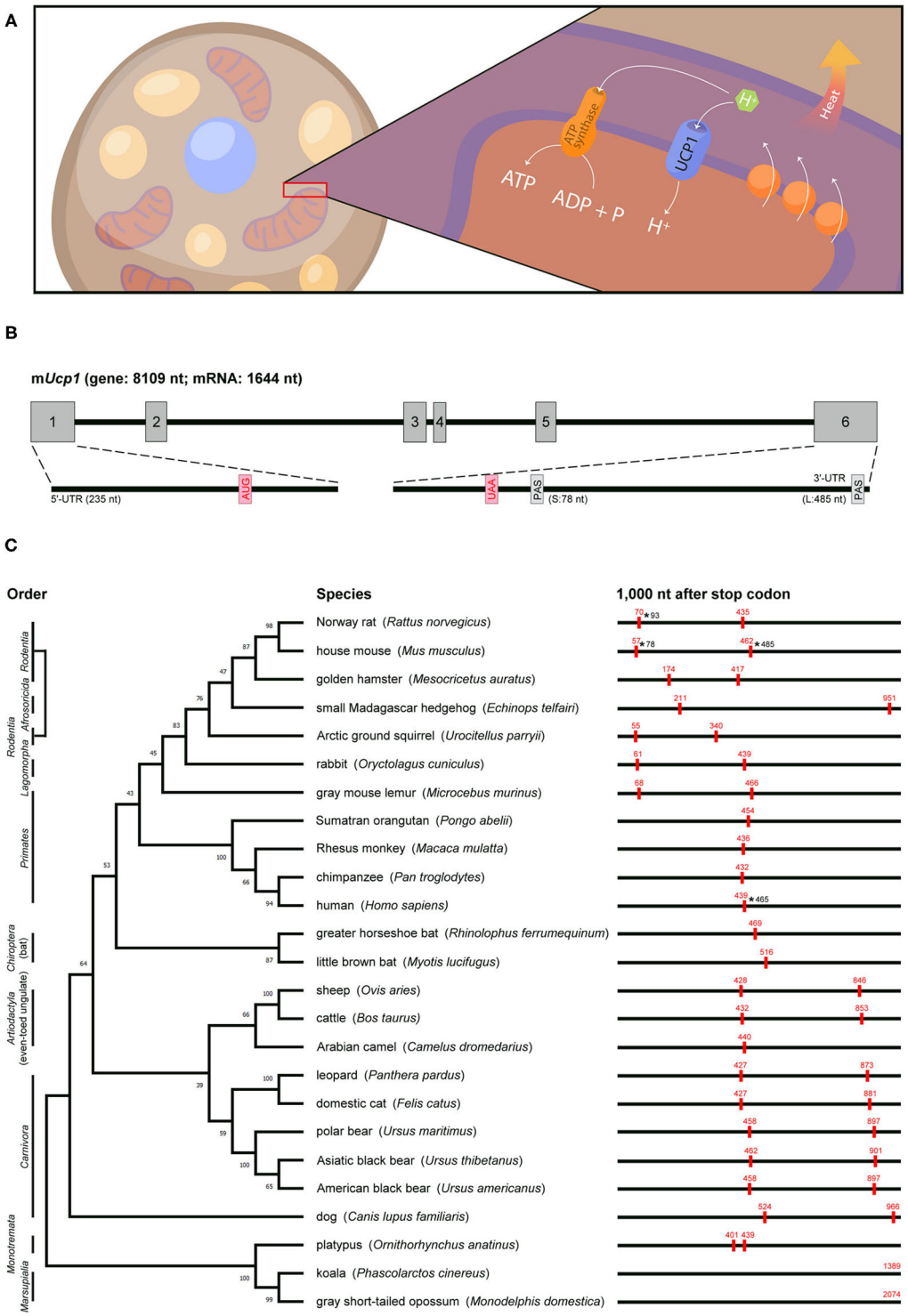

FIGURE 1 | Genomic information, actual or predicted polyadenylation sites in *Ucp1* across species. **(A)** UCP1, a proton channel located in the inner mitochondrial membrane of brown adipocytes, transports protons to the matrix and then disrupts the proton gradient generated by the electron transport chain, thus uncoupling fuel oxidation from ATP synthesis and releasing energy as heat. **(B)** Mouse *Ucp1* spans ~8 kb and contains six exons (gray boxes). The start (AUG) and stop (UAA) codons are labeled in red, and the two polyadenylation sites (PAS) are denoted. The 5′-UTR and 3′-UTR of *Ucp1* are located on exons 1 and 6, respectively. **(C)** Phylogenetic relationship of mammalian *Ucp1* by alignment of the nucleotide sequences of coding regions. Animal orders are marked in the left and the percentage of bootstrap support of minimum evolution is shown on the corresponding branch. The BLAST search of 1,000 nt after the *Ucp1* stop codon across species involved the Ensembl or NCBI database. The poly(A) signals (AAUAAA) are marked as red boxes with their positions in red. The ends of 3′-UTRs confirmed by cDNA sequencing in rat, mouse, and human are labeled with asterisks and numbers.

of rat *Ucp1* gene (28, 31). Many studies used *in vitro* assays to identify the *trans*-acting factors for *Ucp1* transcription, including protein kinase A-activated cAMP-response element binding protein (CREB) to compete with the negative regulator, Jun, for binding to the −139- to −122-bp region, and CCAAT/enhancer-binding protein α and β (C/EBPα and β) to activate through regions −457 to −440 and −335 to −318 bp [for details, see the review (32)]. Zfp516 binds to the promoter region (−70 to −45) with PR domain-containing 16 (PRDM16), which is essential for BAT differentiation and promotes browning of beige adipocytes to increase body temperature and energy expenditure (33) (**Figure 2A**). Notably, the absence of the CCAAT sequence in the human *UCP1* promoter suggests evolutionary variations in regulating *Ucp1* transcription (22).

When fused to a thymidine kinase promoter-driven reporter in a transgenic mouse line, the 212-bp enhancer sufficiently conferred BAT-specific expression and cold- and β3AR agonist-induced expression (34). Peroxisome proliferator-activated receptor α and γ (PPARα and PPARγ), PPARγ-coactivator 1α (PGC-1α), and CREB-binding protein (CBP) bind to the enhancer (−2,485 to −2,458) together to upregulate *Ucp1* transcription (35) (**Figure 2A**). Phosphorylation of PGC-1α and activating transcription factor 2 (ATF2) by cold exposure-activated p38 mitogen-activated protein kinase can directly or indirectly potentiate *Ucp1* transcription because *Ppargc1a* (i.e., the gene name of PGC-1α) transcription is also enhanced by phosphorylated ATF2 (35, 36). Moreover, PRDM16 recruited by PGC-1α interacts with thyroid receptor/retinoid X receptor heterodimers, and MED1, a component of the Mediator complex, is then recruited by PRDM16 to form a pre-initiation complex on the enhancer to facilitate *Ucp1* transcription (37, 38) (**Figure 2A**). Therefore, the enhancer region of *Ucp1* is regulated by signaling of multiple *trans*-acting factors through β3ARs.

In addition to positive regulators, receptor-interacting protein 140 (RIP140) mediates methylation at CpG sites in both the promoter and enhancer to silence *Ucp1* transcription (39) (**Figure 2A**). In pregnant mice with streptozotocin-induced diabetes, intrauterine exposure to hyperglycemia impaired fetal BAT development by altering methylation in several metabolic genes including *Ucp1*. Such epigenetic changes can last and affect BAT function and glucose homeostasis later in life (40). Besides CpG methylation, epigenetic modification of histone 3 (H3) affects chromatin structure to regulate transcription. Histone deacetylase 3 (HDAC3) controls the acetylation of H3 on lysine 27 (H3K27ac) in the enhancers of *Ucp1* and *Pparα* (**Figure 2B**), so its deficiency facilitates *Ucp1* and *Pparα* transcription and browning in beige adipocytes (41). Of note, pregnant mice fed omega-3 polyunsaturated fatty acids resulted in epigenetic changes, including decreased tri-methylation of H3 on lysine 27 (H3K27me3), increased H3K27ac and di-methylation of H3 on lysine 9 (H3K9me2) to affect BAT development, and increased *Ucp1* and *Ppargc1a* transcription in mouse offspring even at 8 weeks after post-weaning (42). H3K9me2 is usually defined as a repressed histone mark (43), so increased H3K9me2 may not directly associate with the *Ucp1* promoter. Nevertheless, this study reported that a maternal dietary effect could have long-lasting benefits on the development and function of the offspring's BAT. Moreover, the silencing mark, H3K9me2, was identified at the *Ucp1* enhancer in white adipose tissue (WAT), whereas the active mark, H3K4me3, at the *Ucp1* promoter was found in BAT in response to cold stimulation (43). The ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX) is a histone demethylase recruited to *Ucp1* upon the activation of β3ARs to reduce H3K27me3 level (**Figure 2B**), which consequently leads to increased H3K27ac level *via* unidentified histone acetyltransferases (44). Therefore, epigenetic changes on *Ucp1* are dynamically regulated for temporal and spatial control of UCP1 synthesis, and intrauterine nutrient exposure has a long-term effect on metabolic gene expression *via* epigenetic regulation.

Posttranscriptional Regulation of *Ucp1* mRNA

A pre-mRNA undergoes several processing steps, including splicing, 3′-end cleavage, and polyadenylation, to become a mature mRNA. Alternative polyadenylation generates *Ucp1* mRNA carrying long (*Ucp1L*, ∼10%) or short (*Ucp1S*, ∼90%) 3′-UTR in mice and rats, but rabbits and humans express only the long form. The regulatory sequences in the 3′-UTR dictate posttranscriptional efficiency for protein production (45), but the effect of different *Ucp1* 3′-UTRs on UCP1 synthesis and thermogenesis has not been addressed until recently. The study of *Ucp1*Δ*L* mice, with 10% *Ucp1L* converted to *Ucp1S*, reported that the protein but not the mRNA level of UCP1 was reduced ∼50–60% in *Ucp1*Δ*L* BAT, so *Ucp1L* is translated ∼15 times more efficiently than *Ucp1S* in BAT (21). Moreover, the same study also identified that cytoplasmic polyadenylation element binding protein 2 (CPEB2) signaling through β3ARs binds to and activates polyadenylation-induced translation of only *Ucp1L* but not *Ucp1S* (**Figure 2C**), so both CPEB2-knockout (KO) and *Ucp1*Δ*L* mice show reduced UCP1 level and impaired BAT thermogenesis (21).

The other posttranscriptional mechanisms identified to date inhibit UCP1 synthesis. Reporter assay revealed that insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2/IMP2) is a type 2 diabetes- and obesity-associated gene that suppresses *Ucp1* mRNA translation likely *via* both 5′- and 3′-UTRs. Thus, IMP2-null mice show increased energy expenditure, insulin sensitivity, and glucose tolerance and defend against cold temperature better than wild-type mice (46). HFD reduces the amount of UCP1 in obese adipose tissues by diminishing the stability of mouse *Ucp1* mRNA *via* the CCR4–NOT deadenylase complex. Moreover, HFD induces the expression of several subunits of the CCR4–NOT complex, including Cnot1–3, Cnot6 and Cnot7, and Cnot7-interacting Tob in inguinal WAT (iWAT) of obese mice. The binding of butyrate response factor 1 (BRF1) to the 3′-UTR of *Ucp1L* mRNA recruits Tob and Cnot7 to cause deadenylation-induced *Ucp1* decay (**Figure 2C**). Thus, Cnot7- or Tob-KO mice are resistant to HFD-induced obesity with increased *Ucp1* mRNA level in iWAT and BAT (47). Although IMP2 and BRF1 suppressed the translation and stability, respectively, of a reporter appended to the *Ucp1L* 3′-UTR in HEK293 cells, both studies did not examine *Ucp1L* and *Ucp1S* separately when comparing polysomal distribution (46) or mRNA stability of *Ucp1* (47) between wild-type and KO adipose tissues.

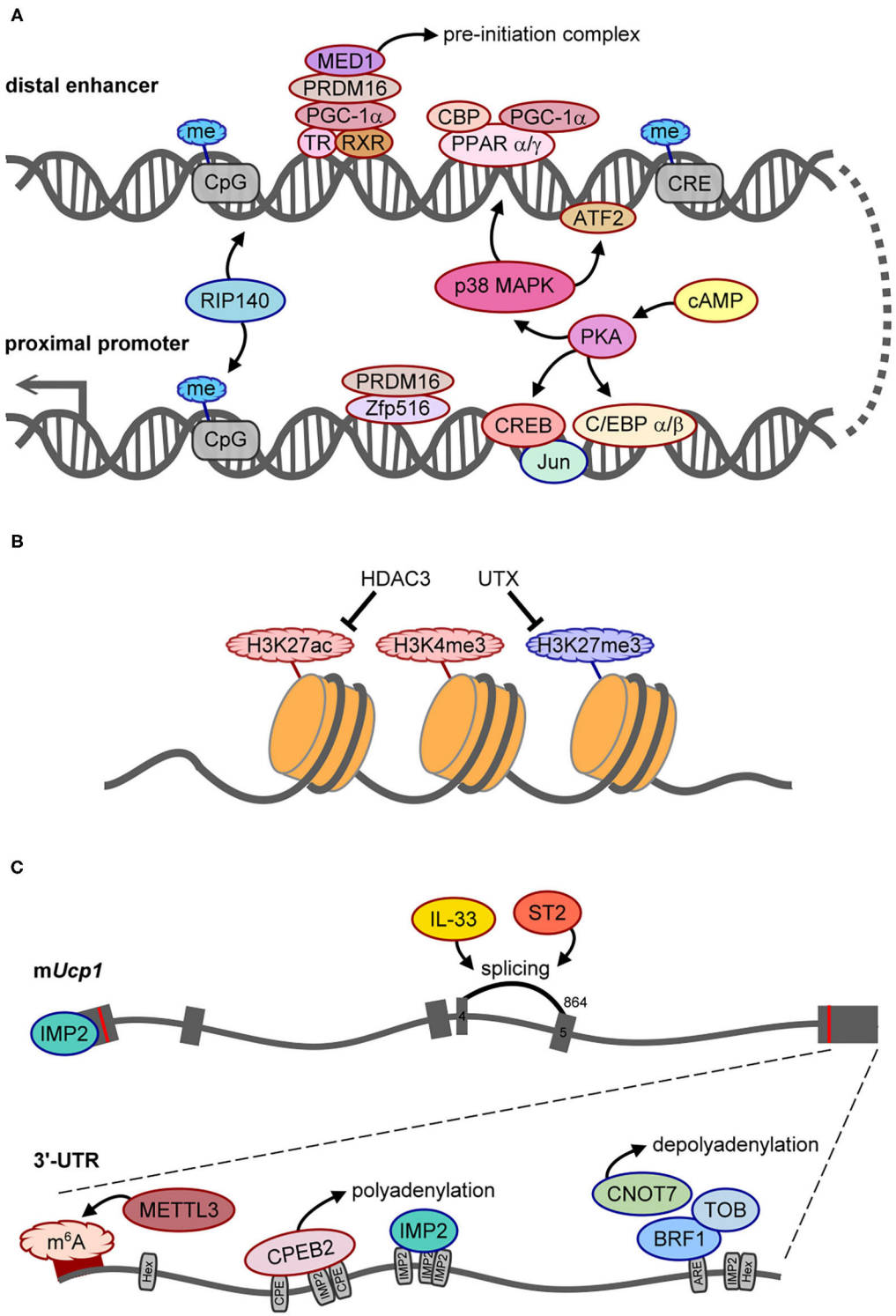

FIGURE 2 | The molecular mechanisms control UCP1 synthesis. Positive and negative regulators of UCP1 expression are outlined in red or blue, respectively. **(A)** Scheme of regulatory factors acting on the distal enhancer and proximal promoter of *Ucp1*. **(B)** The histone modifications, HDAC3 and UTX demethylase, involved in epigenetic regulation of *Ucp1* transcription. **(C)** Illustration of splicing and posttranscriptional regulation of *Ucp1* RNA. The gray boxes are six exons of *Ucp1* with the start (AUG) and stop (UAA) codons labeled as red lines. The number 864 above exon 5 is the splice acceptor site for generating functional UCP1. The bottom gray line represents the 3′-UTR of mouse *Ucp1* with 2 hexanucleotides (Hex, AAUAAA polyadenylation site), denoted regulatory sequences, including CPE (CPEB2-binding site), ARE (AU-rich element for BRF1 binding), and predicted IMP2-binding sites. The dark red band indicates the hot spot area for m^6A modification.

MicroRNAs (miRNAs) are a conserved class of small RNAs of about 20–22 nt that regulate the expression of their target mRNAs *via* the miRNA-induced silencing complex (48, 49). Despite the relatively short 3′-UTR (485 bp) of mouse *Ucp1L* mRNA, a previous study used miRanda to identify miR-9 and miR-338-3p as putative miRNAs targeting the 3′-UTR of *Ucp1L* mRNA (50). Activation of β3AR signaling decreased both miRNA levels and increased *Ucp1* mRNA level in epididymal WAT (eWAT) but not iWAT and BAT. Despite a negative correlation between miR-9 and miR-338-3p and *Ucp1* expression in eWAT, no further experiments were performed to determine whether both miRNAs affect *Ucp1* mRNA stability by binding to the predicted target sites in the 3′-UTR (50). Although cold exposure did not change the proportion of *Ucp1L* and *Ucp1S* in BAT (21), no studies have measured the proportion of *Ucp1L* and *Ucp1S* in WAT and whether it can be changed under HFD or cold temperature. The alternative use of proximal and distal poly(A) signals in mouse *Ucp1* mRNA may vary in different adipose tissues.

A recent paper demonstrated that methyltransferase like 3 (METTL3)-catalyzed m^6A modifications in *Ucp1* mRNA increased its mRNA and protein levels during postnatal BAT development (**Figure 2C**). In mice with BAT-specific ablation of *Mettl3*, the loss of m^6A-enhanced UCP1 expression reduced energy expenditure and cold tolerance (51). Moreover, these mice showed accelerated HFD-induced obesity, impaired glucose intolerance, and insulin resistance (51). Depending on the reader, m^6A modification on mRNA can affect translation efficiency or mRNA stability (52), but how this epitranscriptomic modification mechanistically affects UCP1 expression has not yet been answered.

Besides these posttranscriptional regulations, interleukin 33 (IL-33) and ST2 (also known as IL-1 receptor 4, a receptor of IL-33) are required for accurate splicing of *Ucp1* mRNA between exons 4 and 5 by using the splice site at nucleotide 864 on exon 5 (**Figure 2C**). Without IL-33 or ST2, the splicing machinery prefers the cryptic nucleotide 972 site on exon 5 to produce a non-functional transcript (53). Depletion of either protein results in the loss of UCP1 in brown and beige adipocytes, so IL-33/ST2 signaling is important to register brown and beige adipocytes for uncoupled respiration and thermogenesis during the perinatal period (53).

DISCUSSION

Treating lean healthy men with a specific β3AR agonist, CL316243, increased insulin sensitivity and fat oxidation without affecting body weight (54), but 4-week administration of another β3AR agonist, L796568, in obese men did not confer evident metabolic benefits (55). The lack of chronic effect on shifting energy balance for weight loss may be due to few β3AR-responsive adipose tissues in humans. Indeed, two recent studies found a very low level of β3AR mRNA in human BAT (56, 57). Using cultured human brown adipocytes, one study reported that β2AR activation increased UCP1 level and lipolysis (56), but the other study identified β1AR signaling as responsible for upregulating thermogenic gene expression (57). Because the activation of β1AR and β2AR has a profound impact on cardiopulmonary function, the positive regulatory mechanisms downstream of β-adrenergic signaling must be targeted to promote UCP1 synthesis, energy expenditure, and metabolic fitness in humans. Notably, the mouse model has been used to reveal mechanisms controlling UCP1 synthesis, thermogenesis, and possible implications for human obesity. However, the loss of the CCAAT sequence and Sp1-binding motif in the proximal promoter (22) and alternative polyadenylation in human *UCP1* (21) supports that UCP1 expression is regulated by species-dependent variations. For example, despite comparable UCP1 activity in mouse and human BAT (58), we reported that the expression of human *Ucp1* mRNA in five different BAT samples is only 1/30–1/300 the level in mice (21). Our result agrees with a Northern blot study, which detected 1.9-kb *UCP1* mRNA by using 5 μg poly(A) RNA from human perirenal BAT. Thus, absolute RT-qPCR was used to compare the amount of *Ucp1* mRNA between human and mouse BAT (21). Because *Ucp1L* is translated 15 times more efficiently than *Ucp1S* in mouse BAT, we predicted that CPEB2-mediated translational activation, if fully functioning in human BAT, can make up the protein level to ∼1/2–1/20 of that in mouse BAT (21). Similarly, if the mechanism of BRF1-mediated deadenylation-induced *Ucp1L* decay (47) is conserved in humans, it is expected that HFD-induced *UCP1* mRNA degradation in human beige adipocytes should become more prominent. Thus, humans, and perhaps also other primates, may rely more on posttranscriptional regulation to control UCP1 synthesis because they express a low level of only long 3′-UTR *UCP1* mRNA. Moreover, although ectopic expression of mouse *Ucp1* in white adipocytes of pigs also enhances thermogenic capacity and lipolysis to decrease fat deposition (59), why UCP1-mediated metabolic plasticity is lost in some mammals through evolution remains unclear. Therefore, the results from studying gene-modified mice in BAT thermogenesis need to be translated with caution into humans and other species.

AUTHOR CONTRIBUTIONS

W-HL wrote the manuscript and illustrated the figures. Y-MC analyzed the poly(A) sites in *Ucp1* across species. Y-SH co-wrote the manuscript and is responsible for its content. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. (2008) 454:961–7. doi: 10.1038/nature07182
2. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. (2009) 360:1509–17. doi: 10.1056/NEJMoa0810780
3. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med*. (2009) 360:1500–8. doi: 10.1056/NEJMoa0808718
4. Dulloo AG. Translational issues in targeting brown adipose tissue thermogenesis for human obesity management. *Ann N Y Acad Sci*. (2013) 1302:1–10. doi: 10.1111/nyas.12304
5. Brondani LA, Assmann TS, Duarte GC, Gross JL, Canani LH, Crispim D. The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 diabetes mellitus. *Arq Bras Endocrinol Metabol*. (2012) 56:215–25. doi: 10.1590/S0004-27302012000400001
6. Chathoth S, Ismail MH, Vatte C, Cyrus C, Al Ali Z, Ahmed KA, et al. Association of Uncoupling Protein 1 (UCP1) gene polymorphism with obesity: a case-control study. *BMC Med Genet*. (2018) 19:203. doi: 10.1186/s12881-018-0715-5
7. Mori H, Okazawa H, Iwamoto K, Maeda E, Hashiramoto M, Kasuga M. A polymorphism in the 5' untranslated region and a Met229–>Leu variant in exon 5 of the human UCP1 gene are associated with susceptibility to type II diabetes mellitus. *Diabetologia*. (2001) 44:373–6. doi: 10.1007/s001250051629
8. Nicoletti CF, de Oliveira AP, Brochado MJ, de Oliveira BP, Pinhel MA, Marchini JS, et al. UCP1−3826 A>G polymorphism affects weight, fat mass, and risk of type 2 diabetes mellitus in grade III obese patients. *Nutrition*. (2016) 32:83–7. doi: 10.1016/j.nut.2015.07.016
9. Lin CS, Klingenberg M. Isolation of the uncoupling protein from brown adipose tissue mitochondria. *FEBS Lett*. (1980) 113:299–303. doi: 10.1016/0014-5793(80)80613-2
10. Nicholls DG. Hamster brown-adipose-tissue mitochondria. Purine nucleotide control of the ion conductance of the inner membrane, the nature of the nucleotide binding site. *Eur J Biochem*. (1976) 62:223–8. doi: 10.1111/j.1432-1033.1976.tb10151.x
11. Zhu R, Rupprecht A, Ebner A, Haselgrubler T, Gruber HJ, Hinterdorfer P, et al. Mapping the nucleotide binding site of uncoupling protein 1 using atomic force microscopy. *J Am Chem Soc*. (2013) 135:3640–6. doi: 10.1021/ja312550k
12. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature*. (2000) 404:652–60. doi: 10.1038/35007527
13. Morrison SF, Madden CJ, Tupone D. Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. *Cell Metab*. (2014) 4:1677–713. doi: 10.1002/cphy.c140013
14. Hankir MK. Loading and firing the brown adipocyte. *Adipocyte*. (2018) 7:4–11. doi: 10.1080/21623945.2017.1405879
15. Bouillaud F, Ricquier D, Thibault J, Weissenbach J. Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein. *Proc Natl Acad Sci USA*. (1985) 82:445–8. doi: 10.1073/pnas.82.2.445
16. Jacobsson A, Stadler U, Glotzer MA, Kozak LP. Mitochondrial uncoupling protein from mouse brown fat. Molecular cloning, genetic mapping, and mRNA expression. *J Biol Chem*. (1985) 260:16250–4. doi: 10.1016/S0021-9258(17)36228-2
17. Bouillaud F, Raimbault S, Ricquier D. The gene for rat uncoupling protein: complete sequence, structure of primary transcript and evolutionary relationship between exons. *Biochem Biophys Res Commun*. (1988) 157:783–92. doi: 10.1016/S0006-291X(88)80318-8
18. Kozak LP, Britton JH, Kozak UC, Wells JM. The mitochondrial uncoupling protein gene. Correlation of exon structure to transmembrane domains. *J Biol Chem*. (1988) 263:12274–7. doi: 10.1016/S0021-9258(18)37751-2
19. Ridley RG, Patel HV, Gerber GE, Morton RC, Freeman KB. Complete nucleotide and derived amino acid sequence of cDNA encoding the mitochondrial uncoupling protein of rat brown adipose tissue: lack of a mitochondrial targeting presequence. *Nucleic Acids Res*. (1986) 14:4025–35. doi: 10.1093/nar/14.10.4025
20. Balogh AG, Ridley RG, Patel HV, Freeman KB. Rabbit brown adipose tissue uncoupling protein MRNA: use of only one of two polyadenylation signals in its processing. *Biochem Biophys Res Commun*. (1989) 161:156–61. doi: 10.1016/0006-291X(89)91574-X
21. Chen HF, Hsu CM, Huang YS. CPEB2-dependent translation of long 3'-UTR Ucp1 mRNA promotes thermogenesis in brown adipose tissue. *EMBO J*. (2018) 37:e99071. doi: 10.15252/embj.201899071
22. Cassard AM, Bouillaud F, Mattei MG, Hentz E, Raimbault S, Thomas M, et al. Human uncoupling protein gene: structure, comparison with rat gene, and assignment to the long arm of chromosome 4. *J Cell Biochem*. (1990) 43:255–64. doi: 10.1002/jcb.240430306
23. Jastroch M, Wuertz S, Kloas W, Klingenspor M. Uncoupling protein 1 in fish uncovers an ancient evolutionary history of mammalian nonshivering thermogenesis. *Phys Genom*. (2005) 22:150–6. doi: 10.1152/physiolgenomics.00070.2005
24. Saito S, Saito CT, Shingai R. Adaptive evolution of the uncoupling protein 1 gene contributed to the acquisition of novel nonshivering thermogenesis in ancestral eutherian mammals. *Gene*. (2008) 408:37–44. doi: 10.1016/j.gene.2007.10.018
25. Jastroch M, Oelkrug R, Keipert S. Insights into brown adipose tissue evolution and function from non-model organisms. *J Exp Biol*. (2018) 221(Pt Suppl 1):jeb169425. doi: 10.1242/jeb.169425
26. Gaudry MJ, Jastroch M, Treberg JR, Hofreiter M, Paijmans JLA, Starrett J, et al. Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades. *Sci Adv*. (2017) 3:e1602878. doi: 10.1126/sciadv.1602878
27. Berg F, Gustafson U, Andersson L. The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: a genetic explanation for poor thermoregulation in piglets. *PLoS Genet*. (2006) 2:e129. doi: 10.1371/journal.pgen.0020129
28. Cassard-Doulcier AM, Gelly C, Fox N, Schrementi J, Raimbault S, Klaus S, et al. Tissue-specific and beta-adrenergic regulation of the mitochondrial uncoupling protein gene: control by cis-acting elements in the 5'-flanking region. *Mol Endocrinol*. (1993) 7:497–506. doi: 10.1210/mend.7.4.8388995
29. Kozak UC, Kopecky J, Teisinger J, Enerback S, Boyer B, Kozak LP. An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. *Mol Cell Biol*. (1994) 14:59–67. doi: 10.1128/MCB.14.1.59
30. Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, McKnight GS. Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature*. (1996) 382:622–6. doi: 10.1038/382622a0
31. Cassard-Doulcier AM, Larose M, Matamala JC, Champigny O, Bouillaud F, Ricquier D. *In vitro* interactions between nuclear proteins and uncoupling protein gene promoter reveal several putative transactivating factors including Ets1, retinoid X receptor, thyroid hormone receptor, and a CACCC box-binding protein. *J Biol Chem*. (1994) 269:24335–42. doi: 10.1016/S0021-9258(19)51087-0
32. Villarroya F, Peyrou M, Giralt M. Transcriptional regulation of the uncoupling protein-1 gene. *Biochimie*. (2017) 134:86–92. doi: 10.1016/j.biochi.2016.09.017
33. Dempersmier J, Sambeat A, Gulyaeva O, Paul SM, Hudak CS, Raposo HF, et al. Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white fat and development of brown fat. *Mol Cell*. (2015) 57:235–46. doi: 10.1016/j.molcel.2014.12.005
34. Cassard-Doulcier AM, Gelly C, Bouillaud F, Ricquier D. A 211-bp enhancer of the rat uncoupling protein-1 (UCP-1) gene controls specific and regulated expression in brown adipose tissue. *Biochem J*. (1998) 333(Pt 2):243–6. doi: 10.1042/bj3330243
35. Barbera MJ, Schluter A, Pedraza N, Iglesias R, Villarroya F, Giralt M. Peroxisome proliferator-activated receptor alpha activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J Biol Chem*. (2001) 276:1486–93. doi: 10.1074/jbc.M006246200
36. Cao W, Daniel KW, Robidoux J, Puigserver P, Medvedev AV, Bai X, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol*. (2004) 24:3057–67. doi: 10.1128/MCB.24.7.3057-3067.2004
37. Harms MJ, Lim HW, Ho Y, Shapira SN, Ishibashi J, Rajakumari S, et al. PRDM16 binds MED1 and controls chromatin architecture to determine

a brown fat transcriptional program. *Genes Dev.* (2015) 29:298–307. doi: 10.1101/gad.252734.114
38. Iida S, Chen W, Nakadai T, Ohkuma Y, Roeder RG. PRDM16 enhances nuclear receptor-dependent transcription of the brown fat-specific Ucp1 gene through interactions with Mediator subunit MED1. *Genes Dev*. (2015) 29:308–21. doi: 10.1101/gad.252809.114
39. Kiskinis E, Hallberg M, Christian M, Olofsson M, Dilworth SM, White R, et al. RIP140 directs histone and DNA methylation to silence Ucp1 expression in white adipocytes. *EMBO J*. (2007) 26:4831–40. doi: 10.1038/sj.emboj.7601908
40. Yu DQ, Lv PP, Yan YS, Xu GX, Sadhukhan A, Dong S, et al. Intrauterine exposure to hyperglycemia retards the development of brown adipose tissue. *FASEB J*. (2019) 33:5425–39. doi: 10.1096/fj.201801818R
41. Ferrari A, Longo R, Fiorino E, Silva R, Mitro N, Cermenati G, et al. HDAC3 is a molecular brake of the metabolic switch supporting white adipose tissue browning. *Nat Commun.* (2017) 8:93. doi: 10.1038/s41467-017-00182-7
42. Fan R, Toney AM, Jang Y, Ro SH, Chung S. Maternal n-3 PUFA supplementation promotes fetal brown adipose tissue development through epigenetic modifications in C57BL/6 mice. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2018) 1863:1488–97. doi: 10.1016/j.bbalip.2018.09.008
43. Shore A, Karamitri A, Kemp P, Speakman JR, Lomax MA. Role of Ucp1 enhancer methylation and chromatin remodelling in the control of Ucp1 expression in murine adipose tissue. *Diabetologia*. (2010) 53:1164–73. doi: 10.1007/s00125-010-1701-4
44. Zha L, Li F, Wu R, Artinian L, Rehder V, Yu L, et al. The histone demethylase UTX promotes brown adipocyte thermogenic program via coordinated regulation of H3K27 demethylation and acetylation. *J Biol Chem*. (2015) 290:25151–63. doi: 10.1074/jbc.M115.662650
45. Moore MJ. From birth to death: the complex lives of eukaryotic mRNAs. *Science*. (2005) 309:1514–8. doi: 10.1126/science.1111443
46. Dai N, Zhao L, Wrighting D, Kramer D, Majithia A, Wang Y, et al. IGF2BP2/IMP2-Deficient mice resist obesity through enhanced translation of Ucp1 mRNA and Other mRNAs encoding mitochondrial proteins. *Cell Metab*. (2015) 21:609–21. doi: 10.1016/j.cmet.2015.03.006
47. Takahashi A, Adachi S, Morita M, Tokumasu M, Natsume T, Suzuki T, et al. Post-transcriptional Stabilization of Ucp1 mRNA protects mice from diet-induced obesity. *Cell Rep*. (2015) 13:2756–67. doi: 10.1016/j.celrep.2015.11.056
48. Ameres SL, Zamore PD. Diversifying microRNA sequence and function. *Nat Rev Mol Cell Biol*. (2013) 14:475–88. doi: 10.1038/nrm3611
49. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol.* (2014) 15:509–24. doi: 10.1038/nrm3838
50. Zheng Z, Liu X, Zhao Q, Zhang L, Li C, Xue Y. Regulation of UCP1 in the browning of epididymal adipose tissue by beta3-adrenergic agonist: A Role for MicroRNAs. *Int J Endocrinol.* (2014) 2014:530636. doi: 10.1155/2014/530636
51. Wang Y, Gao M, Zhu F, Li X, Yang Y, Yan Q, et al. METTL3 is essential for postnatal development of brown adipose tissue and energy expenditure in mice. *Nat Commun.* (2020) 11:1648. doi: 10.1038/s41467-020-15488-2
52. Huang YS, Lu WH. Decoding hidden messages in neurons: insights from epitranscriptome-controlled and specialized ribosome-controlled translation. *Curr Opin Neurobiol.* (2018) 48:64–70. doi: 10.1016/j.conb.2017.10.018
53. Odegaard JI, Lee MW, Sogawa Y, Bertholet AM, Locksley RM, Weinberg DE, et al. Perinatal licensing of thermogenesis by IL-33 and ST2. *Cell*. (2016) 166:841–54. doi: 10.1016/j.cell.2016.06.040
54. Weyer C, Tataranni PA, Snitker S, Danforth E, Ravussin E. Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. *Diabetes*. (1998) 47:1555–61. doi: 10.2337/diabetes.47.10.1555
55. Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH, et al. Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr*. (2002) 76:780–8. doi: 10.1093/ajcn/76.4.780
56. Blondin DP, Nielsen S, Kuipers EN, Severinsen MC, Jensen VH, Miard S, et al. Human brown adipocyte thermogenesis is driven by beta2-AR stimulation. *Cell Metab*. (2020) 32:287–300.e7. doi: 10.1016/j.cmet.2020.07.005
57. Riis-Vestergaard MJ, Richelsen B, Bruun JM, Li W, Hansen JB, Pedersen SB. Beta-1 and not beta-3 adrenergic receptors may be the primary regulator of human brown adipocyte metabolism. *J Clin Endocrinol Metab.* (2020) 105:e994–1005. doi: 10.1210/clinem/dgz298
58. Porter C, Herndon DN, Chondronikola M, Chao T, Annamalai P, Bhattarai N, et al. Human and mouse brown adipose tissue mitochondria have comparable UCP1 function. *Cell Metab*. (2016) 24:246–55. doi: 10.1016/j.cmet.2016.07.004
59. Zheng Q, Lin J, Huang J, Zhang H, Zhang R, Zhang X, et al. Reconstitution of UCP1 using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity. *Proc Natl Acad Sci USA*. (2017) 114:E9474–82. doi: 10.1073/pnas.1707853114

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frontiers in Pediatrics

BRIEF RESEARCH REPORT
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Comparison of Growth Velocity Among School Age Children With Different Body Mass Index From Childhood Into Early Adolescence in Hualien County, Taiwan: A Retrospective Cohort Study

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Objective: This study aimed to investigate the contribution of high body mass index (BMI) to growth velocity among school-aged children who remained in the same BMI categories for a 6-year period.

Methods: This retrospective cohort study included children who enrolled in the school year 2009 and remained in the same BMI categories during their 1st, 4th, and 7th grades (6–7, 9–10, 12–13 years of age). Annual linear growth velocity and weight gain were calculated and compared between sexes, BMI groups, and different times. Risk analysis and repeated measures analysis of variance were performed to identify the impact of BMI on growth velocity.

Results: Of the 1,637 subjects, 53.0% were male, and 2.5% and 10.9% belonged to BMI groups of overweight and obese, respectively. In students between 6 and 13 years of age, obesity was associated with higher annual weight gain and height gain. Risk analysis showed that obese subjects had higher linear growth velocity than normal BMI groups of both sexes between 6 and 9 years of age. Unexpectedly, overweight and obese girls between 9 and 13 years of age had less linear growth velocity than underweight girls at the same interval. Repeated measures analysis of variance in both sexes showed a significant statistical association between BMI and different times of growth. However, the effect was less in girls between 9 and 13 years of age.

Conclusion: Puberty may dominate over BMI as the main contributor to high growth velocity in girls with underweight BMI emerging into pubertal age.

Keywords: obesity, puberty, growth velocity, body mass index, anthropometry

INTRODUCTION

Childhood obesity continues to be a significant public health concern globally in recent decades. In the United States, a report warned that the levels of severe obesity in all children aged 2–19 years and populations have increased in the past 18 years (1). Many studies have reported that the prevalence of severe obesity has increased quickly (2–4). In Taiwan, an increasing trend in overweight and obesity was observed among Taiwanese children and adolescents over a 2-year period (5). The Nutrition and Health Survey in Taiwan 2013–2016 report showed that the prevalence of childhood overweight and obesity among children aged 7–12 years was 28.4%. In Hualien County, the prevalence of overweight or obese was 25% among children aged 7–12 years after analyzing the dataset of the school year 2010 (6). In Taiwan, routine health examinations for students are a suitable way to identify health problems, such as obesity, early and refer them for further evaluation (6). Childhood obesity has increased the risk of adulthood obesity and its related complications, including cardiovascular and metabolic diseases, which will reduce quality of life (2). Associations of growth velocity and pubertal timing are possible predictors of health problems, including a higher risk of early cardiovascular and metabolic morbidity and mortality (5–7).

The worldwide pandemic of childhood obesity has increased interest in the relationship between obesity and growth patterns during childhood and adolescence. Growth velocity and puberty are associated with complex mechanisms that involve the neuroendocrine system, and obesity has a significant impact on the neuroendocrine system (7). Rapid weight gain in infants has been associated with a higher rate of childhood obesity, which has been associated with a higher risk of obesity in adulthood (8, 9). The number of adipocytes present in the body in adulthood is related to obesity and correlates with the rate of increase in adipocyte counts in childhood and adolescence (10). Increased risk of obesity-related complications is significantly associated with childhood obesity (11, 12). Thus, early intervention to prevent childhood obesity is needed (13).

Children with early-onset obesity seem to have higher growth velocity before puberty and earlier onset of puberty than normal-weight or underweight children (14). In 2014, De Leonibus et al. (15) showed that pre-pubertal obese boys and girls were predisposed to an early-onset of puberty and achievement of pubertal maturation. In addition, obesity has been associated with taller pre-pubertal stature, which is subsequently lost, leading to similar adult heights between obese and normal-weight children (15). Recent longitudinal studies in the United States (16) and Sweden (17) have observed inverse associations between high BMI for age in childhood and linear growth during adolescence. Furthermore, recent evidence has suggested a relationship between childhood obesity and the timing of pubertal milestones in boys and girls (18, 19), which is known to influence adolescent linear growth. In one study conducted in both Belarus and the United States, the authors supported the role of higher body mass index (BMI) in accelerating linear growth in early childhood (i.e., taller stature and longer trunk length in those aged 3–6 years), but earlier pubertal development and slower linear growth during adolescence (12–17 years of age) (11).

Thus, this study aimed to investigate the contribution of high BMI on growth velocity by retrospectively tracking multiple anthropometric measurements among children 6–13 years old who remained in the same BMI categories for a 6-year period. We hypothesized that obesity would contribute primarily and equally to high growth velocity in both sexes from childhood into early adolescence.

Abbreviations: BMI, body mass index; GH, growth hormone; IGF, insulin-like growth factor.

MATERIALS AND METHODS

Subjects

The dataset in this retrospective cohort study was collected from the annual health examination records of school-aged children between 2009 and 2015 in Hualien County. The Hualien County Government Education Bureau reviewed and approved this study. This study was approved by the Research Ethics Committee of Hualien Tzu Chi Hospital for data review (REC No. IRB105-52-B). The students' parents or guardians provided written informed consent for participating in the health examinations.

Measurement and Procedures

Well-trained pediatricians, family physicians, and nurses from the medical center in Hualien County conducted health examinations of all students during their 1st, 4th, and 7th grade school years. Physical exam items recorded on student health examination records included eyes, teeth ears-nose-throat (ENT), head-neck, heart, chest-lung, abdomen, spine-limbs, male genitalia (hydrocele and varicocele), and skin systems according to the regulations set by the Taiwan Ministry of Education (20). Private parts such as female breasts and genitalia were not examined in school physical checkups. Tanner stages of both sexes were not evaluated and recorded. Anthropometrics including height, weight, and visual acuity were measured by school nurses prior to the physical examination dates. The portable stadiometer for height and weight measurement were mostly Seca digital column scale with integrated measuring rod (Seca, Germany) in school settings. Demographic data on health examination records included age, sex, and aboriginal and urban residential status, which was categorized into three groups: urban, suburban, and rural, according to the population densities of the residential areas (21). The flowchart of our study design is shown in **Figure 1**. Demographic data and anthropometric measurements of first graders enrolled in the 2009 school year, and their follow-up dataset as fourth (in 2012) and seventh (in 2015) graders were collected from the dataset of student health examination records. Students with missing data, such as age, sex, aboriginal and urban residential status, and BMI in 2012 and 2015 were excluded. Students with complete follow-up data were categorized into underweight, normal weight, overweight, and obese groups according to the age-sex-specific BMI cut-off values from the new growth charts for Taiwanese children

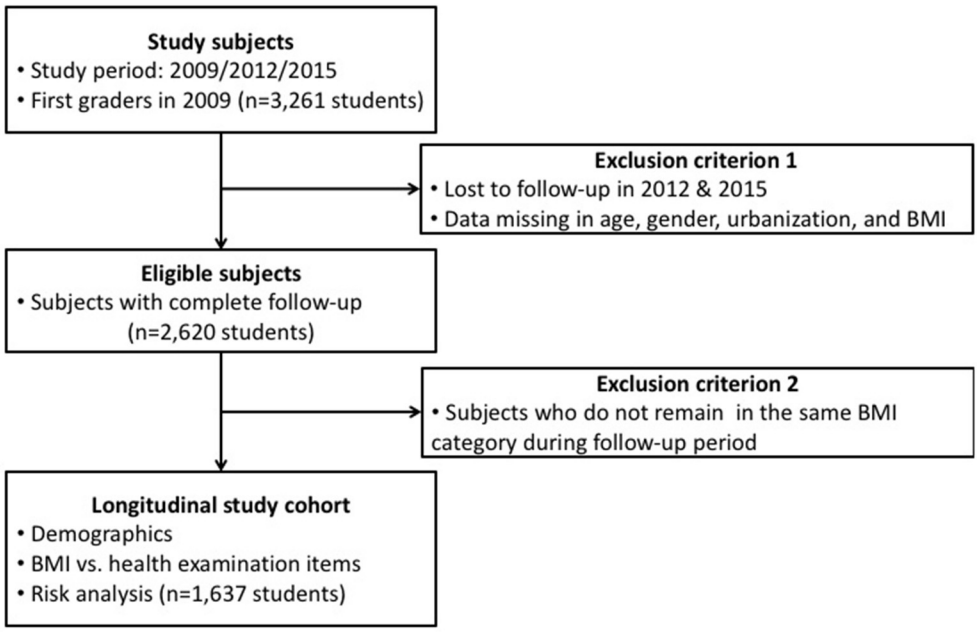

FIGURE 1 | Flowchart of participant recruitment. BMI, body mass index.

and adolescents, based on the World Health Organization (WHO) standards, and health-related physical fitness, which was developed by the Department of Health in Taiwan in 2010 (6).

Statistical Analysis

According to their BMIs, the students were categorized into four different groups: underweight, normal weight, overweight, and obesity groups (11). Annual linear growth velocity and weight gain were calculated and compared between sexes, BMI groups, and times. Multiple linear regression and repeated measures analysis of variance were performed to identify the impact of BMI on growth velocity since all subjects were examined three times during the follow-up period.

Descriptive statistics for sex, aboriginal and urban residential status are presented as frequencies or proportions. Annual height gain and annual weight gain are presented as mean ± standard deviation or median (lower quartile, upper quartile). The chi-square test was performed to evaluate the associations between categorical examination components and BMI groups. Analysis of variance was used to determine differences in means of continuous variables among different BMI groups. Multiple comparisons were performed using Bonferroni correction. Multiple linear regression models were used to simultaneously analyze the association between constant examination components and risk factors encountered.

Multiple logistic regression was performed to analyze categorical variables. Adjusted beta coefficients, odds ratios, and 95% confidence intervals were also calculated. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed with Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic Data

In total, 3,261 1st graders enrolled in the school year 2009 and their follow-up dataset as fourth graders (in 2012) and seventh graders (in 2015) were also collected. Two thousand six hundred twenty students who had complete follow-up data were categorized into underweight, normal weight, overweight, and obese groups according to the age-sex-specific BMI cut-off points from the new growth charts for Taiwanese children and adolescents as mentioned previously. Those 1,637 subjects who remained in the same BMI category over the 6-year period (2009–2015) were included for data analysis in the study (**Figure 1**). The demographic data including age, sex, urban residential status, aboriginal status, height, weight, and BMI are shown in **Supplementary Tables 1**, **2**. The average height and weight among male and female groups of the same age did not show any significant difference (**Supplementary Figure 1**).

In **Table 1**, obesity was associated with higher annual weight gain ($p < 0.001$ in both sexes) and higher height gain in male students alone ($p < 0.001$ in male subjects and $p = 0.09$ in female subjects) between 6 and 13 years of age. There was no association between obesity and urban residential status.

Associated Factors With Physical Growth

Table 2 illustrates the factors associated with height gain and weight gain for male and female students, including BMI and different periods of growth (6–9 and 9–13 years of age). In female subjects aged 6–9 years, the growth showed higher linear growth velocity and weight gain in overweight and obese groups than in the normal BMI groups; at the same time, underweight girls had less linear growth velocity and weight gain than girls with

TABLE 1 | Demographics among BMI groups (2009–2015) (*N* = 1,637).

Variable	Underweight	Normal	Overweight	Obese	Total	*P*-value	*Post-hoc* test
Male subjects (*N* = 867)							
N	33	700	24	110	867		
Urbanization						0.084	
Urban, n(%)	29 (87.9)	511 (73.0)	17 (70.8)	82 (74.5)	639 (73.7)		
Suburban, n(%)	4 (12.1)	124 (17.7)	7 (29.2)	23 (20.9)	158 (18.2)		
Rural, n(%)	0 (0.0)	65 (9.3)	0 (0.0)	5 (4.5)	70 (8.1)		
Aborigine						0.025*	
No, n(%)	22 (91.7)	337 (68.9)	12 (80.0)	60 (78.9)	431 (71.4)		
Yes, n(%)	2 (8.3)	152 (31.1)	3 (20.0)	16 (21.1)	173 (28.6)		
Female subjects (*N* = 770)							
N	32	653	17	68	770		
Urbanization						0.712	
Urban, n(%)	24 (75.0)	467 (71.5)	14 (82.4)	48 (70.6)	553 (71.8)		
Suburban, n(%)	8 (25.0)	153 (23.4)	2 (11.8)	15 (22.1)	178 (23.1)		
Rural, n(%)	0 (0.0)	33 (5.1)	1 (5.9)	5 (7.4)	39 (5.1)		
Aborigine						0.262	
No, n(%)	22 (91.7)	333 (74.2)	10 (71.4)	38 (77.6)	403 (75.2)		
Yes, n(%)	2 (8.3)	116 (25.8)	4 (28.6)	11 (22.4)	133 (24.8)		
Annual height gain (AHG) and Annual weight gain (AWG) of male subjects							
AHG 1st−4th grade (mean ± SD, cm)	5.20 ± 0.63 5.13 (4.93, 5.50)	5.49 ± 0.76 5.50 (5.07, 5.93)	5.96 ± 0.64 5.93 (5.38, 6.49)	6.28 ± 0.73 6.25 (5.83, 6.78)	5.59 ± 0.80 5.60 (5.13, 6.03)	<0.001*	Obese, overweight > normal, underweight
AHG 4th−7th grade (mean ± SD, cm)	6.09 ± 1.45 5.93 (4.78, 7.20)	6.74 ± 1.47 6.60 (5.57, 7.90)	6.93 ± 1.25 7.12 (5.68, 8.16)	6.83 ± 1.03 6.80 (6.19, 7.51)	6.73 ± 1.42 6.67 (5.60, 7.87)	0.050	
AHG 1st−7th grade (mean ± SD, cm)	5.64 ± 0.84 5.53 (4.90, 6.10)	6.12 ± 0.87 6.08 (5.50, 6.80)	6.44 ± 0.80 6.66 (5.59, 6.98)	6.55 ± 0.69 6.53 (6.07, 7.08)	6.16 ± 0.87 6.17 (5.53, 6.83)	<0.001*	Obese, overweight > normal > underweight
AWG 1st−4th grade (mean ± SD, kg)	1.84 ± 0.37 1.87 (1.67, 2.05)	2.79 ± 0.82 2.63 (2.27, 3.23)	4.72 ± 0.78 4.45 (4.20, 5.38)	6.41 ± 1.34 6.32 (5.53, 7.38)	3.26 ± 1.54 2.77 (2.30, 3.77)	<0.001*	Obese > overweight > normal > underweight
AWG 4th−7th grade (mean ± SD, kg)	3.08 ± 1.01 2.87 (2.27, 3.77)	4.52 ± 1.30 4.43 (3.53, 5.43)	5.97 ± 1.08 5.82 (4.94, 6.87)	7.85 ± 2.21 7.65 (6.08, 9.31)	4.93 ± 1.85 4.70 (3.60, 5.83)	<0.001*	Obese > overweight > normal > underweight
AWG 1st-7th grade (mean ± SD, kg)	2.46 ± 0.58 2.38 (2.03, 2.78)	3.65 ± 0.86 3.61 (3.05, 4.25)	5.34 ± 0.69 5.33 (4.82, 5.86)	7.13 ± 1.47 6.84 (6.18, 8.06)	4.10 ± 1.54 3.78 (3.08, 4.70)	<0.001*	Obese > overweight > normal > underweight
Annual height gain (AHG) and Annual weight gain (AWG) of female subjects							
AHG 1st−4th grade (mean ± SD, cm)	5.48 ± 0.99 5.57 (5.26, 5.83)	5.95 ± 1.08 5.83 (5.33, 6.50)	6.87 ± 1.02 6.97 (6.05, 7.65)	6.87 ± 0.92 6.72 (6.33, 7.33)	6.03 ± 1.11 5.88 (5.37, 6.63)	<0.001*	Obese, overweight > normal, underweight
AHG 4th−7th grade (mean ± SD, cm)	6.54 ± 1.02 6.78 (5.53, 7.30)	6.33 ± 1.10 6.50 (5.73, 7.10)	5.47 ± 1.56 5.80 (4.43, 6.63)	5.07 ± 1.39 5.15 (4.21, 6.07)	6.21 ± 1.20 6.40 (5.57, 7.03)	<0.001*	Normal, underweight > obese, overweight

(Continued)

TABLE 1 | Continued

Variable	Underweight	Normal	Overweight	Obese	Total	*P*-value	*Post-hoc* test
AHG 1st–7th grade (mean ± SD, cm)	6.01 ± 0.67 6.08 (5.76, 6.36)	6.14 ± 0.58 6.18 (5.83, 6.50)	6.17 ± 0.62 6.13 (5.83, 6.55)	5.97 ± 0.62 6.08 (5.53, 6.30)	6.12 ± 0.59 6.17 (5.82, 6.48)	0.090	
AWG 1st–4th grade (mean ± SD, kg)	1.96 ± 0.41 1.97 (1.65, 2.27)	2.95 ± 0.90 2.80 (2.30, 3.47)	5.12 ± 0.75 5.13 (4.52, 5.78)	6.55 ± 1.32 6.57 (5.54, 7.52)	3.28 ± 1.43 2.90 (2.33, 3.87)	<0.001*	Obese > overweight > normal > underweight
AWG 4th–7th grade (mean ± SD, kg)	3.52 ± 0.76 3.70 (2.88, 4.09)	4.58 ± 1.07 4.60 (3.90, 5.30)	5.18 ± 1.28 4.87 (4.00, 6.45)	7.19 ± 2.29 7.07 (5.45, 8.77)	4.78 ± 1.45 4.63 (3.90, 5.43)	<0.001*	Obese > overweight, normal > underweight
AWG 1st–7th grade (mean ± SD, kg)	2.74 ± 0.42 2.79 (2.53, 3.01)	3.77 ± 0.69 3.75 (3.30, 4.24)	5.15 ± 0.72 5.08 (4.56, 5.87)	6.87 ± 1.47 6.78 (5.68, 7.88)	4.03 ± 1.22 3.83 (3.30, 4.40)	<0.001*	Obese > overweight > normal > underweight

*Values beneath mean ± SD are median (lower quartile, upper quartile). *p < 0.05 was considered statistically significant after test. AWG, annual weight gain; AHG, annual height gain; BMI, body mass index; SD, standard deviation.*

normal BMI. Among female subjects between 9 and 13 years of age, overweight and obese girls had less linear growth than girls with normal BMI. Underweight boys between 6 and 13 years of age had less linear growth velocity and weight gain than boys with normal BMI. The obese boys between 6 and 9 years of age had higher linear growth velocity than boys with normal BMI. However, the overweight and obese boys aged 9–13 years did not show a significant difference in linear growth velocity compared to boys with normal BMI.

Supplementary Tables 3, **4** show the results of repeated measures analysis of variance for male and female subjects. In both sexes, a significant statistical association was observed between BMI and different times of growth (**Figure 2**). High BMI to the range of overweight and obese had strong effects on high linear growth in subjects between 6 and 13 years of age (1st to 7th grade); on the contrary, this effect was less in female subjects between 9 and 13 years of age (4th to 7th grade).

DISCUSSION

This study revealed that obese boys and girls had higher linear growth velocity between 6 and 9 years of age (1st to 4th grade) on risk analysis, but the finding was different in children aged 9–13 years (4th to 7th grade). The growth pattern among boys and girls was not the same—the obese and overweight girls had less linear growth velocity than underweight girls between 9 and 13 years old, which was not consistent with our hypothesis that obesity would contribute equally to high growth velocity in both sexes from childhood into early adolescence. It has been found that the average age of menarche in Taiwanese girls is about 12 years of age (general range, between 11 and 13 years of age) (22). Additionally, the age of female pubertal growth spurt was 2–3 years before menarche occurred. Obesity has also been related to the early onset of puberty including early menarche (19, 23, 24). Peripheral adipose tissue and interaction of leptin with the kisspeptin system may be associated with obesity and puberty (19). Many studies have found that the trend of obesity and early puberty onset is more prominent in girls than in boys (19, 23, 25, 26). Although we did not collect data on the onset of puberty or age of menarche, we speculated that overweight and obese girls in our study had an earlier onset of puberty and earlier age of menarche than normal and underweight BMI girls based on our clinical observation. Overweight and obese girls had earlier timing of growth spurt when compared with underweight BMI girls within the same age interval, between 9 and 13 years old, which may explain the low linear growth velocity of obese and overweight girls observed in the present study. However, the maximum growth velocity of boys was generally 2 years behind the growth spurt of girls, e.g., 10–12 years in girls vs. 12–14 years in boys.

Overweight and obese boys between 9 and 13 years of age did not show a significant difference in linear growth velocity when compared with boys with normal BMI. On the other hand, boys with underweight BMI between 6 and 13 years of age revealed less growth velocity than boys with normal BMI in the present study. Although overweight and obese boys between 6

TABLE 2 | Factors associated with height gain and weight gain.

Variable	AHG (6–9 years of age)		AHG (9–13 years of age)		AWG (6–9 years of age)		AWG (9–13 years of age)	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value
MALE STUDENTS (*N* = 867)								
BMI group§								
Normal	Reference		Reference		Reference		Reference	
Underweight	−0.333 (−0.624, −0.043)	0.025*	−0.816 (−1.395, −0.237)	0.006*	−0.972 (−1.321, −0.622)	<0.001*	−1.365 (−1.953, −0.776)	<0.001*
Overweight	0.345 (−0.018, 0.708)	0.063	0.033 (−0.691, 0.757)	0.928	1.766 (1.328, 2.203)	<0.001*	1.405 (0.670, 2.140)	<0.001*
Obese	0.886 (0.715, 1.057)	<0.001*	0.196 (−0.144, 0.537)	0.258	3.805 (3.600, 4.011)	<0.001*	3.517 (3.171, 3.863)	<0.001*
FEMALE STUDENTS (*N* = 770)								
BMI group§								
Normal	Reference		Reference		Reference		Reference	
Underweight	−0.432 (−0.840, −0.024)	0.038*	0.170 (−0.289, 0.629)	0.468	−1.029 (−1.408, −0.650)	<0.001*	−1.152 (−1.658, −0.647)	<0.001*
Overweight	1.043 (0.516, 1.570)	<0.001*	−0.852 (−1.445, −0.259)	0.005*	2.222 (1.733, 2.710)	<0.001*	0.576 (−0.077, 1.228)	0.084
Obese	0.920 (0.628, 1.213)	<0.001*	−1.303 (−1.632, −0.974)	<0.001*	3.705 (3.434, 3.977)	<0.001*	2.828 (2.466, 3.190)	<0.001*

**P < 0.05 was considered statistically significant. §Adjusted for aborigine status and urbanization. AWG, annual weight gain; AHG, annual height gain; CI, confidence interval; BMI, body mass index.*

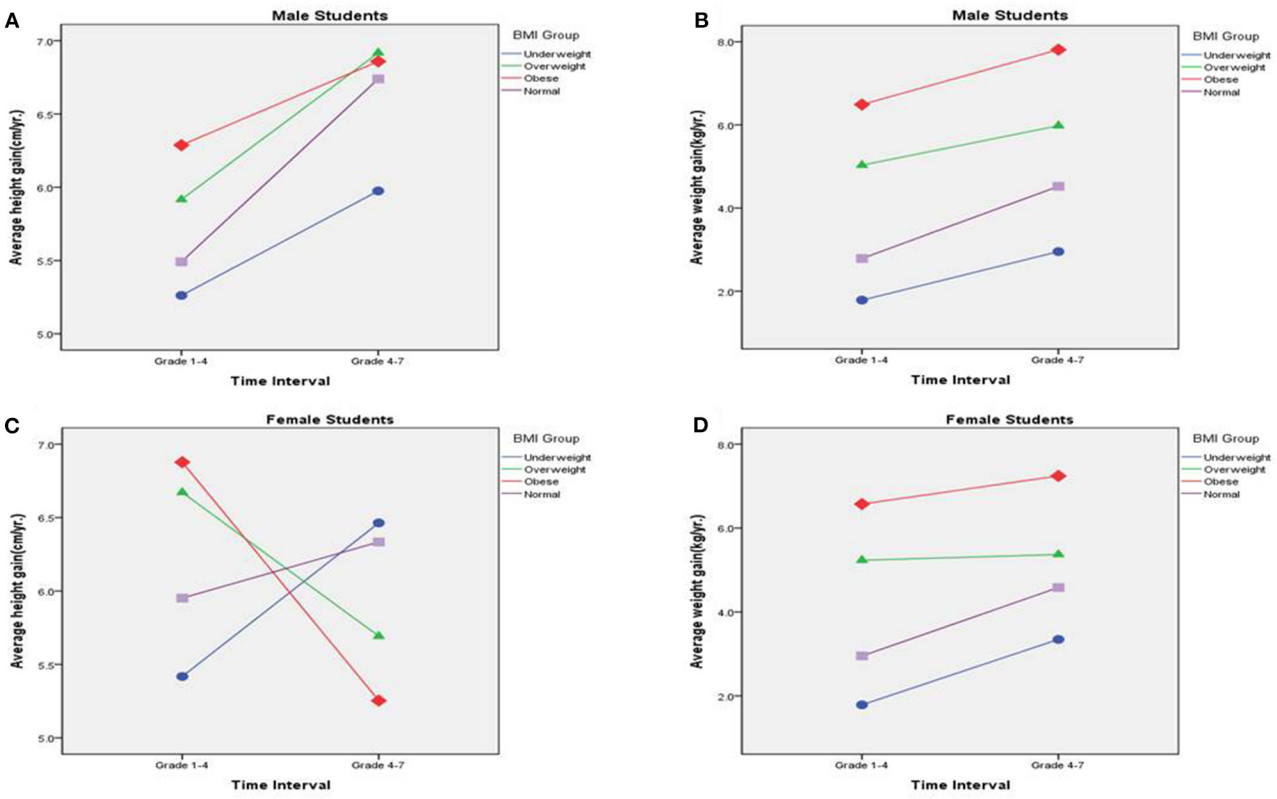

FIGURE 2 | (A–D) Results of repeated measures analysis of variance for male and female subjects.

and 9 years of age had significant high growth velocity compared to boys with normal BMI, high BMI did not have a strong impact on growth velocity in boys between 9 and 13 years of age when emerging into puberty. It is still controversial whether obesity has an impact on the early onset of puberty in boys (11). The present study illustrated that low BMI still reduced the growth velocity in underweight boys aged 9–13 years. However, longitudinal growth data from previous studies revealed that adult heights were not different among different BMI groups (17). In addition, taller standing heights during early adolescence (10–15 years of age) were observed among children with higher BMIs during mid-childhood (6–9 years of age). Furthermore,

the greater heights in early adolescence were mainly attributable to longer trunks, rather than longer legs (11). De Leonibus et al. (15) reported that mean ages at the onset of pubertal boys were about 11.66 years in the obese group and 12.12 years in the normal-weight group. Similarly, late puberty was reached at a younger age in obese children. Mean ages at late puberty of boys were 13.33 years in obese children and 14.47 years in normal-weight children (15). Although the present study lacks data on the physical findings of Tanner stages in both sexes, we could still speculate that obese and overweight boys may have lower growth velocity than boys with normal or underweight BMI after 13 years of age because most of the obese and overweight boys would complete their growth spurt by then. More data on the growth velocity of male students after 13 years of age should be collected in the future to confirm this hypothesis.

Nutrition status and BMI have been associated with growth velocity, as well as weight and height in childhood, and obese subjects are taller than non-obese ones in childhood (11, 14, 27). Some possible mechanisms have been proposed as responsible for this association, and correlations of growth hormone-binding protein, fasting insulin level, and insulin-like growth factor (IGF)-1 level with BMI were mentioned in some studies (11, 14, 27–29). The regulation of the growth hormone (GH)–IGF-1 axis has also been associated with prepuberty BMI (29). GH secreted from the anterior pituitary gland not only promotes the growth of bone and muscle but also has an essential role in the regulation of lipids. GH stimulates the secretion of IGF-1, and both GH and IGF-1 have been proven to be related to linear growth (30). In obese children, decreased GH secretion has been noted (28). However, hyperinsulinemia due to obesity-related insulin resistance may also be related to elevated free IGF-1 levels in obese children. An increase in free IGF-1 level is thought to be associated with rapid linear growth and skeletal maturation (14, 31). Thus, the above mechanisms may explain our finding of higher linear growth velocity in pre-pubertal obese children aged 6–10 years of age.

In Taiwan, parents and grandparents are usually proud of the high linear growth velocity of their obese children, rather than of obesity itself. They have a strong belief in our traditional proverb "childhood obesity is not obese at all" and often seek medical assistance for their children late when obesity-related complications, such as hypertension and high blood sugar or cholesterol, occur; however, delayed intervention for obesity leads to more harm to health. To achieve good family health, physicians should engage the concerns of parents to empower their knowledge of healthy literacy in nutrition, exercise, and parenting skills. A family-based exercise plan, healthy diet plan, and structural daily schedule will provide a supportive environment for children after leaving the school campus. Reward options should not include snacks, sweetened beverages, or high calorie foods; instead, outdoor sports or tours are recommended to promote physical activity. Management of childhood obesity is progressive and age-dependent, and it should be initiated as early as possible (32, 33). Based on the report of the Commission on Ending Childhood Obesity by the WHO in 2016, comprehensive programs that promote healthy school environments, health and nutrition literacy, and physical activity among school-age children and adolescents should be established and assisted by family-based, multicomponent lifestyle weight management services (34).

LIMITATIONS

This study has some limitations that need to be addressed. First, the retrospective data in our study were collected from the annual health examination dataset of elementary school students in Hualien County; we did not have information of the subjects outside the period evaluated. Thus, we did not know their physical measurements after birth, Tanner stage, and final height in adulthood. Second, many factors affect the linear growth velocity in addition to obesity, such as exercise, diet, underlying diseases, and parents' heights, which were not collected during the regular school health examinations in our dataset. Further studies should be conducted to evaluate the associations between these factors and growth velocity. Third, the timing of puberty onset is essential to evaluate the change of growth velocity, especially in subjects with different BMIs. They should have different times for the onset of puberty, but no reliable physical findings of puberty were recorded in the present study.

CONCLUSIONS

We support current models that higher BMI accelerates linear growth in childhood, but slows linear growth during early adolescence. Our findings illustrated that obese subjects tended to have higher linear growth velocity than subjects with normal BMI. However, underweight girls had higher linear growth between 9 and 13 years of age, which coincided with the onset of puberty and a growth spurt. Thus, we propose that puberty dominates over BMI as the main contributor to high growth velocity in underwent early adolescent girls.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of Hualien Tzu Chi Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

C-HC, Y-HC, J-SC, R-HJ, S-HY, M-CC, S-YC, P-CL, C-FC, P-YC, and Y-HL contributed to data collection. J-HW and Y-CC analyzed and interpreted the subjects' data. Y-CH and Y-CC were

major contributors to writing the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.599730/full#supplementary-material

REFERENCES

1. Skinner AC, Ravanbakht SN, Skelton JA, Perrin EM, Armstrong SC. Prevalence of obesity and severe obesity in US children, 1999–2016. *Pediatrics*. (2018) 141:e20173459. doi: 10.1542/peds.2017-3459
2. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet*. (2017) 390:2627–42. doi: 10.1016/s0140-6736(17)32129-3
3. Chang HC, Yang HC, Chang HY, Yeh CJ, Chen HH, Huang KC, et al. Morbid obesity in Taiwan: prevalence, trends, associated social demographics, and lifestyle factors. *PLoS ONE*. (2017) 12:e0169577. doi: 10.1371/journal.pone.0169577
4. Nam HK, Kim HR, Rhie YJ, Lee KH. Trends in the prevalence of extreme obesity among Korean children and adolescents from 2001 to 2014. *J Pediatr Endocrinol Metab*. (2017) 30:517–23. doi: 10.1515/jpem-2016-0456
5. Chen LJ, Fox KR, Haase A, Wang JM. Obesity, fitness and health in Taiwanese children and adolescents. *Eur J Clin Nutr*. (2006) 60:1367–75. doi: 10.1038/sj.ejcn.1602466
6. Chu CH, Wang JH, Jan RH, Huang CH, Cheng CF. Association between health examination items and body mass index among school children in Hualien, Taiwan. *BMC Public Health*. (2013) 13:975. doi: 10.1186/1471-2458-13-975
7. Vazquez MJ, Velasco I, Tena-Sempere M. Novel mechanisms for the metabolic control of puberty: implications for pubertal alterations in early-onset obesity and malnutrition. *J Endocrinol*. (2019) 242:R51–65. doi: 10.1530/JOE-19-0223
8. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. *Pediatrics*. (2002) 109:194–9. doi: 10.1542/peds.109.2.194
9. Winter JD, Langenberg P, Krugman SD. Newborn adiposity by body mass index predicts childhood overweight. *Clin Pediatr*. (2010) 49:866–70. doi: 10.1177/0009922810369698
10. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. *Nature*. (2008) 453:783–7. doi: 10.1038/nature06902
11. Aris IM, Rifas-Shiman SL, Zhang X, Yang S, Switkowski K, Fleisch AF, et al. Association of BMI with linear growth and pubertal development. *Obesity*. (2019) 27:1661–70. doi: 10.1002/oby.22592
12. Marinkovic T, Toemen L, Kruithof CJ, Reiss I, van Osch-Gevers L, Hofman A, et al. Early infant growth velocity patterns and cardiovascular and metabolic outcomes in childhood. *J Pediatr*. (2017) 186:57–63.e4. doi: 10.1016/j.jpeds.2017.02.004
13. Militello LK, Kelly S, Melnyk BM, Smith L, Petosa R. A review of systematic reviews targeting the prevention and treatment of overweight and obesity in adolescent populations. *J Adolesc Health*. (2018) 63:675–87. doi: 10.1016/j.jadohealth.2018.07.013
14. He Q, Karlberg J. BMI in childhood and its association with height gain, timing of puberty, and final height. *Pediatric Res*. (2001) 49:244–51. doi: 10.1203/00006450-200102000-00019
15. De Leonibus C, Marcovecchio ML, Chiavaroli V, de Giorgis T, Chiarelli F, Mohn A. Timing of puberty and physical growth in obese children: a longitudinal study in boys and girls. *Pediatr Obes*. (2014) 9:292–9. doi: 10.1111/j.2047-6310.2013.00176.x
16. Sunder M. Shrinking due to corpulence? BMI in childhood predicts subsequent linear growth among US children and youth, 1963–1970. *Ann Hum Biol*. (2008) 35:432–8. doi: 10.1080/03014460802232706
17. Holmgren A, Niklasson A, Nierop AF, Gelander L, Aronson AS, Sjöberg A, et al. Pubertal height gain is inversely related to peak BMI in childhood. *Pediatric Res*. (2017) 81:448–54. doi: 10.1038/pr.2016.253
18. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of menarche. *Arch Dis Child*. (1971) 46:695–701.
19. Reinehr T, Roth CL. Is there a causal relationship between obesity and puberty? *Lancet Child Adolesc Health*. (2019) 3:44–54. doi: 10.1016/S2352-4642(18)30306-7
20. Liu W, Lin R, Liu A, Du L, Chen Q. Prevalence and association between obesity and metabolic syndrome among Chinese elementary school children: a school-based survey. *BMC Public Health*. (2010) 10:780. doi: 10.1186/1471-2458-10-780
21. Liu CY, Hung YT, Chuang YL, Chen YJ, Weng WS, Liu JS. Incorporating development stratification of Taiwan townships into sampling design of large scale health interview survey. *J Health Manage*. (2006) 4:1–22. doi: 10.29805/JHM.200606.0001
22. Lee C-T, Tsai M-C, Lin C-Y, Strong C. Longitudinal effects of self-report pubertal timing and menarcheal age on adolescent psychological and behavioral outcomes in female youths from Northern Taiwan. *Pediatr Neonatal*. (2016) 58:313–20. doi: 10.1016/j.pedneo.2016.04.004
23. Li W, Liu Q, Deng X, Chen Y, Liu S, Story M. Association between obesity and puberty timing: a systematic review and meta-analysis. *Int J Environ Res Public Health*. (2017) 14:1266. doi: 10.3390/ijerph14101266
24. Nokoff N, Thurston J, Hilkin A, Pyle L, Zeitler PS, Nadeau KJ, et al. Sex differences in effects of obesity on reproductive hormones and glucose metabolism in early puberty. *J Clin Endocrinol Metab*. (2019) 104:4390–7. doi: 10.1210/jc.2018-02747
25. Bygdell M, Kindblom JM, Celind J, Nethander M, Ohlsson C. Childhood BMI is inversely associated with pubertal timing in normal-weight but not overweight boys. *Am J Clin Nutr*. (2018) 108:1259–63. doi: 10.1093/ajcn/nqy201
26. Lian Q, Mao Y, Luo S, Zhang S, Tu X, Zuo X, et al. Puberty timing associated with obesity and central obesity in Chinese Han girls. *BMC Pediatr*. (2019) 19:1. doi: 10.1186/s12887-018-1376-4
27. De Simone M, Farello G, Palumbo M, Gentile T, Ciuffreda M, Olioso P, et al. Growth charts, growth velocity and bone development in childhood obesity. *Int J Obes Relat Metab Disord*. (1995) 19:851–7.
28. Juul A, Dalgaard P, Blum WF, Bang P, Hall K, Michaelsen KF, et al. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. *J Clin Endocrinol Metab*. (1995) 80:2534–42. doi: 10.1210/jcem.80.8.7543116

29. Mauras N. Growth hormone and sex steroids. Interactions in puberty. *Endocrinol Metab Clin North Am*. (2001) 30:529–44. doi: 10.1016/s0889-8529(05)70200-0
30. Dehkhoda F, Lee CMM, Medina J, Brooks AJ. The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. *Front Endocrinol*. (2018) 9:35. doi: 10.3389/fendo.2018.00035
31. Artz E, Haqq A, Freemark M. Hormonal and metabolic consequences of childhood obesity. *Endocrinol Metab Clin North Am*. (2005) 34:643–58. doi: 10.1016/j.ecl.2005.04.004
32. Cuda SE, Censani M. Pediatric obesity algorithm: a practical approach to obesity diagnosis and management. *Front Pediatr*. (2019) 6:431. doi: 10.3389/fped.2018.00431
33. Imoisili OE, Goodman AB, Dooyema CA, Park S, Harrison M, Lundeen EA, et al. Referrals and management strategies for pediatric obesity—docstyles survey 2017. *Front Pediatr*. (2018) 6:431. doi: 10.3389/fped.2018.00367
34. World Health Organization. *Report of the Commission on Ending Childhood Obesity*. (2016). Available online at: https://www.who.int/end-childhood-obesity/news/launch-final-report/en/ (accessed January 2, 2021).

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Liver Enzymes Correlate With Metabolic Syndrome, Inflammation, and Endothelial Dysfunction in Prepubertal Children With Obesity

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Background: Metabolic syndrome (MetS) can start in children with obesity at very young ages. Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic component of metabolic syndrome. If left untreated, the clinical course of NAFLD can be progressive and can become chronic if not detected at an early stage.

Objective: We aimed to quantify the differences in liver enzymes between prepubertal children with obesity and children with normal weight to determine any associations between them and parameters related to MetS, adipokines, or markers of endothelial dysfunction and inflammation.

Methods: This cross-sectional study included 54 prepuberal children with obesity (aged 6–9 years) and 54 children with normal weight, matched by age and sex. Liver enzymes, C-reactive protein (CRP), interleukin-6, soluble intercellular adhesion molecule-1 (sICAM-1), adipokines, and parameters related to metabolic syndrome (MetS) were all measured.

Results: Alanine aminotransferase (ALT) levels, serum butyryl cholinesterase (BChE), leptin, CRP, sICAM-1, triglycerides, blood pressure, and homeostasis model assessment for insulin resistance were significantly higher in children with obesity, while Apolipoprotein A-1, HDL-cholesterol, and adiponectin were significantly lower. In the children with obesity group, ALT and BChE levels correlated with anthropometric measurements, insulin resistance, and lipid parameters, leptin, interleukin-6, CRP, and sICAM-1 while BChE levels negatively correlated with adiponectin.

Conclusions: Compared to children with normal weight, prepubertal children with obesity had elevated values for liver enzymes, leptin, markers of insulin resistance, inflammation, and endothelial dysfunction, and variables associated with MetS. There was also a correlation between these disorders and liver enzyme levels.

Keywords: liver enzymes, prepuberal age, obesity, inflammation, metabolic syndrome, endothel dysfunction

INTRODUCTION

Metabolic syndrome (MetS) can start in obese children at very young ages (1), and a not negligible proportion of prepubertal children were even classified as affected by metabolic syndrome in the IDEFICS study (2). The presentation of this syndrome in children is associated with a high risk for diabetes and atherosclerotic cardiovascular disease during adulthood (3).

Together with the disorders that define metabolic syndrome, a subclinical inflammation state indicative of alterations producing endothelial dysfunction, as well as changes in adipokine levels, have been documented in prepubertal children with obesity (4–6).

Non-alcoholic fatty liver disease (NAFLD) is associated with important components of MetS such as obesity (7, 8), some authors consider these to be hepatic manifestations of this syndrome (9) and its prevalence increases in line with that of MetS and its components, particularly obesity (10). NAFLD has now become the most frequent cause of chronic liver disease, both in children and adults (10, 11).

The pathogenesis of NAFLD is multifactorial, dietary factors, insulin resistance (IR), inflammation, adipocytokines, lipotoxicity, and a genetic predisposition, are all factors that may be involved (12).

Elevated Alanine aminotransferase (ALT) levels are associated with the incidence of MetS, diabetes mellitus, and cardiovascular disease and have been shown to be a predictive factor for non-alcoholic steatosis (13, 14). Elevated serum butyryl cholinesterase (BChE) levels have also been described in individuals with obesity and MetS (15) and there is an association between BChE activity, lipid metabolism, and MetS in adults (16).

Adipose tissue plays an important role in the pathogenesis of NAFLD by contributing to the low-grade inflammation strongly related to this disease (17). In a subsample of European children forming part of the IDEFICS study, the highest levels of leptin were associated with metabolic syndrome, regardless of body mass. The measurement of leptin could help to discriminate overweight/obese children with an increased risk of cardio-metabolic complications while still at an early age (18).

The available data indicate that obesity-associated metabolic disorders, including NAFLD, IR, and inflammation, as well as altered levels of adipokines, can onset at very early ages. Although limited, the data on the natural history of pediatric NAFLD show that a few children rapidly progress from NAFLD to clinical events, some of which are even more severe than those diagnosed in adults (19, 20). This emphasizes the potential importance of applying preventive interventions in patients while they are still young, and consequently, the need for diagnostic tools that are easy to use in daily clinical practice.

Abbreviations: MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; IR, insulin resistance; ALT, alanine aminotransferase; BChE, butyryl cholinesterase; BMI, body mass index; AST, aspartate aminotransferase; sICAM-1, soluble intercellular adhesion molecule-1; CRP, C-reactive protein; IL-6, Interleukin- 6; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model formula for IR; Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; FFAs, free fatty acids; SEM, standard error mean.

Limited information is available about the possible association between liver-function enzymes, obesity-related disorders, and NAFLD in prepubescent children. There is currently a lack of predictors of NAFLD among young children with obesity. The seriousness of these pathologies and the consequences of their clinical evolution without treatment serve to highlight the need for their early diagnosis and treatment. Therefore, we must try to develop diagnostic tools that can be applied in general clinical practice.

Thus, in this study we aimed to analyse the possible differences between children with obesity and prepubertal children with normal weight in liver enzyme values to determine any associations between them and parameters related to MetS, adipokines, and markers of endothelial dysfunction or inflammation.

MATERIALS AND METHODS

Study Design

We carried out a cross-sectional study in prepubertal (Tanner stage 1) children with obesity of both sexes. To reduce selection bias, both groups, cases and controls were selected from the same population, and case control ratio observed was of 1:1, matched by age and sex.

Inclusion criteria were the same for the study group and control group with the exception for BMI: prepubertal (Tanner stage 1) children of both sexes and aged 6–9 years. The children were placed into one or the other group according to whether their BMI categorized them as with obesity (cases) or normoweight (control).

Exclusion criteria for both groups: children with diabetes (fasting glucose $\geq$ 7.0 mmol/L), impaired fasting glucose (fasting glucose $\geq$ 6.1 mmol/L or $<$ 7.0 mmol/L), primary hyperlipidaemia, hypertension, aspartate aminotransferase $>$40 U/L, or secondary obesity were excluded from both the study. None of the participants were receiving any regular treatments with any medications. Children with CRP levels $>$ 10 mg/L (which thus indicates the presence of clinically relevant inflammatory conditions), were excluded from both the children with obesity group and the control group. ALT levels must be interpreted in the context of the gender specific upper limits of normality in children (22 U/L for girls and 26 U/L for boys) and not upon the upper limits of normality provided by individual laboratories (14). Reference curves for leptin and adiponectin in the IDEFICS population may be useful when trying to assess the limits of their normality (21).

Study Population

All the children in this study were Spanish. Both, children with obesity and children with normal weight were selected from the same schools, and had similar lifestyles. We informed in four schools in the area about this study in order to recruit the participants (Córdoba, Spain). All the parents of the children included in this study gave their written consent, and the study was authorized by the local hospital ethics committee.

First, parents were invited to a briefing on this study and asked to participate in it. All parents with children between the ages of

6 and 9 were summoned. All students whose parents signed the informed consent were included in the study consecutively, by order of registration.

One group included 54 children with obesity [with a body mass index (BMI) exceeding the 95th percentile in the reference tables for the Spanish population] (22), and the control group included an equal number of children with a normal weight (under the 85th percentile) matched by age and sex (aged 6–9 years). Taking ALT as the main variable, and given that the standard deviations for the two groups were 0.061 and 0.083, respectively, and, the expected mean difference was 0.040, to achieve a power of 80% at a confidence level of 95%, we calculated that 54 patients would be required in each of the two groups.

Anthropometric Measurements and Blood Sampling and Analysis

After a fasting for 12 h, blood samples were collected without venous occlusion from a vein in the antecubital fossa. All the samples were collected between 8:00 a.m. and 9:00 a.m., and were divided into aliquots and immediately frozen at −45°C until their analysis. Serum ALT, aspartate aminotransferase (AST), serum cholinesterase, leptin, adiponectin, soluble intercellular adhesion molecule-1 (sICAM), C-reactive protein (CRP), and interleukin-6 (IL-6) levels, as well as a range of MetS-related variables (glucose, insulin, lipids, and blood pressure) were measured in all the children.

Serum glucose, ALT, AST, BChE, creatine kinase (CK), total cholesterol, and triglycerides (TG) concentrations were measured using a random-access analyser (ADVIA 1800, Siemens Healthcare Diagnostics) with reagents from the same manufacturer. Insulin was quantified using an Access 2-Immunoassay System (Beckman Coulter, Brea, CA, USA). High-density lipoprotein cholesterol (HDL-c) was measured after precipitation of chylomicrons, very low-density lipoproteins, and low-density lipoprotein cholesterol (LDL-c) with phosphotungstic acid and magnesium ions. The LDL-c concentration was calculated using the Friedewald formula.

IR was assessed using the homeostasis model formula for IR (HOMA-IR) based on fasting glucose and insulin concentrations: resistance = [insulin (mU/L) × glucose (mmol/L)]/22.5. Apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B), and CRP were measured by nephelometry (N Antisera to Human Apo AI, Apo B, and N high-sensitivity CRP reagent; Behringwerke AG, Marburg, Germany) in a Dade Behring Analyzer II Nephelometer (Dade Behring, Inc., Deer field, IL, USA).

Antigenic immunoassay methods were used to quantify adiponectin (Quantikine human adiponectin, R&D Systems, Wiesbaden-Norderstedt, Germany), sICAM-1 (IBL Immuno-Biological Laboratories, Hamburg, Germany), leptin (Quantikine human leptin, R&D Systems, Wiesbaden-Norderstedt, Germany), and IL-6 (Quantikine Human IL-6; RD Systems, Wiesbaden-Norderstedt, Germany) in a microtiter plate analyser (Personal LAB, Phadia Spain SL, Barcelona, Spain).

Free fatty acids (FFAs) were quantified by a colorimetric enzyme assay (NEFA C ACS-ACOD Method, Woko Chemicals GmbH, Neuss, Germany). Blood pressure was measured with a mercury sphygmomanometer (Pymah Corporation, Sommerville, NJ, USA) after resting for 20 min in a supine position. The measurements were performed on three consecutive days and the mean was used in our analyses. The sphygmomanometer cuff width had to cover 2/3 of the length of the child's arm and so three cuff sizes were available for use (9 cm × 32 cm, 11 cm × 36 cm, and 12 cm × 41 cm). Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. BMI was calculated as the weight (kg)/height $(m)^2$.

Statistical Analysis

Statistical assessment was performed using Microstat (Ecosoft, Indianapolis, IN, USA) or GraphPad InStat software (GraphPad Software, San Diego, CA, USA). Abnormal values (outliers) were excluded by applying Reed's method. The distribution of each variable was tested to check for deviance from the Gaussian distribution, and the equality of the variance was checked by using Snedecor's *F*-test. The mean values of the groups were compared by applying Student's unpaired *t*-tests. All the results were expressed as a mean ± standard error mean (*SEM*) with a 95% confidence interval (95% CI). Statistical significance was set at $p < 0.05$. The correlation between the variables was evaluated using Pearson's correlation coefficient and regression analysis. Multivariate regression analysis was performed using the stepwise method. For each variable, potential confounders ($0.05 < p < 0.2$) were evaluated by analyzing the raw and adjusted regression coefficients.

RESULTS

Liver Enzymes, Insulin Resistance, and Parameters Related to MetS

The clinical, anthropometric, and biochemical parameters related to MetS were measured in both groups (**Table 1**). The mean ALT levels were significantly higher in children with obesity, at 19.57 U/L (95% CI [18.21–20.93]) compared to 17.20 U/L in the control group (95% CI [16.21–18.19]; **Table 2**). Although the BChE levels were higher in the children with obesity group at 11,178.5 U/L (95% CI [10,888.9–11,468.2]) compared to 10,636.3 U/L in the control group (95% CI [10,201.1–11,072.1]; **Table 2**). No significant differences were found in the AST values.

The univariate correlation analysis for MetS-related parameters for the children with obesity group is summarized in **Table 3** and **Figure 1**. In the single linear correlation, ALT and BChE levels correlated with anthropometric measurements, IR, and lipid parameters related to MetS, while the ALT/AST indices correlated only with the anthropometric measurements.

In the children with obesity group, age, and sex-corrected multivariate regression analysis showed that the BMI (P partial = 0.0074), waist circumference (P partial = 0.0057), serum insulin (P partial = 0.0014), HOMA-IR (P partial = 0.0009), TG (P partial = 0.0287), HDL-c (P partial = 0.0369), and Apo A1 (P partial = 0.032) were independent predictive factors for ALT. For serum BChE, the age and sex-corrected BMI

TABLE 1 | Comparison between children with obesity and children with normal weight.

	Children with obesity ($n = 54$)	Children with normal weight ($n = 54$)	p
Age (years)	7.95 ± 0.14	7.89 ± 0.12	0.7414
Male/Female	25/29	25/29	
BMI (Kg/m^2)	23.34 ± 0.28	16.76 ± 0.19	<0.0001
BMI *z*-score	3.30 ± 0.15	0.06 ± 0.01	<0.0001
Waist circumference (cm)	73.39 ± 0.89	57.74 ± 1.16	<0.0001
SBP (mm Hg)	100.99 ± 1.49	89.36 ± 1.23	<0.0001
DBP (mm Hg)	62.34 ± 1.12	52.36 ± 1.07	<0.0001
Total cholesterol (mmol/L)	4.49 ± 0.08	4.41 ± 0.08	0.5006
Triglycerides (mmol/L)	0.76± 0.04	0.59 ± 0.16	0.0002
HDL-cholesterol (mmol/L)	1.32 ± 0.03	1.47 ± 0.04	0.0013
Apolipoprotein A1 (μmol/L)	53.5 ± 0.71	57.86 ± 1.07	0.0001
Apolipoprotein B (μmol/L)	26.43 ± 0.71	23.93 ± 0.36	0.0038
Free fatty acids (mmol/L)	0.548 ± 0.03	0.502 ± 0.02	<0.0001
Glucose (mmol/L)	5.05 ± 0.06	4.78 ± 0.04	0.0003
Insulin (pmol/L)	47.5 ± 2.64	38.68 ± 1.80	0.0066
HOMA-IR	1.554 ± 0.095	1.187 ± 0.059	0.0013

Clinical and anthropometric measurements, and biochemical parameters related to metabolic syndrome.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment for insulin resistance. Results are expressed as the mean ± S.E.M.

TABLE 2 | Comparison between children with obesity and children with normal weight.

	Children with obesity (n = 54)	Children with normal weight (n = 54)	p
ALT (U/L)	19.57 ± 0.68	17.20 ± 0.49	0.0058
AST (U/L)	24.81 ± 0.43	24.51± 0.53	0.655
BChE (U/L)	11178.5 ± 144.3	10636.3± 217.6	0.0413
IL-6 (pg/mL)	1.71 ± 0.14	1.43 ± 0.27	0.2526
CRP (mg/L)	2.34 ± 0.25	0.89 ± 0.22	<0.0001
sICAM-1 (ng/mL)	284.9 ± 8.84	250.3 ± 6.33	0.0006
Leptin (ng/mL)	19.39 ± 1.37	4.16 ± 0.54	<0.0001
Adiponectin (ng/mL)	9.74 ± 0.68	11.59 ± 0.67	0.0161
Creatine kinase (U/L)	113.39 ± 4.50	117.18 ± 5.98	0.6142

Hepatic enzymes, adipokines, inflammation, and endothelial biomarkers.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BChE, butyryl cholinesterase; IL-6, interleukin-6; CRP, C-reactive protein; sIACM-1, soluble intercellular adhesion molecule-1. Results are expressed as the mean ± S.E.M.

(P partial = 0.0005), waist circumference (P partial = 0.0251), TG (P partial = 0.0045), and Apo A1 (P partial = 0.0327), but not insulin (P partial = 0.1071), HOMA-IR (P partial = 0.1138), or HDL-c (P partial = 0.0599) were independent predictive factors.

In the combined group (children with obesity group and children with normal weight together), serum ALT positively correlated with BMI ($p = 0.0007$), waist circumference ($p = 0.0009$), insulin ($p < 0.0001$), HOMA-IR ($p < 0.0001$), and TG ($p = 0.0010$), and negatively correlated with HDL-c ($p = 0.0121$), and Apo A1 ($p = 0.0126$) while serum BChE positively correlated with BMI ($p = 0.0007$), waist circumference ($p = 0.0143$), insulin ($p = 0.0494$), HOMA-IR ($p = 0.0320$), and TG ($p = 0.0015$), and negatively with HDL-c ($p = 0.0416$).

Liver Enzymes, Inflammation, Endothelial Dysfunction, and Adipokines

Table 2 shows liver enzymes, adipokines, inflammation, and endothelial biomarkers for children with obesity and children with normal weight. The mean values for CRP, sICAM, and leptin were significantly higher in the children with obesity and adiponectin levels were significantly lower.

The univariate correlation analysis for liver enzymes, adipokines, inflammation, and endothelial biomarkers for children with obesity is summarized in **Table 3** and **Figure 2**. The ALT levels and the ALT/AST index positively correlated with IL-6, CRP, sICAM-1, and leptin, but not with adiponectin values. Serum BChE levels positively correlated with inflammation

TABLE 3 | Single correlation coefficients (*r*) between different variables in the children with obesity group.

	ALT		AST		ALT/AST		BChE	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
BMI	0.3705	0.0063	−0.1319	0.3466	0.4286	0.0013	0.4733	0.0003
BMI *z*-score	0.3112	0.0247	−0.0616	0.6578	0.2752	0.044	0.3409	0.0124
Waist circumference	0.4057	0.0035	−0.2489	0.0773	0.4057	0.0035	0.3019	0.0257
Glucose	0.3007	0.0286	−0.0597	0.668	0.3128	0.0224	0.0765	0.5822
Insulin	0.4452	0.0007	−0.1293	0.3512	0.4583	0.0005	0.2816	0.0414
HOMA-IR	0.4581	0.0005	−0.1220	0.3795	0.4696	0.0003	0.2739	0.0471
SBP	−0.2270	0.1098	−0.1848	0.1937	−0.0864	0.5463	0.0837	0.5592
DBP	−0.1469	0.3098	−0.1814	0.2012	−0.1090	0.4461	0.1051	0.4629
Total cholesterol	0.07	0.6148	−0.0639	0.6458	0.1116	0.4219	0.0659	0.6391
Triglycerides	0.3327	0.0156	0.0304	0.8318	0.2404	0.0887	0.4199	0.0017
HDL cholesterol	−0.2998	0.0287	−0.0474	0.7394	−0.1990	0.1574	−0.3024	0.0279
Apo A1	−0.3125	0.0237	−0.0057	0.9694	−0.2416	0.0882	−0.2993	0.0289
Apo B	0.1792	0.1947	0.0222	0.8733	0.1708	0.2168	0.0276	0.8429
IL-6	0.3631	0.007	0.0159	0.669	0.2853	0.0365	0.3075	0.0251
CRP	0.4159	0.0018	0.1965	0.1544	0.2795	0.0407	0.3117	0.0243
sICAM-1	0.323	0.0187	0.1332	0.3468	0.3262	0.0181	0.5333	<0.0001
Leptin	0.3633	0.0069	−0.3562	0.0082	0.5336	<0.0001	0.2522	0.0701
Adiponectin	0.0779	0.6058	−0.1961	0.1876	0.1916	0.1587	−0.4327	0.0006

BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; IL-6, interleukin-6; CRP, C-reactive protein; sIACM-1, soluble intercellular adhesion molecule-1.

and endothelial biomarkers and negatively correlated with the adiponectin concentration.

A multivariate regression analysis was carried out for the children with obesity group. When adjusted for age and sex, levels of IL-6 (P partial = 0.0090), CRP (P partial = 0.0032), sICAM-1 (P partial = 0.0224), and leptin (P partial = 0.0223) were independent predictive factors for ALT. For serum BChE, age and sex-corrected levels of IL-6 (P partial = 0.0481), CRP (P partial = 0.0497), sICAM-1 (P partial < 0.0001), and Apo A1 (P partial = 0.0223) were independent predictive factors.

In the combined children with obesity and children with normal weight group, serum ALT positively correlated with levels of IL-6 ($p = 0.0040$), CRP ($p = 0.0003$), sICAM-1 ($p = 0.0018$), and leptin ($p < 0.0001$), but not with adiponectin (p = 0.5397) while BChE levels positively correlated with IL-6 ($p = 0.0481$), sICAM-1 (P partial = 0.0004), and leptin ($p = 0.0047$) levels, and negatively correlated with adiponectin ($p = 0.0082$).

DISCUSSION

MetS is associated with an increased risk of type 2 diabetes, cardiovascular disease, and all-cause mortality (23). Moreover, children with metabolic syndrome are at an increased risk of cardiovascular disease in adulthood (24). We and other authors have detected low-grade systemic inflammation, alterations indicative of endothelial dysfunction, and altered adipokine levels alongside the disorders that define MetS (4–6) as well as an increase in leptin and decrease in adiponectin in children with obesity. In this work we described an increase in liver-function enzymes in prepubescent children with obesity compared to children with normal weight of an equal age. In addition to the variables related to MetS, we also correlated the values of these enzymes with markers of inflammation, endothelial dysfunction, and adipokine levels.

Liver Enzymes, Insulin Resistance, and Parameters Related to MetS

Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic component of metabolic syndrome (25). In concert with the increase in prevalence rates of obesity and metabolic syndrome, the prevalence of NAFLD has increased dramatically to over 25% of the population worldwide (26).

The prevalence of NAFLD in children overweight or obesity ranges from 29.8 to 34.7% (27) and can cause a broad spectrum of lesions (9). The risk of steatosis and non-alcoholic steatohepatitis increases in children with obesity with MetS (9). Individuals with NAFLD had a significantly higher risk of cardiovascular disease (28).

The high prevalence of NAFLD, and its possible serious health consequences, make its early detection important because simple steatosis is reversible by lifestyle modifications, especially weight loss (29). Some expert committees recommend the use of serum ALT levels to screen for NAFLD in children older than 10 years (14). In this work, we analyzed the biochemical variables related to obesity and NAFLD before puberty. Studying these in this age group also allowed us to study how these parameters affect IR by eliminating the interference produced by hormonal changes during puberty.

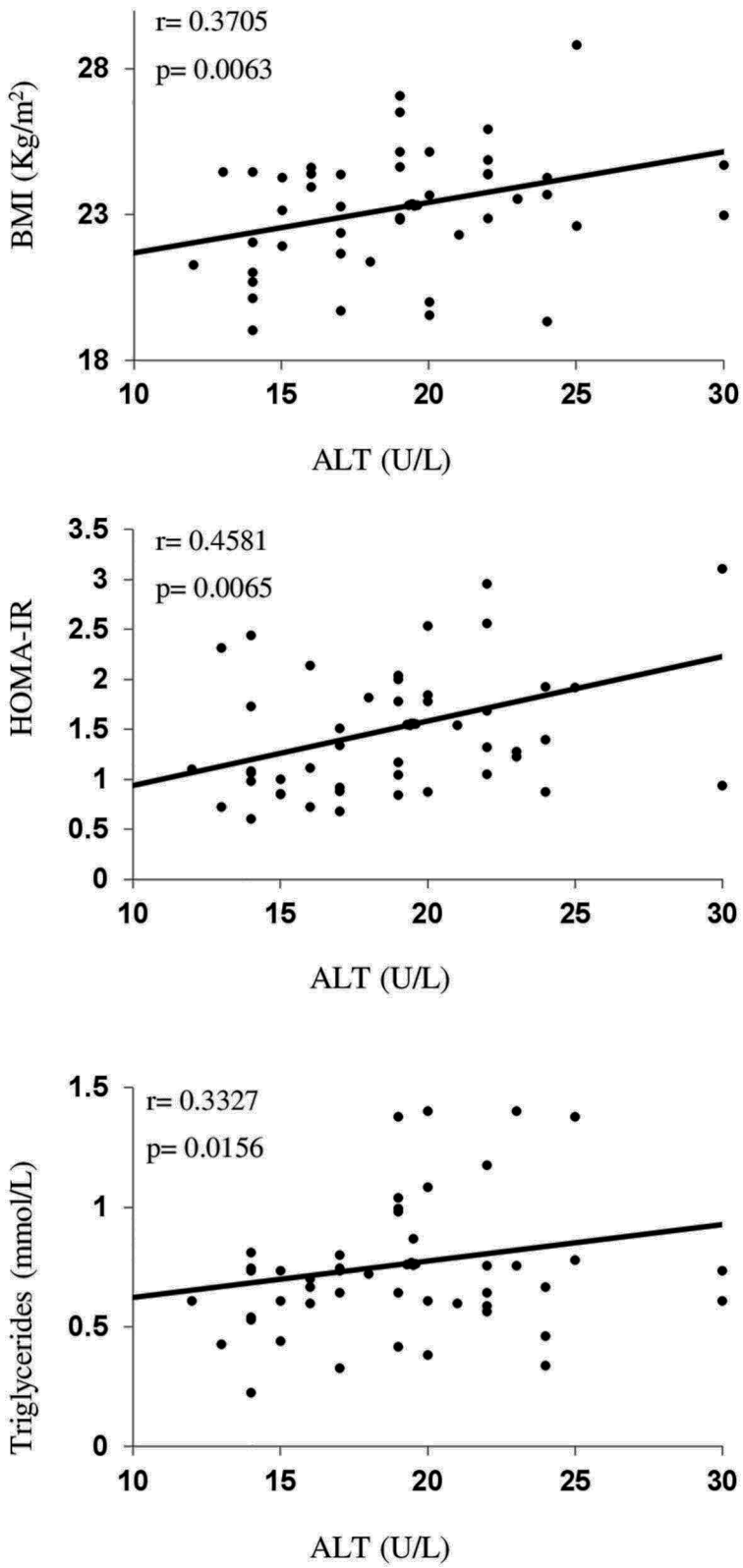

FIGURE 1 | Serum alanine aminotransferase (ALT) levels as a function of body mass index (BMI), homeostasis model assessment for insulin resistance (HOMA-IR), and triglycerides in children with obesity group.

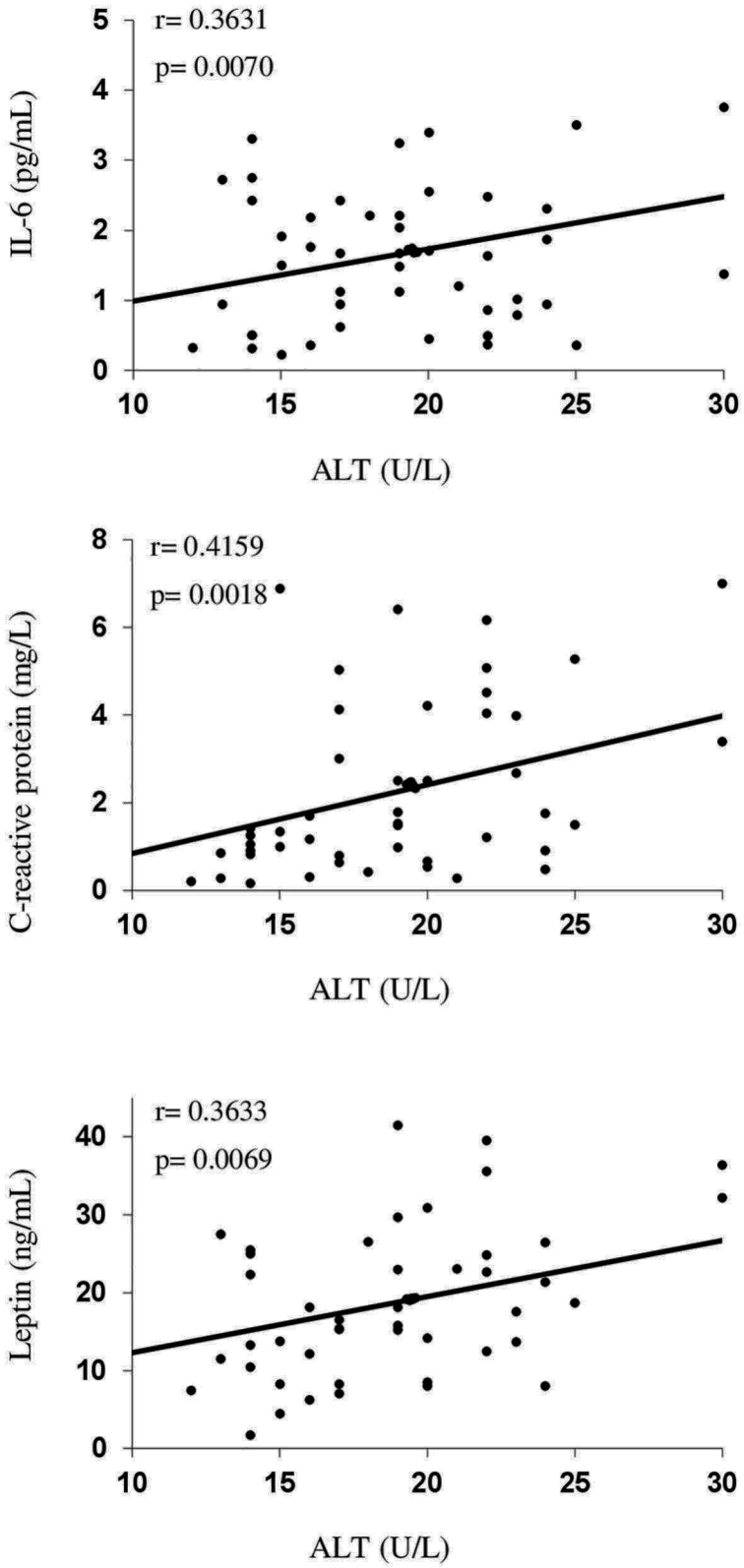

FIGURE 2 | Serum alanine aminotransferase (ALT) levels as a function of interleukin-6 (IL-6), C-reactive protein, and leptin in children with obesity.

ALT is closely related to the accumulation of fat in the liver and is considered a sensitive indicator of liver injury. It has been correlated with obesity and several components of MetS, including dyslipidaemia (30). Its elevation is also associated with the incidence of MetS, diabetes mellitus, and cardiovascular disease (14). Children with increased liver transaminases as surrogates of NAFLD show higher prevalences for prediabetes and type 2 diabetes mellitus as compared to those with normal transaminases (31)

AST and gamma glutamyl transferase (GGT) have not yet been independently tested as screening tools for NAFLD in children. In the context of elevated ALT, higher AST and GGT levels are associated with poorer histology (14). However, elevated AST or GGT in the context of normal ALT can also be representative of conditions other than NAFLD. Different studies describe a high prevalence of NAFLD in children and adolescents aged 10–19 years, even using an upper limit of normality for ALT that is lower than the usual standards (32). They propose that the definition of the upper limit of normal for ALT can be adjusted for each gender and ethnicity in the general population, while the laboratory ALT thresholds used for children should be re-examined. Similar to the results described in adults and adolescents, ALT was increases in prepubescent children with obesity in this study compared to children with normal weight. ALT correlated with anthropometric measurements, IR, and lipid parameters related to MetS and provided evidence that metabolic disorders related to obesity and NAFLD begin at a very early age in children with obesity. However, this association persisted when children with obesity and children with normal weight were jointly analyzed, suggesting that the increase in ALT levels entails a progressive increase in the values of parameters related to MetS.

The ALT test outcome is a continuous variable, and even fluctuation in the normal range also indicates a potential risk of metabolic disorders or cardiovascular disease in a given population (33). Of note, the definition of the upper limit of normality for ALT can be adjusted according to gender and ethnicity in the general population. For some authors, using the gender-specific upper limits of normality in children as a reference, the use of two times the gender-specific ALT in overweight and obese children aged $\geq$ 10 years showed a sensitivity of 88% and a specificity of 26% for the diagnosis of NAFLD (14, 34). Thus, it might be of interest to review the normal limits of ALT, as well as considering the inclusion of liver enzyme assessments, even for children aged under 10 years.

NAFLD is closely related to IR and hyperinsulinemia, which favors an increase in the levels of free fatty acids, TG, and the onset of hepatic steatosis (35). The group of children with obesity studied in this work presented an increase in FFAs, TG, and IR markers and both serum TG and HOMA-IR were correlated with liver enzyme values. The presence of saturated free fatty acids generates reticulocyte stress and hepatocellular lesions (36). Indeed, an increase in plasma levels of BChE has been reported in individuals with abdominal obesity and MetS (15).

Serum BChE levels are associated with components of MetS has also been described in adolescents (37). In our group, BChE values were also correlated with parameters related to MetS, although when corrected for age and sex, insulin, and HOMA-IR were not independent predictive factors. However, this association was also maintained when both groups were analyzed together. The ALT/AST index did not appear to provide more information than derived from ALT and BChE separately. Thus, we can conclude that increased levels of liver enzymes and parameters related to altered MetS are present at an early age in children with obesity and there is a correlation between them.

Liver Enzymes, Inflammation, Endothelial Dysfunction, and Adipokines

Adipose tissue interacts with the liver and releases a series of adipokines involved in processes such as inflammation, insulin sensitivity, and NAFLD. An increase in BChE has also been observed in NAFLD. Another mechanism for these associations may be inflammation (38).

Leptin increases IR and the production of fatty acids in hepatocytes and promotes inflammatory and fibrogenic pathways in the liver (39), mechanisms that can contribute to the development of NAFLD. Higher leptin levels are associated with NAFLD and the serum leptin concentration correlates with its severity (40).

In this age group (6–9 years), we found a significant association both between ALT and BChE with markers of inflammation, endothelial dysfunction, and IR, in turn resulting in a positive correlation between liver enzymes and leptin concentration. Serum CRP levels are predictive of NAFLD and have been related to the presence and severity of liver fibrosis (41).

The transition from simple steatosis to non-alcoholic steatohepatitis is accompanied by an additional decrease in adiponectin levels (42). It may have a key role in the relationship between adipose tissue, IR, and inflammation. Although ALT was not associated with adiponectin levels, we did find a significant inverse correlation between adiponectin and BChE. Thus, from an early age, adipose tissue may be involved in the onset of the metabolic disorders that accompany obesity and its complications such as MetS and NAFLD.

The optimal age for NAFLD screening and the need for repeat screenings remain undetermined because of the lack of pediatric studies on the incidence and natural history of NAFLD in young patients. The North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) guidelines recommend the use of serum ALT levels to screen for NAFLD in children, starting from the age of 9 years (14).

The data provided in this study describe the early onset of metabolic disorders related to obesity and NAFLD, which may suggest that screening should be initiated at ages under 10 years, particularly in children with obesity. Thus, it may be of interest to review the normal limits of ALT and to evaluate liver enzymes from prepubertal ages, as well as assessing the usefulness of other possible biochemical markers in pediatric patients. In this regard, extensive studies will be

necessary in prepubertal children, including the evaluation of whether weight loss positively affects liver enzymes and its impact on other variables that accompany metabolic syndrome and NAFLD.

The design of this current study did not allow us to establish if there were any sex-related differences in our population. Although this was a limitation, even after age and sex-corrected multivariate regression analysis, the main variables we studied for this age group maintained the same correlations. Only prepubertal children were studied in this current research in order to eliminate the influence of the hormonal changes that occur with puberty.

Another limitation of this work was that we did not perform any imaging studies. Nonetheless, we did aim to assess whether any alterations in biochemical parameters related to obesity and NAFLD were present in children with obesity before puberty and to determine any correlations between them.

Despite recent advances in the understanding of pediatric NAFLD, the evolution, and consequences of this condition are still unclear. The information from the future research would help create clinical programs for early diagnosis and intervention before significant vascular disease begins (43). In addition to the determination of liver enzymes, other parameters related to inflammation and adipokines could also add information to its evaluation in children with obesity at an early age (6–9 years).

CONCLUSIONS

We described an increase in liver enzyme values, markers of IR, inflammation, and endothelial dysfunction, together with the variables that define MetS and altered levels of adipocytokines in prepubescent children with obesity compared to age and sex-matched children with normal weight and there was a correlation between these alterations and liver-function enzymes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research and Ethics Commission, North Sanitary Area of Cordoba, Hospital Valle de los Pedroches, C/ Juan del Rey Calero s/n 14400, Pozoblanco, Córdoba, Spain. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MC is responsible for all data collection and samples of all participants at the Hospital Reina Sofía (Córdoba). She wrote the manuscript and approved the final manuscript as presented. RV-M participated in designing the study, data analysis, interpretation, discussion, and collaborated in the writing of the manuscript. MV and RM created the sampling and supervised data collection. They participated in the interpretation and discussion of the data, reviewed, and edited the manuscript. RC is responsible and coordinator for recruiting children at the Hospital Reina Sofía (Córdoba). LJ-R designed the study, collaborated in data analysis, interpretation, and discussion. All authors have read and approved the final manuscript and assume full responsibility for its contents.

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REFERENCES

1. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes.* (2007) 8:299–306. doi: 10.1111/j.1399-5448.2007.00271.x
2. Ahrens W, Moreno LA, Mårild S, Molnár D, Siani A, De Henauw S, et al. Metabolic syndrome in young children: definitions and results of the IDEFICS study. *Int J Obes.* (2014) 38:S4–14. doi: 10.1038/ijo.2014.130
3. Day C. Metabolic syndrome, or what you will: definitions and epidemiology. *Diab Vasc Dis Res.* (2007) 4:32–8. doi: 10.3132/dvdr.2007.003
4. Ezgü FS, Hasanoglu A, Tümer L, ÖZbay F, Aybay C, Gündüz M. Endothelial activation and inflammation in prepubertal obese Turkish children. *Metabolism.* (2005) 54:1384–9. doi: 10.1016/j.metabol.2005.05.003
5. Martos R, Valle M, Morales RM, Cañete R, Gascón F, Urbano MM. Changes in body mass index are associated with changes in inflammatory and endothelial dysfunction biomarkers in obese prepubertal children after 9 months of body mass index SD score loss. *Metabolism.* (2009) 58:1153–60. doi: 10.1016/j.metabol.2009.03.017
6. Valle M, Martos R, Gascón F, Cañete R, Zafra MA, Morales R. Low-grade systemic inflammation, hypoadiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome. *Diabetes Metab.* (2005) 31:55–62. doi: 10.1016/S1262-3636(07)70167-2
7. Cuzmar V, Alberti G, Uauy R, Pereira A, García C, De Barbieri F, et al. Early obesity: risk factor for fatty liver disease. *J Pediatr Gastroenterol Nutr.* (2020) 70:93–8. doi: 10.1097/MPG.0000000000002523
8. Machado MV, Cortez-Pinto H. Management of fatty liver disease with the metabolic syndrome. *Expert Rev Gastroenterol Hepatol.* (2014) 8:487–500. doi: 10.1586/17474124.2014.903798
9. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther.* (2011) 34:274–85. doi: 10.1111/j.1365-2036.2011.04724.x
10. Yu EL, Golshan S, Harlow KE, Angeles JE, Durelle J, Goyal NP, et al. Prevalence of nonalcoholic fatty liver disease in children with obesity. *J Pediatr.* (2019) 207:64–70. doi: 10.1016/j.jpeds.2018.11.021
11. Nobili V, Alkhouri N, Alisi A, Della Corte C, Fitzpatrick E, Raponi M, et al. Nonalcoholic fatty liver disease: a challenge for pediatricians. *JAMA Pediatr.* (2015) 169:170–6. doi: 10.1001/jamapediatrics.2014.2702
12. Clemente MG, Mandato C, Poeta M, Vajro P. Pediatric non-alcoholic fatty liver disease: Recent solutions, unresolved issues,

and future research directions. *World J Gastroenterol.* (2016) 22:8078–93. doi: 10.3748/wjg.v22.i36.8078
13. Goessling W, Massaro JM, Vasan RS, D'Agostino RB Sr, Ellison RC, Fox CS. Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology.* (2008) 135:1935–44. doi: 10.1053/j.gastro.2008.09.018
14. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr.* (2017) 64:319–34. doi: 10.1097/MPG.0000000000001482
15. Duchnowicz P, Ziobro A, Rapacka E, Koter-Michalak M, Bukowska B. Changes in cholinesterase activity in blood of adolescent with metabolic syndrome after supplementation with extract from *Aronia melanocarpa*. *Biomed Res Int.* (2018) 2018:5670145. doi: 10.1155/2018/5670145
16. Vallianou NG, Evangelopoulos AA, Bountziouka V, Bonou MS, Katsagoni C, Vogiatzakis ED, et al. Association of butyrylcholinesterase with cardiometabolic risk factors among apparently healthy adults. *J Cardiovasc Med (Hagerstown).* (2014) 15:377–83. doi: 10.2459/JCM.0b013e3283627700
17. Boutari C, Perakakis N, Mantzoros CS. Association of adipokines with development and progression of nonalcoholic fatty liver disease. *Endocrinol Metab (Seoul).* (2018) 33:33–43. doi: 10.3803/EnM.2018.33.1.33
18. Nappo A, González-Gil EM, Ahrens W, Bammann K, Michels N, Moreno LA, et al. Analysis of the association of leptin and adiponectin concentrations with metabolic syndrome in children: Results from the IDEFICS study. *Nutr Metab Cardiovasc Dis.* (2017) 27:543–51. doi: 10.1016/j.numecd.2017.04.003
19. Holterman AX, Guzman G, Fantuzzi G, Wang H, Aigner K, Browne A, et al. Nonalcoholic fatty liver disease in severely obese adolescent and adult patients. *Obesity (Silver Spring).* (2013) 21:591–7. doi: 10.1002/oby.20174
20. Schwimmer JB, Zepeda A, Newton KP, Xanthakos SA, Behling C, Hallinan EK, et al. Nonalcoholic steatohepatitis clinical research network. Longitudinal assessment of high blood pressure in children with nonalcoholic fatty liver disease. *PLoS One.* (2014) 9:e112569. doi: 10.1371/journal.pone.0112569
21. Erhardt E, Foraita R, Pigeot I, Barba G, Veidebaum T, Tornaritis M, et al. Reference values for leptin and adiponectin in children below the age of 10 based on the IDEFICS cohort. *Int J Obes (Lond).* (2014) 38:S32–8. doi: 10.1038/ijo.2014.133
22. Fernández C, Lorenzo H, Vrotsou K, Aresti U, Rica I, Sánchez E. *Estudio de Crecimiento de Bilbao. Curvas y tablas de crecimiento.* Estudio Transversal. Fundación Faustino Orbegozo (2011).
23. Grundy SM. Metabolic syndrome: a multiplex cardiovascular risk factor. *J Clin Endocrinol Metab.* (2007) 92:399–404. doi: 10.1210/jc.2006-0513
24. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. *Pediatrics.* (2007) 120:340–5. doi: 10.1542/peds.2006-1699
25. Arab JP, Arrese M, Trauner M. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. *Annu Rev Pathol.* (2018) 13:321–50. doi: 10.1146/annurev-pathol-020117-043617
26. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* (2017) 15:11–20. doi: 10.1038/nrgastro.2017.109
27. Crespo M, Lappe S, Feldstein AE, Alkhouri N. Similarities and differences between pediatric and adult nonalcoholic fatty liver disease. *Metabolism.* (2016) 65:1161–71. doi: 10.1016/j.metabol.2016.01.008
28. Motamed N, Rabiee B, Poustchi H, Dehestani B, Hemasi GR, Khonsari MR, et al. Non-alcoholic fatty liver disease (NAFLD) and 10-year risk of cardiovascular diseases. *Clin Res Hepatol Gastroenterol.* (2017) 41:31–8. doi: 10.1016/j.clinre.2016.07.005
29. Hannah WN Jr, Harrison SA. Effect of weight loss, diet, exercise, and bariatric surgery on nonalcoholic fatty liver disease. *Clin Liver Dis.* (2016) 20:339–50. doi: 10.1016/j.cld.2015.10.008
30. Hartman C, Rennert HS, Rennert G, Elenberg Y, Zuckerman E. Prevalence of elevated liver enzymes and comorbidities in children and adolescents with overweight and obesity. *Acta Paediatr.* (2020). doi: 10.1111/apa.15469. [Epub ahead of print].
31. Koutny F, Weghuber D, Bollow E, Greber-Platzer S, Hartmann K, Körner A, et al. Prevalence of prediabetes and type 2 diabetes in children with obesity and increased transaminases in European German-speaking countries. Analysis of the APV initiative. *Pediatr Obes.* (2020) 15:e12601. doi: 10.1111/ijpo.12601
32. Kang Y, Park S, Kim S, Koh H. Normal serum alanine aminotransferase and non-alcoholic fatty liver disease among Korean adolescents: a cross-sectional study using data from KNHANES 2010-2015. *BMC Pediatr.* (2018) 18:215. doi: 10.1186/s12887-018-1202-z
33. Siddiqui MS, Sterling RK, Luketic VA, Puri P, Stravitz RT, Bouneva I, et al. Association between high-normal levels of alanine aminotransferase and risk factors for atherogenesis. *Gastroenterology.* (2013) 145:1271–9. doi: 10.1053/j.gastro.2013.08.036
34. Schwimmer JB, Newton KP, Awai HI, Choi LJ, Garcia MA, Ellis LL, et al. Paediatric gastroenterology evaluation of overweight and obese children referred from primary care for suspected non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* (2013) 38:1267–77. doi: 10.1111/apt.12518
35. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev.* (2002) 23:201–29. doi: 10.1210/edrv.23.2.0461
36. Pardo V, González-Rodríguez Á, Muntané J, Kozma SC, Valverde ÁM. Role of hepatocyte S6K1 in palmitic acid-induced endoplasmic reticulum stress, lipotoxicity, insulin resistance and in oleic acid-induced protection. *Food Chem Toxicol.* (2015) 80:298–309. doi: 10.1016/j.fct.2015.03.029
37. Han Y, Ma Y, Liu Y, Zhao Z, Zhen S, Yang X, et al. Plasma cholinesterase is associated with Chinese adolescent overweight or obesity and metabolic syndrome prediction. *Diabetes Metab Syndr Obes.* (2019) 12:685–702. doi: 10.2147/DMSO.S201594
38. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* (2006) 444:860–7. doi: 10.1038/nature05485
39. Meek TH, Morton GJ. The role of leptin in diabetes: metabolic effects. *Diabetologia.* (2016) 59:928–32. doi: 10.1007/s00125-016-3898-3
40. Rotundo L, Persaud A, Feurdean M, Ahlawat S, Kim HS. The Association of leptin with severity of non-alcoholic fatty liver disease: a population-based study. *Clin Mol Hepatol.* (2018) 24:392–401. doi: 10.3350/cmh.2018.0011
41. Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. *J Gastroenterol.* (2007) 42:573–82. doi: 10.1007/s00535-007-2060-x
42. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism.* (2011) 60:313–26. doi: 10.1016/j.metabol.2010.09.003
43. Karjoo S. Is there an association of vascular disease and atherosclerosis in children and adolescents with obesity and non-alcoholic fatty liver disease?. *Front Pediatr.* (2018) 6:345. doi: 10.3389/fped.2018.00345

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Association Between Obesity and Lower Urinary Tract Symptoms Among Children and Adolescents: A Community-Based Study

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Introduction: Obesity is associated with lower urinary tract symptoms (LUTSs) and dysfunction in adults while its impact on children and adolescents remains unknown. This study aimed to explore the impact of obesity on LUTSs among children and adolescents through a large-scale community-based study.

Methods: From July 2004 to April 2017, children and adolescents aged 5–15 years-old in Xin-Dian District, New Taipei City were invited to participate in our study. The exclusion criteria were a history of congenital genitourinary tract anomalies, neurological anomalies, or a presence of urinary tract infection. After providing informed consent the participant completed a questionnaire, which included their baseline characteristics and dysfunctional voiding symptom score (DVSS); a parent completed the questionnaire with the younger children. Urgency and daytime incontinence were defined as having positive statement for DVSS questions 7 and 1, respectively. Multivariate regression analysis was used to evaluate the predictors of urgency, daytime incontinence and enuresis. A p-value of <0.05 was considered statistically significant.

Results: A total of 2,371 participants were enrolled in the study, and 1,599 were ultimately eligible for analysis. The prevalence of urgency, daytime incontinence, constipation, and enuresis were 37.6, 6.4, 26.1, and 7.7%, respectively. Multivariate analysis revealed that younger age ($p = 0.01$) and obesity ($p = 0.04$) were independent predictors for urgency. Younger age ($p < 0.01$) and constipation ($p = 0.04$) were independent predictors for daytime incontinence but obesity was not. Younger children were more likely to have nocturnal enuresis (95% CI = 0.77–0.88) and obesity did not have a significant impact on enuresis.

Conclusion: Obesity was significantly associated with urgency but it was not significantly associated with daytime incontinence and enuresis in community dwelling children and adolescents.

Keywords: lower urinary tract symptoms, urgency, incontinence, dysfunctional voiding symptom score, children, adolescents, obesity

INTRODUCTION

Obesity has become a major public health issue in the modern era and its prevalence has increased several fold over the past few decades (1, 2). Obese children are known to have an increased risk of fracture and are at higher risk of having adulthood obesity, premature death, hypertension, early markers of cardiovascular disease, insulin resistance, and psychological effects (3). Obesity is also associated with bladder dysfunction. The underlying pathophysiology of how obesity contribute to lower urinary tract dysfunction and symptoms has not yet been clarified. Obesity has been previously shown to be associated with lower urinary tract symptoms (LUTSs) in adulthood (4–6). According to a previous study by the authors that included 838 children (aged 8.0 ± 2.0 years), obesity is associated with higher urgency. However, data regarding the prevalence of LUTSs in adolescents and their association with obesity remains scarce. Therefore, the current study included more adolescents and children to further illustrate the prevalence of LUTS in children and adolescents aged 5 to 15 years old, and to investigate the potential association between obesity and LUTSs.

METHODS

The current study was an observational study which was approved by the Institutional Review Board at Taipei Tzu-Chi Hospital in New Taipei City of Taiwan. Informed consent was obtained from all participants when they were enrolled into the study. Between July 2004 and April 2017, students from the elementary schools and junior high schools in Xin-Dian District, New Taipei City were invited to participate in this study. Individuals were excluded from the study if they had congenital genitourinary tract anomalies, neurological anomalies, or a history of urinary tract infection. The participants took urinary dipsticks tests when they were enrolled into the study and if they had a positive leukocyte esterase or nitrites result on the test, they were also excluded from the analysis. In summary, the inclusion criteria of our study were (1) aging 5–15 years old, and (2) receiving at least one uroflowmetry test. The exclusion criteria of the study were (1) congenital genitourinary tract anomalies, (2) neurologic anomalies, (3) recurrent or active urinary tract infection, and (4) incomplete answer of the questionnaire.

Definition

Body mass index (BMI) is defined as the individual's weight (kg) divided by the square of their height (m^2). The participants were divided into normal weight, overweight, and obese group as determined by the definition given in the Children and Adolescents Growth References, Department of Health, Executive Yuan, Republic of China, published in June 2013. The participants were considered as overweight if their BMI was between the 85th and 95th percentile on gender- and age-specific nomograms. If the participants BMI was over the 95th percentile on gender- and age-specific nomograms, they were considered as obese.

Questionnaire

Elementary school children aged 5–15 years old completed a questionnaire with one of their parents that included their baseline characteristics (age, gender, body weight, and height), presence of snoring or sleep apnea during the past month, dysfunctional voiding symptom score (DVSS), status of nocturnal enuresis (defined as "having at least one episode of wetting in discrete portions while asleep in a child who has passed his or her fifth birthday in the past 1 month"). Junior high school adolescents completed the questionnaire by themselves. The body height and weight of the participants were measured at health check-up of semester and reported by parents or the participants themselves.

Of the 2,371 participants invited to join our study, 1,599 (67.4%) children were finally included for analysis. The DVSS has 10 sections, including seven questions about LUTSs (daytime incontinence, amount of incontinence, infrequent voiding, curtsying, urgency, push to void, and dysuria), two questions about bowel movement (low defecation frequency and difficult defecation), and one question about stressful events (new home, new baby, new school problem, abuse, accidents, home problem, or another special event in the past month). The Chinese version of the DVSS was validated in children with dysfunctional voiding (7). Urgency and daytime incontinence symptom were assessed according to the statement, "When I have to pee, I cannot wait" and "I have had wet clothes or wet underwear during the day," respectively. The symptoms were scored on a scale of 0–3 (0, almost never; 1, less than half of the time; 2, about half of the time; and 3, almost every time). We defined urgency and daytime incontinence as a symptom score of 1, or more than 1, respectively. Defecation conditions and stool form were assessed using the Bristol Stool Form Scale (8). If the answer was type 1 or type 2, the children and adolescents were categorized as having constipation.

Statistical Analysis

Data were expressed as the mean ± standard deviation and analyzed using commercial statistical software (Medcalc1, version 12.7, Software bvba, Ostend, Belgium, and SAS1, version 9.2, SAS Institute, Inc., Cary, NC, USA). Demographic and clinical parameters were compared using an analysis of variance model (continuous demographic variables) and a Chi-squared test (categorical data) and a Kruskal–Wallis test (ordinal data). A Cochran–Armitage trend test was used to evaluate the trend in daytime incontinence and overactive bladder (OAB: defined as a sudden or uncontrollable urge to urinate) proportion across age. A multivariate regression analysis model was used to evaluate the predictors (age in years, gender, non-obese vs. obese, constipated vs. non-constipated) for urgency, daytime incontinence, and nocturnal enuresis. The risk factors were calculated as odds ratios (ORs) with 95% confidence intervals (95% CIs) for enuresis. A P-value of <0.05 was considered statistically significant.

RESULTS

Figure 1 is a flow chart of the participants included within the study. A total of 2,371 questionnaires were distributed to the

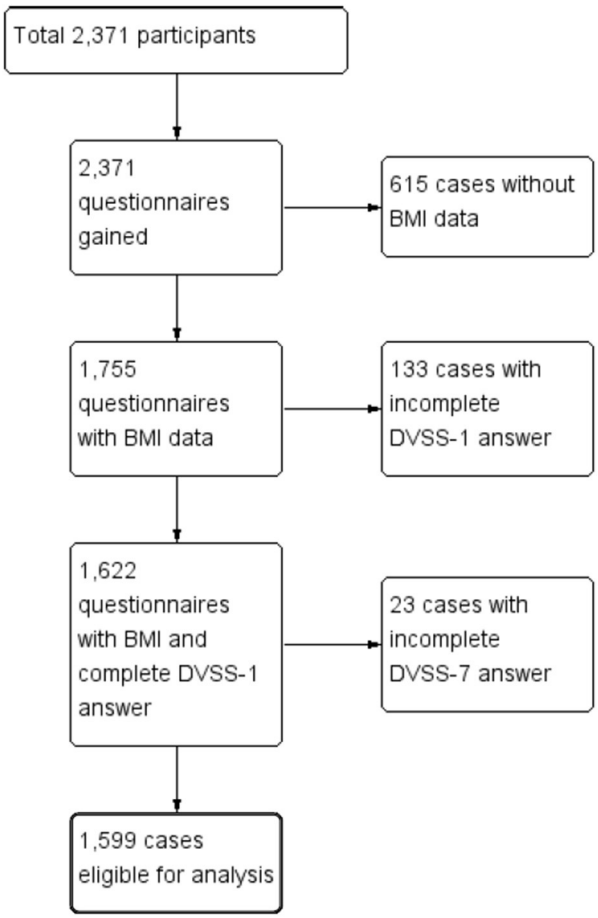

FIGURE 1 | Flow chart of participant inclusion.

participating schools. After we excluded the children who did not meet the inclusion criteria and or had provided incomplete answers to the questionnaire, a total of 1,599 children (769 boys vs. 830 girls, age: 10.04 ± 3.66 years vs. 10.39 ± 3.95 years, $p = 0.06$) were eligible for analysis (the case numbers in each subgroup: 5–7 year-old = 465, 8–10 year-old = 360, 11–13 year-old = 360, and 14–15 year-old = 414). **Table 1** gives a comparison of the baseline characteristics and LUTSs between the boys and the girls. The prevalence of obesity was significantly higher in the boys (15.3%) compared with the girls (9.6%, $p < 0.01$). The rate of constipation was significantly higher in the girls (30.3%) compared with the boys (21.6%, $p < 0.01$). **Table 2** details the prevalence of incontinence, urgency, and constipation between the non-obese and obese groups. The obese group was older than the non-obese group and the prevalence of obesity was higher in the boys. Other risk factors, including urgency, constipation, and incontinence showed no significant differences between these two groups.

Urgency

The urgency score decreased from 0.55 ± 0.09 in 5–7 year-old children to 0.43 ± 0.08 in 14–15 year-old adolescents. **Figure 2A** depicts the prevalence of self-reported urgency (urgency score ≥1 in the DVSS-7) in our study population. There was no significant difference in self-reported urgency between genders ($p = 0.63$) or individuals with different constipation statuses ($p = 0.53$). The children and adolescents with obesity had a significantly higher risk of urgency compared with the non-obese group ($p = 0.04$). Younger age was also a significant predictor of a higher urgency scores ($p = 0.01$).

Incontinence

The incontinence scores decreased from 0.10 ± 0.04 in 5–7 year-old children to 0.02 ± 0.02 in 14–15 year-old adolescents. The self-reported incontinence rate (incontinence score ≥1 in the DVSS-1) over variable ages is shown in **Figure 2B**. The significant risk factors for higher incontinence scores were younger age ($p = 0.0002$) and constipation ($p = 0.04$). Obesity and gender did not have a significant impact on incontinence in children and adolescents.

Nocturnal Enuresis

The prevalence of nocturnal enuresis was 13.0% in 5–7 year-old children and 2.7% in 14–15 year-old adolescents. The only significant protective factor was older age (OR 0.82; 95% CI: 0.77–0.88). Gender, BMI, and constipation status were not significant risk factors.

Constipation

The prevalence of constipation was 29.1% in 5–7 year-old children and 26.4% in 14–15 year-old adolescents (**Figure 2C**). The constipation rate was significantly lower in boys (OR: 0.63, 95% CI: 0.50–0.80) compared with girls. Age and obesity were not risk factors for constipation (OR 0.99; 95% CI: 0.96–1.02, and OR 0.88; 95% CI: 0.61–1.26, respectively).

DISCUSSION

The current study was a large-scale community-based study ($n = 1,599$) that explored the prevalence of LUTSs in community dwelling children and adolescents and evaluated their potential association with obesity. The prevalence of obesity was 12.7% among our study population. The prevalence of urgency, daytime incontinence, and enuresis in children aged 5–7 years old was 34.5, 8.0, and 13.0%, respectively. LUTSs decreased as age increased. The prevalence of urgency, daytime incontinence, and enuresis in adolescents aged 14–15 years was 33.8, 2.4, and 2.8%, respectively. The current study revealed that obesity was associated with higher urgency scores but not significantly associated with incontinence or nocturnal enuresis. The possible underlying pathophysiology of how obesity affects LUTSs and function are listed below.

The increasing number of LUTSs observed in obese children could be a consequence of hormonal, dietary, and even personality/behavioral differences. Children with obesity were observed to have more psychological distress than non-obese children (9–12). Obese children may suffered from increased intra-abdominal pressures which could result in the development of urinary urgency or incontinence (13). Additionally, obesity

TABLE 1 | Average age and prevalences for obesity, incontinence, urgency, and constipation in boys and girls.

	Boys (n = 769)	Girls (n = 830)	p-value
Age (years, mean ± S.D)	10.04 ± 3.66	10.39 ± 3.95	0.06
Body height (cm, mean ± S.D)	140.88 ± 0.15	139.45 ± 0.14	0.50
Body weight (kg, mean ± S.D)	39.49 ± 0.46	37.42 ± 0.42	<0.01
Obese (95% CI)	15.3% (12.9∼18.1%)	9.6% (7.7∼11.9%)	<0.01
Incontinence (95% CI)	7.2% (5.4∼9.2%)	5.7% (4.2∼7.5%)	0.22
Urgency (95% CI)	37.50% (34.4∼41.1%)	37.8% (34.5∼41.2%)	0.90
Constipation (95% CI)	21.6% (18.8∼24.7%)	30.3% (27.1∼33.6%)	<0.01

TABLE 2 | Prevalences for incontinence, urgency, and constipation in non-obese and obese group.

	Non-obese (n = 1,401)	Obese (n = 198)	p-value
Age (Mean ± S.D)	10.11 ± 3.83	10.98 ± 3.60	<0.01
Boys (95% CI)	46.5% (43.8∼49.1%)	59.6% (52.4∼66.5%)	<0.01
Incontinence (95% CI)	6.6% (5.3∼8.0%)	5.1% (2.4∼9.1%)	0.41
Urgency (95% CI)	37.0% (34.5∼39.7%)	42.1% (35.0∼49.3%)	0.18
Constipation (95% CI)	26.5% (24.2∼28.9%)	23.4% (17.6∼29.9%)	0.34

has also been considered to be associated with hyperglycemia (14). Hyperglycemia could cause diuresis, leading to LUTSs. There are several hypotheses that could explain the association between pediatric OAB and constipation. First, stool in the rectum stimulates stretch receptors which transmit a signal to the brain and induced the contraction of external anal sphincter and puborectalis muscles. Second, a distended rectum compresses the adjacent organs, such as the urinary bladder, which results in decreased capacity (15). Our current study confirmed that urgency was the core symptom of lower urinary tract dysfunction in obese children and adolescents. This finding also indicates the importance of body weight control in school-aged children. The benefits could not only be a reduction in cardiovascular disease, insulin resistance and musculoskeletal disorders but also less LUTSs.

Urgency is the core symptom of childhood OAB (16). The International Children Continence Society also mentioned other relative symptoms, including urge incontinence, urinary frequency, and nocturnal enuresis (17, 18). In the current study, we defined urgency and incontinence as when the urgency and incontinence symptom scores were ≥1. A former study by the authors demonstrated that childhood obesity was an independent risk factor for pediatric OAB symptoms (OR 1.97; 95% CI: 1.14–3.40) (14). Similarly, a large scale study in China which was conducted by Xing et al. (19) and evaluated 10,133 anonymous questionnaires, disclosed that children with obesity, a history of urinary tract infection, nocturnal enuresis, a family history of LUTSs, constipation, and fecal incontinence, had a significantly higher prevalence of OAB compare with normal children ($p <$ 0.05). Fraga et al. (20) conducted a cross-sectional study which included 423 children and adolescents aged 5–17 years. There were three factors that were independently and significantly associated with a positive DVSS: age <10 years (95% CI: 0.34–1.18), constipation (95% CI: 0.88–2.70), and obesity (95% CI: 0.25–1.52). The author reported that only the bladder-filling symptoms of lower urinary tract dysfunction appeared to be associated with obesity. To further explore the impact of obesity on LUTSs among children and adolescents, we conducted current study. Multivariate regression analysis revealed that obesity was a significant independent risk factor for urgency ($p = 0.04$). Obese patients with severe OAB symptoms may warrant further examination for lower urinary tract dysfunction because both childhood OAB symptoms and obesity are considered to have a negative effect on the social, emotional, and behavioral development of children (12, 21). A previous cohort study also found that children with OAB symptoms were significantly more likely to have OAB symptoms in adulthood (9).

Obesity was also thought be associated with urinary incontinence in children. A Danish study conducted by Warner et al. (22) demonstrated an association between daytime urinary incontinence and BMI. Girls with one OAB symptom had a significantly higher average BMI than girls with no OAB (BMI-SDS 0.18 vs. 0.30 [$p < 0.05$] and 0.17 vs. 0.29 [$p < 0.01$], respectively). Obesity led to a significantly increased risk of nocturia in both adolescent boys and girls (OR 1.74; CI: 1.17–2.60 and OR 2.01; CI: 1.25–3.23, respectively). The study by Wagner et al. (23) also concluded that being overweight or obese gives you a significant higher risk of developing OAB symptoms, especially urinary incontinence ($p = 0.017$). A recent study by von Gontard et al. (24) supported the theory that behavioral symptoms, psychiatric disorders, and being overweight/obese were risk factors associated with urinary incontinence. However, we did not observe a significantly higher risk of daytime incontinence in obese children and adolescents in the current study. Our analysis indicated that younger age and constipation were the two independent risk factors for pediatric incontinence. A possible explanation for the non-significant impact of obesity on daytime incontinence could be the lower incontinence scores in the study

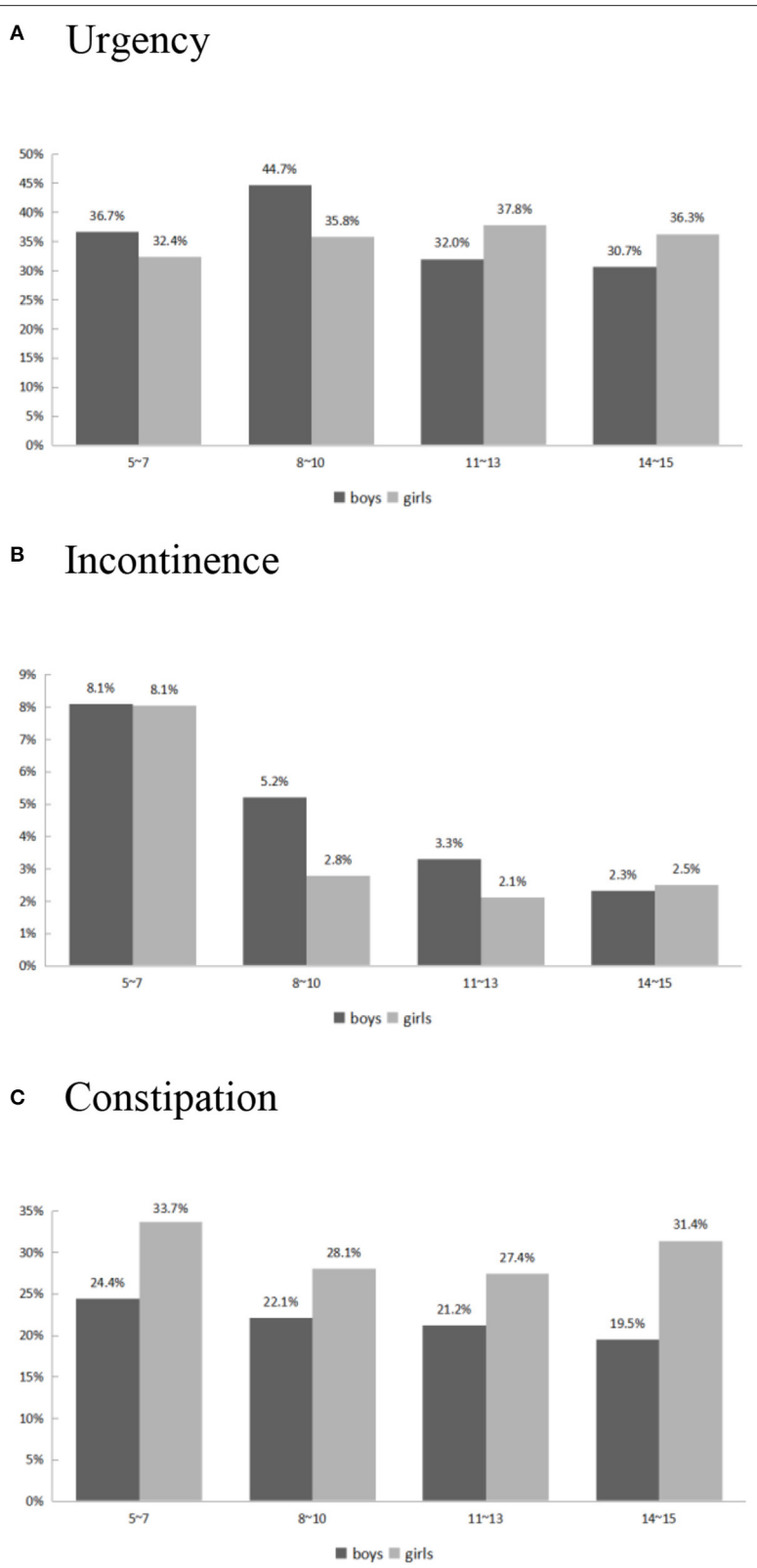

FIGURE 2 | Self-reported Urgency **(A)**, Incontinence **(B)**, and Constipation **(C)** risk vs. age and gender.

populations, especially in the adolescents, (score: 0.03 ± 0.16 in 14–15 year-old adolescents). Also, the case number may not have been large enough to show the differences.

Erdem et al. (25) concluded that most of the children with voiding dysfunction were obese, and that their risk of developing the condition was almost double that of the normal population. A Chinese study by Ma et al. (26) also agreed that risk of severe monosymptomatic nocturnal enuresis (MNE) was higher in obese children. They also found that behavioral therapy was less effective in obese children with MNE. However, in our study, the prevalence of nocturnal enuresis was not significantly increased in the obesity group. We detected a higher nocturnal enuresis rate in younger children, which was compatible with current theories. According to a study from several decades ago, the prevalence of nocturnal enuresis decreases with age (27). Hamed et al. (28) performed a cross-sectional study, which included 4,652 school-age children in Egypt. The younger age categories showed a higher prevalence of MNE compared with the older age categories. The OR ranged from 6-year-olds (OR 3.17, 95% CI: 2.34–4.30) to 11-year-olds (OR 1.47, 95% CI: 1.04–2.09). Further studies needs to be conducted to clarify the association between children's BMI and nocturnal enuresis.

The current study had some limitations. First, in some cases the DVSS questionnaire was answered by the one parent along with the participating child. A bladder diary was not conducted and there may have been a discrepancy between a bladder diary and the questionnaire. Second, among the 2,371 participants, only 1,599 were eligible for inclusion within the study based on their questionnaire responses. A total of 772 children were excluded. The strict inclusion criteria may have resulted in the removal of some suitable cases from our study, which may have impacted the findings. Third, we defined the cut-off point of for urgency and daytime incontinence as having "1 or more" symptoms. However, the cut-off point for the definition of these conditions needs further investigation. The overall incontinence prevalence was 6.4%. The case number would be too scarce to analyze if we used "2 or more" cut-off point and low prevalence made it harder to reach significance in multivariate regression analysis model. The *p*-value was 0.94 in obese vs. non-obese and 0.33 in boy vs. girl group, separately. The study did have several strengths. It enrolled a total of 1,599 children and adolescents, which is a notable increase on the previous study which included only 838 children. We further separated LUTSs into urgency, incontinence and nocturnal enuresis. Each symptom had one corresponding question in DVSS. This is easier to analyze the data and to repeat similar studies in the future.

CONCLUSION

The results of the current study revealed that obesity was significantly associated with urgency but not daytime incontinence and enuresis in community dwelling children and adolescents.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB No: 06-XD29-063. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SY and S-JC: project development, data collection, and manuscript writing. S-GW and S-JC: manuscript writing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. de Onis M, Lobstein T. Defining obesity risk status in the general childhood population: which cut-offs should we use? *Int J Pediatr Obes*. (2010) 5:458–60. doi: 10.3109/17477161003615583
2. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. (2007) 85:660–7. doi: 10.2471/BLT.07.043497
3. Antwi F, Fazylova N, Garcon MC, Lopez L, Rubiano R, Slyer JT. The effectiveness of web-based programs on the reduction of childhood obesity in school-aged children: a systematic review. *JBI Libr Syst Rev*. (2012) 10(Suppl. 42):1–14. doi: 10.11124/jbisrir-2012-248
4. Gacci M, Vignozzi L, Sebastianelli A, Salvi M, Giannessi C, De Nunzio C, et al. Metabolic syndrome and lower urinary tract symptoms: the role of inflammation. *Prostate Cancer Prostatic Dis*. (2013) 16:101–6. doi: 10.1038/pcan.2012.44
5. He Q, Wang Z, Liu G, Daneshgari F, MacLennan GT, Gupta S. Metabolic syndrome, inflammation, and lower urinary tract symptoms: possible translational links. *Prostate Cancer Prostatic Dis*. (2016) 19:7–13. doi: 10.1038/pcan.2015.43
6. Zacche MM, Giarenis I, Thiagamoorthy G, Robinson D, Cardozo L. Is there an association between aspects of the metabolic syndrome and overactive bladder? A prospective cohort study in women with lower urinary tract symptoms. *Eur J Obstet Gynecol Reprod Biol*. (2017) 217:1–5. doi: 10.1016/j.ejogrb.2017.08.002
7. Chang SJ, Chen TH, Su CC, Yang SS. Exploratory factory analysis and predicted probabilities of a Chinese version of Dysfunctional Voiding Symptom Score (DVSS) questionnaire. *Neurourol Urodyn*. (2012) 31:1247–51. doi: 10.1002/nau.22254
8. Thabit AK, Nicolau DP. Lack of correlation between bristol stool scale and quantitative bacterial load in clostridium difficile infection. *Infect Dis*. (2015) 8:1–4. doi: 10.4137/IDRT.S23079
9. Fitzgerald MP, Thom DH, Wassel-Fyr C, Subak L, Brubaker L, Van Den Eeden SK, et al. Childhood urinary symptoms predict

adult overactive bladder symptoms. *J Urol*. (2006) 175(3 Pt. 1):989–93. doi: 10.1016/S0022-5347(05)00416-7
10. Barone JG, Hanson C, DaJusta DG, Gioia K, England SJ, Schneider D. Nocturnal enuresis and overweight are associated with obstructive sleep apnea. *Pediatrics*. (2009) 124:e53–9. doi: 10.1542/peds.2008-2805
11. Braga A, Veiga MLT, Ferreira M, Santana HM, Barroso U Jr. Association between stress and lower urinary tract symptoms in children and adolescents. *Int Braz J Urol*. (2019) 45:1167–79. doi: 10.1590/s1677-5538.ibju.2019.0128
12. Oliver JL, Campigotto MJ, Coplen DE, Traxel EJ, Austin PF. Psychosocial comorbidities and obesity are associated with lower urinary tract symptoms in children with voiding dysfunction. *J Urol*. (2013) 190(Suppl. 4):1511–5. doi: 10.1016/j.juro.2013.02.025
13. Cummings JM, Rodning CB. Urinary stress incontinence among obese women: review of pathophysiology therapy. *Int Urogynecol J Pelvic Floor Dysfunct*. (2000) 11:41–4. doi: 10.1007/s001920050008
14. Chang SJ, Chiang IN, Lin CD, Hsieh CH, Yang SS. Obese children at higher risk for having overactive bladder symptoms: a community-based study. *Neurourol Urodyn*. (2015) 34:123–7. doi: 10.1002/nau.22532
15. Kim JH, Lee JH, Jung AY, Lee JW. The prevalence and therapeutic effect of constipation in pediatric overactive bladder. *Int Neurourol J*. (2011) 15:206–10. doi: 10.5213/inj.2011.15.4.206
16. Fonseca EG, Bordallo AP, Garcia PK, Munhoz C, Silva CP. Lower urinary tract symptoms in enuretic and nonenuretic children. *J Urol*. (2009) 182(Suppl. 4):1978–83. doi: 10.1016/j.juro.2009.04.083
17. Chung JM, Lee SD, Kang DI, Kwon DD, Kim KS, Kim SY, et al. Prevalence and associated factors of overactive bladder in Korean children 5-13 years old: a nationwide multicenter study. *Urology*. (2009) 73:63–7; discussion 8–9. doi: 10.1016/j.urology.2008.06.063
18. Vaz GT, Vasconcelos MM, Oliveira EA, Ferreira AL, Magalhaes PG, Silva FM, et al. Prevalence of lower urinary tract symptoms in school-age children. *Pediatr Nephrol*. (2012) 27:597–603. doi: 10.1007/s00467-011-2028-1
19. Xing D, Wang YH, Wen YB, Li Q, Feng JJ, Wu JW, et al. Prevalence and risk factors of overactive bladder in Chinese children: a population-based study. *Neurourol Urodyn*. (2020) 39:688–94. doi: 10.1002/nau.24251
20. Fraga LGA, Sampaio A, Boa-Sorte N, Veiga ML, Nascimento Martinelli Braga AA, Barroso U. Obesity and lower urinary tract dysfunction in children and adolescents: Further research into new relationships. *J Pediatr Urol*. (2017) 13:387.e1–6. doi: 10.1016/j.jpurol.2017.03.014
21. Ramsay S, Bolduc S. Overactive bladder in children. *Can Urol Assoc J*. (2017) 11(Suppl. 1):S74–S9. doi: 10.5489/cuaj.4337
22. Warner TC, Baandrup U, Jacobsen R, Boggild H, Aunsholt Ostergaard PS, Hagstrom S. Prevalence of nocturia and fecal and urinary incontinence and the association to childhood obesity: a study of 6803 Danish school children. *J Pediatr Urol*. (2019) 15:225 e1–8. doi: 10.1016/j.jpurol.2019.02.004
23. Wagner C, Equit M, Niemczyk J, von Gontard A. Obesity, overweight, and eating problems in children with incontinence. *J Pediatr Urol*. (2015) 11:202–7. doi: 10.1016/j.jpurol.2015.05.019
24. von Gontard A, Mattheus H, Anagnostakou A, Sambach H, Breuer M, Kiefer K, et al. Behavioral comorbidity, overweight, and obesity in children with incontinence: an analysis of 1638 cases. *Neurourol Urodyn*. (2020) 39:1985–93. doi: 10.1002/nau.24451
25. Erdem E, Lin A, Kogan BA, Feustel PJ. Association of elimination dysfunction and body mass index. *J Pediatr Urol*. (2006) 2:364–7. doi: 10.1016/j.jpurol.2006.05.002
26. Ma Y, Shen Y, Liu X. Association between enuresis and obesity in children with primary monosymptomatic nocturnal enuresis. *Int Braz J Urol*. (2019) 45:790–7. doi: 10.1590/s1677-5538.ibju.2018.0603
27. Collet JP, Simore MF, Cochat P. Prevalence of nocturnal enuresis in school-age children. *Pediatrie*. (1993) 48:701–4.
28. Hamed A, Yousf F, Hussein MM. Prevalence of nocturnal enuresis and related risk factors in school-age children in Egypt: an epidemiological study. *World J Urol*. (2017) 35:459–65. doi: 10.1007/s00345-016-1879-2

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ORIGINAL RESEARCH
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Sex-Specific Temporal Trends in Overweight and Obese Among Schoolchildren From 2009 to 2018: An Age Period Cohort Analysis

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Background: Our study examined the age, period, and cohort effects on overweight and obesity in children using a 10-year dataset collected from schoolchildren in Hualien, Taiwan.

Methods: We used data from the annual health checkup of a total of 94,661 schoolchildren in primary schools and junior high schools in Hualien from 2009 to 2018. Children were defined as overweight or obese by the gender- and age-specific norm of the body mass index. We conducted the age-period-cohort (APC) analysis in boys and girls separately.

Results: From 2009 to 2018, the rates of children overweight and obese were 12.78 and 14.23%, respectively. Boys had higher rates of overweight and obesity than girls (29.73 vs. 24.03%, $P < 0.001$). Based on APC analysis results, positive age effect existed regardless of gender. The risk of overweight or obesity of children aged 9 or 12 years was significantly higher compared to the average rate. As for period effect, a fluctuating downward trend in overweight was evident in 2016, and a similar trend in obesity was seen in 2017 across gender groups. The birth cohort of 2007 to 2009 had a significant higher proportion of overweight and obese than other birth cohorts. This indicated that the proportion of children overweight and obese in the young generation is higher than that in the old generation.

Conclusion: An increased risk of children overweight or obese was associated with age and later birth cohort. For the period effect, the trend in the prevalence of overweight and obesity fluctuated downward slowly from 2016 to 2017.

Keywords: age-period-cohort analysis, body mass index, childhood obesity, gender, poisson regression

INTRODUCTION

The prevalence of overweight and obesity among children and adolescents increased globally. The World Health Organization (WHO) estimated that the number of overweight children under the age of 5 years was over 41 million, and over 340 million children and adolescents aged 5–19 years were overweight or obese in 2016. Persisted-early childhood obesity was associated with an increased risk of high blood pressure (1). The risk of early-onset diabetes in children and adolescents was greatly magnified with increasing severity of obesity (2). In the United States, scholars warned that childhood obesity continues to be a significant concern and the levels of severe obesity in all children aged 2–19 years and populations have seen an increase in the past 18 years (3). In Taiwan, an increasing trend in overweight and obesity was observed among Taiwanese children and adolescents in a 2-year period (4). The Nutrition and Health Survey in Taiwan (NAHSIT) 2013–2016 report showed that the prevalence of childhood overweight and obesity among children aged 7–12 years was 28.4%. In Hualien, the overall prevalence of overweight and obesity was 25% in 2010. The prevalence of overweight and obese was also increased with higher grades (5).

Due to the complexity of developing childhood overweight and obesity, Dr. Davison and Birch proposed the Ecological System Theory (EST) in 2001 which highlighted the importance of ecological niches including the family, school, community, and society. Specifically, child characteristics that place children at risk of the development of overweight (including dietary intake, physical activity, and sedentary behavior) should be embedded in this EST model and tested through causal processes (6). Dr. McCrindle defined a birth cohort between 1997 and 2010 as "generation z" and children born between 2010 and 2025 as "generation alpha or i" (7). Apple Inc., released the first generation of iPad in 2010 which placed children after generation z under the convenient environment fulfilled with high technologic services such as internet and social media. Children and adolescents of generation z and alpha were tight up with virtual activities such as online chat, gaming, shopping, and entertainment and became more sedentary in their daily routine.

With changing environment and time period, to our knowledge, few studies explored the multifactorial causations of childhood obesity through age, period, and cohort (APC) analysis which distinguished three types of time-varying phenomena: age, period, and cohort effects. A previous study used data of children and adolescents aged 2–25 from 1989 to 2009 in China and found a U-shape of age effects, positive period effects, and cohort effects in males (8). Another APC analysis of Iranian children and adolescents indicated a decreasing trend of age, positive period effects, and cohort effects (9). Regarding the magnitude of BMI, a study conducted in Hong Kong, China, found that BMI increased with age and period, but a higher BMI was seen in older cohorts (10). In Taiwan, few papers derived through APC analysis in childhood overweight and obesity, with only one APC model of overweight and obesity in Taiwanese adolescents (aged 12–18), found a decreasing trend with age and period, and a positive birth cohort effect (11). However, the effects of age, period, and cohort on the rising temporal trends of children overweight and obesity remain unclear.

The Taiwan Ministry of Health and Welfare and Ministry of Education launched the Health-Promoting School (HPS) program in 2002, and the HPS program gradually expanded to all counties in Taiwan (12). In 2010, the Ministry of Education initiated a program to provide empowerment strategies for training school faculty, staff, and students to participate actively in school health management; linkage to community resources was also disseminated. In light of current evidence and the recent HPS program in Taiwan, it is important to understand the effects age, period, and cohort may have had on the childhood overweight and obesity. In this study, we used a 10-year long-term dataset (2009–2018) of schoolchildren aged 6–12 in Hualien county in Taiwan to investigate the temporal trends of the age, period, and cohort effects in childhood overweight and obesity.

MATERIALS AND METHODS

Study Samples

We recruited a population-based sample of schoolchildren in Hualien county between 2009 and 2018. Hualien, the largest county of Taiwan by area with a population of 350,000, has implemented a physical health checkup program once per year. Students from the first (aged 6 years, termed grade 1), fourth (aged 9 years, termed grade 4), and seventh grades (aged 12 years, termed grade 7) were participated into the program as per the regulations by the Taiwan Ministry of Education. A total of 95,749 students from 107 primary schools and 25 junior high schools were eligible. The annual completion rates ranged from 98.8 to 99.3%. Children without complete data of birth date, height, or weight were excluded ($n = 1{,}088$). Thus, a total of 94,661 eligible schoolchildren were recruited. The highest proportion of children was in 2009 (12.61%) and declined annually to 8.53% in 2018 because of a decreased population size (**Table 1**). This study was approved by the Institutional Review Board of Hualien Tzu Chi Hospital in Hualien, Taiwan (REC No.: IRB109-061-B).

Data Measurements

Annual physical examination items included body composition, vision, oral health, and urine test. This study used a part of the data measurements from the physical examination. Study variables were examination year, demographic variables (gender, age, grade, and residential area), and body composition such as height and weight. Age was calculated by subtracting the examination data from the birth date. Height and weight were measured by trained school nurses at school according to the standard procedures and instruments. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Child overweight or obesity was defined as BMI between the 85th and 95th percentiles or more than the 95th percentile among children of the same age and sex according to the age–sex-specific BMI cutoff values from the new growth charts for Taiwanese children and adolescents, based on the World Health Organization (WHO) standards, which were developed by the Department of Health in Taiwan in 2010

TABLE 1 | Sample sizes based on age and gender at each examination year ($n = 94,661$).

	Age, 6 y		Age, 9 y		Age, 12 y				
Period	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Total sample size**	**Percentage of total (%)**	**Population size**
2009	1,762	1,481	2,025	1,972	2,439	2,261	11,940	12.61	12,055
2010	1,613	1,454	1,981	1,795	2,211	2,017	11,071	11.70	11,202
2011	1,463	1,397	1,710	1,632	2,137	1,909	10,248	10.83	10,361
2012	1,501	1,265	1,715	1,510	2,090	2,000	10,081	10.65	10,200
2013	1,360	1,234	1,570	1,434	2,032	1,852	9,473	10.01	9,565
2014	1,359	1,227	1,481	1,393	1,793	1,709	8,962	9.47	9,021
2015	1,332	1,200	1,463	1,282	1,813	1,585	8,675	9.16	8,733
2016	1,243	1,112	1,359	1,245	1,687	1,533	8,179	8.64	8,236
2017	1,252	1,098	1,347	1,254	1,537	1,470	7,958	8.41	8,030
2018	1,403	1,241	1,339	1,235	1,514	1,342	8,074	8.53	8,133

(13). Residential area was classified into industrial, sub-industrial, and remote area based on level of development and social-economic status (14). The collected dataset in our retrospective cohort study was from the annual health examination records. Previous BMI category and daily dietary and activity records were not available for each schoolchild in our dataset.

Statistical Analysis

Descriptive statistics were presented for examination year, demographic variables, height, weight, BMI, and weight status by sex group. For comparison of sex group, the independent sample *t*-test and Chi-square test were applied when appropriate. We investigate sex-specific temporal trends of the prevalence of overweight and obesity in terms of three temporal factors: age (6, 9, 12 years old), period (examination year), and cohort (birth year). The prevalence rates of sex-specific overweight and obesity between 2009 and 2018 were modeled by the Poisson log-linear model. All models were analyzed separately for boys and girls. The Poisson log-linear model is commonly used in epidemiology where the counts of events such as overweight or obesity are assumed to follow Poisson distributions and the rates of overweight or obesity are estimated by the log-linear model. The model can be expressed as:

$$\log r_{ij} = \mu + \alpha_i + \beta_j + \gamma_k, i = 1, \ldots, a. j = 1, \ldots, p$$

where r_{ij} denotes the expected prevalence of overweight or obesity at the i-th age group and the j-th examination year; μ denotes the overall population mean; α_i denotes the effect of the i-th age group ($a = 3$); β_j denotes the effect of the j-th examination year ($p = 10$); and $\gamma_k$denotes the effect of the k-th cohort. We estimated the age, period, and cohort effects by using the intrinsic estimator method based on the intention-to-collapse method (15, 16). In other words, because the age groups are 3 years apart, three continuous periods were collapsed into one to have the same time span of age groups. The birth cohorts by the intention-to-collapse method were categorized into the birth years 1997, 1998–2000, 2001–2003, 2004–2006, 2007–2009, and 2010–2012 (**Supplementary Table 1**). Notice that the intention-to-collapse method does not change the coding of the age and period effects. For an intuitive interpretation, we calculated the rate ratio (RR) as the exponential values of the regression coefficients (17, 18). It means the risk of overweight or obesity at a particular age, period, or cohort compared to the overall average rate. Analysis was implemented by APCG1 package in R language (19).

RESULTS

Prevalence of Overweight and Obesity

Overall, the percentages of boys and girls were 52.32 and 47.68%, respectively. Boys and girls had similar age, grade, and period percentages (**Table 2**). Boys had a significantly greater height (138.37 vs. 137.83 cm, $p < 0.001$), weight (37.18 vs. 35.59 kg, $p < 0.001$), and BMI (18.64 vs. 18.07, $p < 0.001$) compared to girls. The percentages of overweight (13.75 vs. 11.72%) and obesity (15.98 vs. 12.31%) were found higher in boys than in girls ($p < 0.001$). Our data showed that the prevalence of overweight declined from 13.17% in 2009 to 12.63% in 2018. In contrast, the prevalence of obesity increased from 13.19% in 2009 to 14.97% in 2018.

Figure 1 presents the prevalence of overweight and obesity based on three age groups from 2009 to 2018. The lowest percentage of overweight was found at the age of 6 years, followed by similar prevalence rates at the age of 9 and 12 years. However, the percentage of obesity increased with age regardless of time period. In general, the prevalence of overweight/obesity was higher in the older age group except that in 2015. Among different age groups of both boys and girls, the prevalence of overweight and obesity increased progressively, especially in children aged 9 and 12 years, respectively. **Figure 2** presents the sex difference of the prevalence of overweight and obesity. From 2009 to 2018, boys had a higher prevalence of overweight than girls, as well as a trend of obesity. In both genders, the prevalence of overweight declined in 2016. Additionally, the prevalence of obesity decreased in 2017. It is worthy of mentioning that the time trend of obesity in girls was more fluctuating than that in boys.

TABLE 2 | Sample characteristics among boys and girls.

Characteristics	Boys (n = 49,522)	Girls (n = 45,139)	p-value
Age (yrs)	9.96 ± 2.47	9.98 ± 2.46	0.063
Grade, *n* (%)			0.058
1	14,288 (28.85)	12,709 (28.15)	
4	15,990 (32.29)	14,752 (32.68)	
7	19,244 (38.86)	17,678 (39.16)	
Height (cm)	138.37 ± 16.77	137.83 ± 15.96	<0.001
Weight (kg)	37.18 ± 15.13	35.59 ± 13.42	<0.001
BMI	18.64 ± 4.10	18.07 ± 3.70	<0.001
BMI group, *n* (%)			<0.001
Underweight	3,043 (6.14)	2,988 (6.62)	
Normal	31,755 (64.12)	31305 (69.35)	
Overweight	6,812 (13.75)	5290 (11.72)	
Obese	7,912 (15.98)	5556 (12.31)	
Living area, *n* (%)			0.017
Industrial area	31,365 (63.33)	28,320 (62.74)	
Semi-industrial area	3,211 (6.48)	3,123 (6.92)	
Remote area	14,950 (30.19)	13,697 (30.34)	
Period, *n* (%)			0.730
2009	5,714 (47.86)	6,226 (52.14)	
2010	5,266 (47.57)	5,805 (52.43)	
2011	4,938 (48.19)	5,310 51.81)	
2012	4,775 (47.37)	5,306 (52.63)	
2013	4,520 (47.71)	4,953 (52.29)	
2014	4,329 (48.30)	4,633 (51.70)	
2015	4,067 (46.88)	4,608 (53.12)	
2016	3,890 (47.56)	4,289 (52.44)	
2017	3,822 (48.03)	4,136 (51.97)	
2018	3,818 (47.29)	4,256 (52.71)	

Table 3 lists the prevalence of overweight and obesity across six birth cohorts and periods in both genders. Across three age groups, the prevalence of overweight increased at the age of 9 years and then became stable until 12 years of age for each cohort. For instance, the prevalence of overweight among the boys, evaluated in 2016–2018, was higher in boys born in 2007–2009, compared with those born in 2010–2012. The prevalence of obesity increased by age for each cohort. For example, boys born in 2004–2006 had the highest prevalence of obesity (18.68%), evaluated in 2016–2018, followed by those born in 2007–2009 (15.5%) and 2010–2012 (12.29%). Across different birth cohorts, boys who were born in the recent cohort (2004–2006 or 2007–2009) had a higher percentage of obesity than those born in the earlier cohort. A similar pattern was also observed among girls.

Age, Period, and Cohort Effect

Results from the Poisson log-linear model using the intrinsic estimator method are shown in **Table 4**. The RR and 95% confidence interval of overweight and obesity are depicted in **Figures 3**, **4**. The age effect was significantly associated with the prevalence of overweight (**Figures 3A,D**) and obesity (**Figures 4A,D**) in both genders. Children at the age of 6 years had a significant lower risk of overweight and obesity in both genders. Boys aged 9 years had a higher risk of overweight, but the risk of obesity was not significant at the age of 12. Among girls, the elevated risk of overweight and obesity was seen at the age of 9 and 12 years.

The period effect of overweight was similar for boys and girls. The downward trends of overweight in both genders occurred in 2016 (**Figures 3B,E**). The estimated coefficient of period effect of overweight in 2016 was −0.083 (RR = 0.92, $p < 0.05$) and −0.138 (RR = 0.87, $p < 0.05$) for boys and girls, respectively (**Table 4**). In addition, boys had a higher risk of overweight in 2009 compared to the overall rate of overweight. The trends of obesity were quite different in both genders. Boys had a significant greater risk of obesity from 2011 to 2012 and a lower risk of obesity in 2017 (**Figure 4B**). The estimated coefficient of period effect of obesity in 2017 was −0.086 (RR = 0.92, $p < 0.05$) for boys. There was a greater fluctuation in period effect of obesity among girls. Higher RR of obesity in girls was found in 2010, 2013, 2015, and 2018. The risks of obesity in 2014 (coefficient = −0.071, RR = 0.93, $p < 0.05$) and 2017 (coefficient = −0.109, RR = 0.90, $p < 0.05$) were significantly lower than the average rate of obesity in girls (**Table 4**; **Figure 4E**).

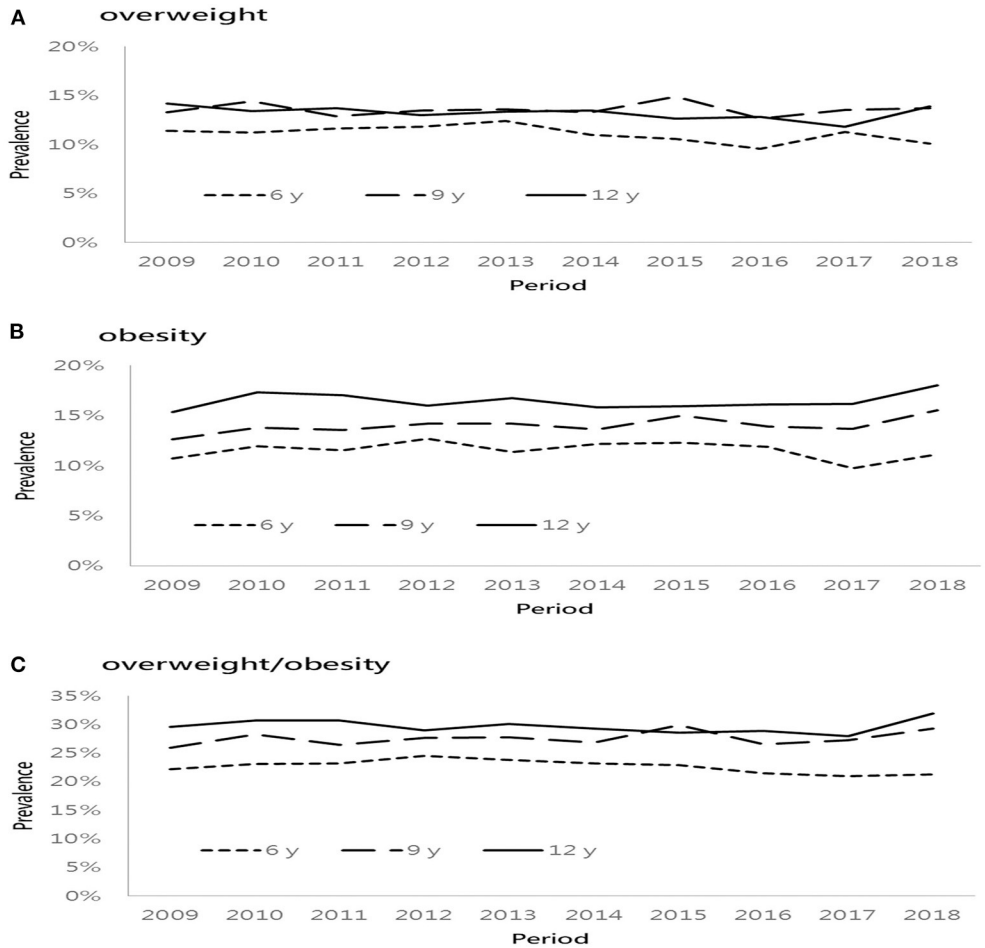

FIGURE 1 | Age-specific prevalence of overweight and obese from 2009 to 2018. **(A)** overweight, **(B)** obesity, and **(C)** overweight/obesity.

With regard to birth cohort effects, results indicated that recent cohorts had a significantly higher risk of overweight or obesity in both genders. Boys from birth cohorts of 2007 to 2009 had a significantly higher risk of overweight and obesity (**Figures 3C**, **4C**). Girls from birth cohorts of 2004 to 2009 had significantly greater risk of obesity. Interestingly, girls who were born in 1997 had a significant low risk of obesity (**Figure 4F**).

DISCUSSION

This is the first study to examine the APC effects in childhood overweight and obesity in Taiwan. Using data collected from schoolchildren between 2009 and 2018, our results from the APC analysis showed a significant positive age effect to the rising overweight and obesity trends. The age effect to overweight and obesity among children aged 6 years in both genders was significantly lower than that of the overall groups. In contrast, the age effect to overweight and obesity among children aged 12 years was significantly higher than that of the overall groups in our study. Among children aged 9 years for both boys and girls, the age effect also had a significant contribution to overweight and obesity (**Figures 3A,D**, **4D**), except for the effect to obesity in boys (**Figure 4A**). This finding is similar to other reports that the prevalence of overweight and obesity increased gradually with increasing age (5, 10, 20). Pubertal physical growth including height and weight contributed to the progressively rising BMI in this age group.

We proposed the difference of age effect in this age group between boys and girls, possibly due to the different timing of pubertal growth spurt, which is 10–12 years of age in girls and 12–14 years of age in boys. Aris et al. demonstrated that the associations with greater standing height and trunk length and earlier pubertal development in adolescence were more pronounced for BMI measures in later childhood (21). Also, children with higher BMI during infancy and childhood demonstrated faster subsequent growth in stature, for both standing height and trunk length. Additionally, children with higher BMI showed earlier pubertal development and slower linear growth during adolescence. Thus, they speculated that factors known to influence linear growth and positively correlate with BMI, such as insulin-like growth factor 1 (IGF-1) and fasting

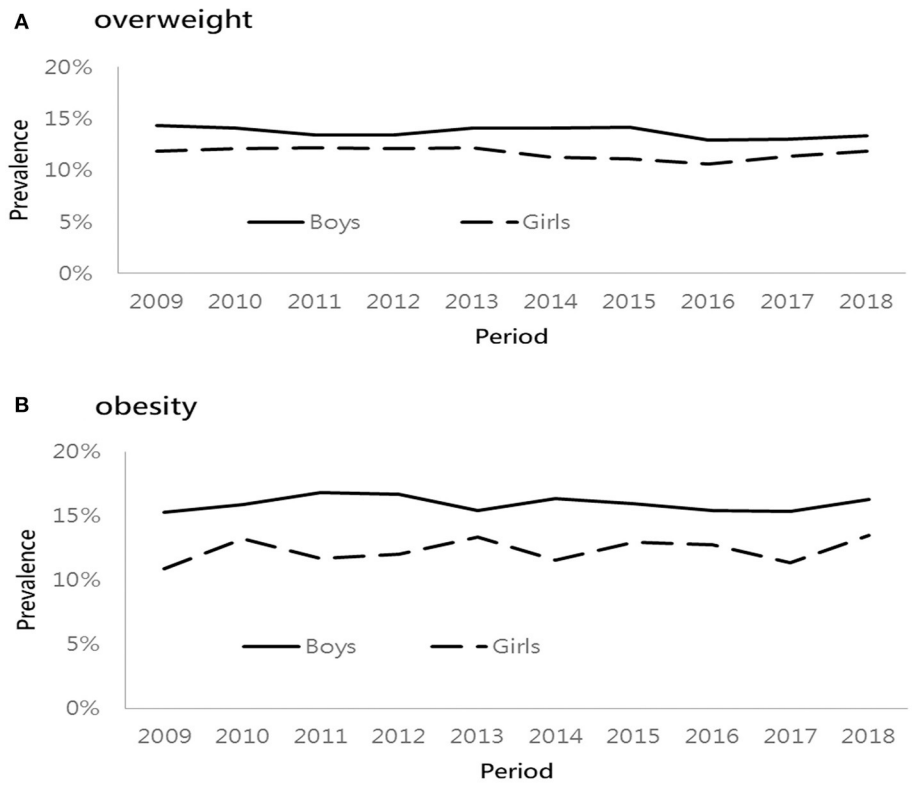

FIGURE 2 | Sex-specific prevalence of overweight and obese from 2009 to 2018. **(A)** overweight and **(B)** obesity.

TABLE 3 | Prevalence (%) of overweight and obesity by period and birth cohort, 2009–2018.

Birth cohort	Period							
	2009		2010–2012		2013–2015		2016–2018	
	Overweight	Obesity	Overweight	Obesity	Overweight	Obesity	Overweight	Obesity
Boys								
1997	14.92	18.90						
1998–2000	14.52	13.78	13.93	19.28				
2001–2003	13.28	12.09	14.41	15.57	14.00	17.60		
2004–2006			12.52	13.61	14.67	15.24	13.55	18.68
2007–2009					12.99	13.43	14.41	15.50
2010–2012							11.24	12.29
Girls								
1997	13.45	11.54						
1998–2000	12.12	11.46	12.77	14.12				
2001–2003	9.25	9.12	12.80	11.95	11.07	13.07		
2004–2006			10.47	10.30	13.09	13.19	12.11	14.57
2007–2009					9.56	10.32	12.13	13.18
2010–2012							9.30	9.39

insulin, may play an important role in the relationship between higher BMI in early life and accelerate subsequent linear growth. The regulation of the growth hormone (GH)–IGF-1 axis has also been associated with prepuberty BMI (22). Rafael et al. illustrated that muscle mass increased greatly in boys as did fat mass in girls within the pubertal period. It has been hypothesized that part of these changes can be justified by the action of sex hormones, which begin to rise during puberty (23).

TABLE 4 | Age period cohort analysis of children overweight and obesity by sex group, 2009–2018.

		Overweight				Obesity			
		Boys		Girls		Boys		Girls	
		Coefficient	SE	Coefficient	SE	Coefficient	SE	Coefficient	SE
Age	6	**−0.114***	0.02	**−0.195***	0.03	**−0.206***	0.02	**−0.206***	0.01
	9	**0.053***	0.02	**0.101***	0.03	−0.020	0.02	**0.038***	0.01
	12	**0.060***	0.02	**0.094***	0.03	**0.226***	0.02	**0.169***	0.01
Period	2009	**0.087***	0.03	0.030	0.04	−0.009	0.03	−0.043	0.02
	2010	0.052	0.04	0.052	0.05	0.021	0.04	**0.087***	0.02
	2011	0.003	0.04	0.081	0.05	**0.086***	0.04	−0.052	0.02
	2012	0.008	0.04	0.075	0.05	**0.096***	0.04	−0.013	0.02
	2013	0.017	0.04	0.050	0.05	−0.052	0.04	**0.053***	0.02
	2014	0.002	0.04	−0.025	0.05	0.020	0.04	**−0.071***	0.02
	2015	0.020	0.04	−0.053	0.05	−0.005	0.04	**0.054***	0.02
	2016	**−0.083***	0.03	**−0.138***	0.05	−0.058	0.04	0.013	0.02
	2017	−0.063	0.03	−0.047	0.05	**−0.086***	0.04	**−0.109***	0.02
	2018	−0.042	0.04	−0.025	0.05	−0.013	0.04	**0.081***	0.03
Birth cohort	1997	−0.041	0.04	0.033	0.06	−0.013	0.05	**−0.134***	0.03
	1998–2000	−0.049	0.04	−0.063	0.05	−0.074	0.04	0.010	0.02
	2001–2003	0.004	0.03	−0.068	0.04	−0.037	0.03	−0.014	0.02
	2004–2006	0.017	0.02	0.032	0.03	0.018	0.02	**0.069***	0.02
	2007–2009	**0.072***	0.02	0.017	0.03	**0.077***	0.02	**0.081***	0.02
	2010–2012	−0.003	0.04	0.050	0.06	0.029	0.04	−0.012	0.03

*Bold values with * are significant at p-value < 0.05.*

For period effect in our APC analysis, the risk of overweight in 2016 and obesity in 2017 declined significantly among both boys (overweight: RR = 0.92; obesity: RR = 0.92) and girls (overweight: RR = 0.87; obesity: RR = 0.90) within this 10-year study period (**Table 4**; **Figures 3B,E**, **4B,E**). The decreasing trend with period is not consistent with previous studies (8–10) other than that in Taiwan (11). We speculated two possible reasons for the declining trend. First, the Ministry of Health and Welfare in Taiwan launched the national weight-loss campaign since 2011, and the Hualien County Health Bureau strongly reinforced the awareness and strategy of better health to support weight-loss campaigns in 2016. Second, the school nutrition policy "Beverage and snack selling rule in school, 2016" by the Ministry of Education in Taiwan since 2016/11/21 banned the soft drink in the senior high school campus, and only the following commercial items could be sold in the elementary and junior high school campuses: 100% fruit or vegetable juice, plain water, fresh milk, extended shelf-life milk, soymilk, yogurt, etc., which should reduce the opportunity to purchase beverage and high-calorie snack at daytime in school. Although in 2018 the rebounded prevalence of overweight and obese did not reach a significant level, the obesogenic environment such as unhealthy high-energy food and daily sedentary behavior should be transformed to support healthful decision and prevent the progression of childhood obesity (24–26).

As for cohort effect in our APC analysis, we demonstrated that later birth cohorts (2004–2006 and 2007–2009) had significant positive effects to increased risk of overweight and obesity among both boys (**Figures 3C**, **4C**) and girls (**Figure 4F**). This is comparable with the findings of previous reports (8, 9, 11). Chu et al. represented the national population-based study related to APC analysis for Taiwanese adolescent aged 12–18 years between 2006 and 2014 (11). They concluded the birth cohorts of the 1990s: the younger cohorts had greater odds of being overweight and obese than the older cohorts when they reached adolescence. Birth cohort effects reflect early-life factors and environmental changes, such as maternal increased food consumption and sedentary behaviors, or during the economic transition (10). Children born between 2004 and 2009 (i.e., generation z) grew up in a convenient environment fulfilled with high technologic services; thus, they are currently exposed to obesogenic environments. Moreover, further studies should be conducted to examine the causal relationship of positive birth cohort effects in relation to child obesity.

Ogden et al. reported that the percentage of obese children in the United States aged 6 to 11 increased from 7% in 1980 to 18% in 2012, and at the same time, the percentage of obese teenagers aged 12–19 increased from 5 to 21% which could represent the influence of a convenient environment to children and adolescents in generation z (27). An obesogenic environment could be modified if teachers and parents worked together to build up healthy food intake and physical activity habits. They illustrated that healthy dietary habits (eating breakfast at home, bringing a school lunch, and not bringing money to purchase food) had a lower risk of obesity. The quality of the eaten food was associated with a risk of obesity: fruit was inversely associated

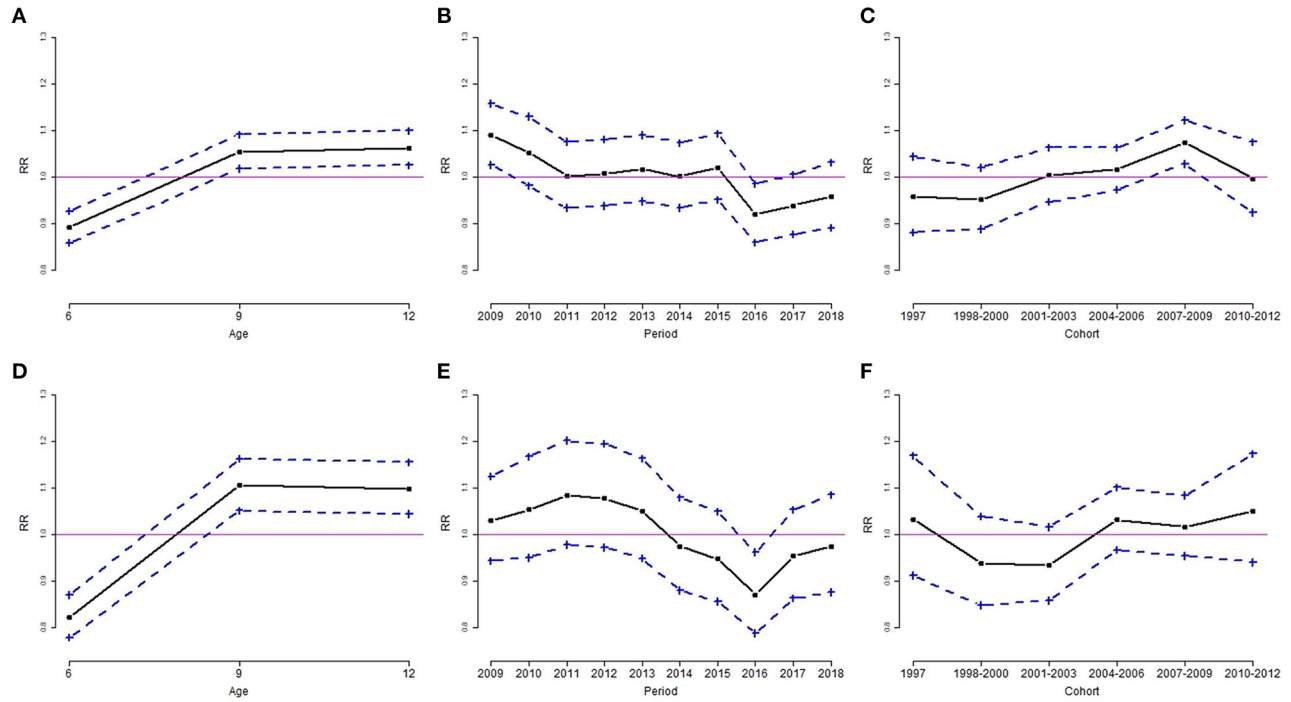

FIGURE 3 | Age, period, and cohort effect of the rate ratio (RR) of overweight by sex group, for boys **(A–C)** and girls **(D–F)**. **(A,D)**: age effect; **(B,E)**: period effect; **(C,F)**: cohort effect. Results are RR (solid line) and 95% confidence interval (dotted line). The solid horizontal line represents RR = 1.

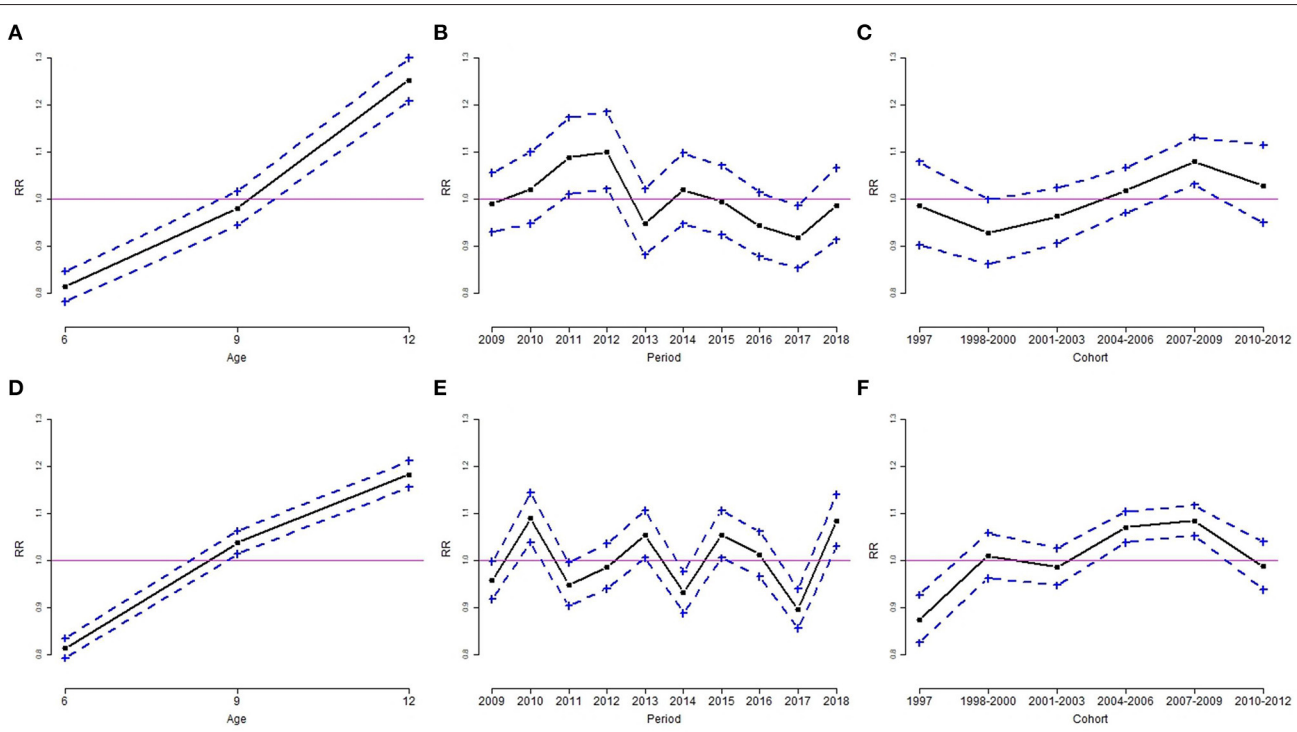

FIGURE 4 | Age, period, and cohort effect of the rate ratio (RR) of obesity by sex group, for boys **(A–C)** and girls **(D–F)**. **(A,D)**: age effect; **(B,E)**: period effect; **(C,F)**: cohort effect. Results are RR (solid line) and 95% confidence interval (dotted line). The solid horizontal line represents RR = 1.

with the risk of obesity; on the contrary, sweetened beverages and refined carbohydrates with added fat were associated with increased risk of obesity (26). Hsiao et al. (28) mentioned that to achieve good family health, physicians should engage the concerns of parents to empower their knowledge of healthy literacy in nutrition, exercise, and parenting skills. A family-based exercise plan, healthy diet plan, and structural daily schedule will provide a supportive environment for children after leaving the school campus. Reward options should not include snacks, sweetened beverages, or high-calorie foods; instead, outdoor sports or tours are recommended to promote physical activity. Management of childhood obesity is progressive and age-dependent, and it should be initiated as early as possible (29, 30).

Based on the report of the Commission on Ending Childhood Obesity by the World Health Organization (WHO) in 2016, their recommendations were to promote the following: intake of healthy food; physical activity; preconception and pregnancy care; early childhood diet and physical activity; health, nutrition, and physical activity for school-aged children; and weight management. Comprehensive programs that promote healthy school environments, health and nutrition literacy, and physical activity among school-age children and adolescents should be established and assisted by family-based, multicomponent lifestyle weight management services (31). In 2018, the same commission by WHO illustrated another action report on childhood obesity and set global targets for halting the increase in obesity, including no increase in overweight among children under age 5, school-age children, or adolescents by 2025 (from 2010 levels). An action to reverse the epidemic is the focus of the recommendations made by the WHO Commission on Ending Childhood Obesity and is one of the main objectives of the Decade of Action on Nutrition (32). The collaboration between school, family, and community is crucial to establishing supportive infrastructure for ending childhood obesity especially in school-age children.

According to our results, we proposed that the timing for weight intervention of schoolchildren could be launched as early as the first grade of elementary school. The outdoor recess between lessons in school should be emphasized to increasing physical activity. "Emptying the classroom at recess" and "Zero hour PE" are good options for school-age children to become mobile in their daily schedule. A recent report (33) concluded that school- and community-based physical activity interventions as part of an obesity prevention or treatment program can benefit executive functions of children with obesity or overweight specifically. Similarly, school-based dietary interventions may benefit general school achievement in children with obesity. Cuda et al. illustrated an algorithm that breaks down intake guidelines for age groups between infancy and adulthood. In considering how to modify the food intake of a child with obesity, there is no universally accepted approach. An understanding of an appropriate intake for a normal-weight child is necessary as a starting point. Children with obesity should not be given any sugar-sweetened beverage or any fast food or desserts (29). School authority should collaborate with shops and restaurants nearby the campus to provide healthy food options for breakfasts and afternoon snacks based on the school nutrition policy "Beverage and snack selling rule in school, 2016" by the Ministry of Education in Taiwan. Among the overweight and obese children detected by the regular health checkup, the notification letter should be given to their parents and enhance their awareness of the consequence of childhood obesity.

The strength of this study is that we used a total of 94,661 schoolchildren recruited from 13 townships in Hualien county between 2009 and 2018. Three age groups of the participants included the first (aged 6 years), fourth (aged 9 years), and seventh grades (aged 12 years). The composition of our dataset showed no significant difference in terms of annual subject number and ratio of boys vs. girls; therefore, our dataset could represent this target age population. Furthermore, body height and weight were actual data measured individually by trained staffs, other than self-reported, which could minimize recall bias. There were some limitations in this study that need to be addressed. First, metabolic status and daily physical activity, dietary records, and anthropometrics such as previous BMI status and waist circumference were not available in the annual health examination dataset. In the future, we should try to collect these data to analyze the effects of these factors on overweight and obesity among schoolchildren. Second is the discontinuous age, e.g., 6, 9, and 12 years, in our dataset, which limits the understanding of age effects with continuous aspects. Third, our data may only illustrate the trend among the schoolchildren of Hualien county, not in other regions of Taiwan.

CONCLUSIONS

In summary, our study showed a significant association of age, period, and cohort effects on the risk of overweight and obesity among school-age children. With increasing age, regardless of fluctuations, the trend in the prevalence of overweight and obesity increased in both genders. For the period effect, the trend in the prevalence of overweight and obesity fluctuated downward slowly from 2016 to 2017 in boys and girls. For cohort effects in both genders, the birth cohort of 2007 to 2009 was correlated with the increased risk of overweight and obesity.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the datasets used in this study will not be made publicly available. The Ethics committee did not give permission for the data to be made publicly available. Requests to access the datasets should be directed to Y-CC, tonyijane@hotmail.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hualien Tzu Chi Hospital. Written informed consent to participate in this study was

provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Y-CC and S-HW: study design and coordination of data collection and manuscript writing/editing. S-HW: statistical analysis. W-HH, S-FH, and HH: data interpretation and revision of the manuscript. Y-WW and C-HC: critical review of the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.615483/full#supplementary-material

REFERENCES

1. Ortiz-Pinto MA, Ortiz-Marrón H, Ferriz-Vidal I, Martínez-Rubio MV, Esteban-Vasallo M, Ordobás-Gavin M, et al. Association between general and central adiposity and development of hypertension in early childhood. *Eur J Prev Cardiol.* (2019) 26:1326–34. doi: 10.1177/2047487319839264
2. Tanamas SK, Reddy SP, Chambers MA, Clark EJ, Dunnigan DL, Hanson RL, et al. Effect of severe obesity in childhood and adolescence on risk of type 2 diabetes in youth and early adulthood in an American Indian population. *Pediatr Diabetes.* (2018) 19:622–9. doi: 10.1111/pedi.12627
3. Skinner AC, Ravanbakht SN, Skelton JA, Perrin EM, Armstrong SC. Prevalence of obesity and severe obesity in US children, 1999–2016. *Pediatrics.* (2018) 141:e20173459. doi: 10.1542/peds.2017-3459
4. Chen L-J, Fox KR, Haase A, Wang J-M. Obesity, fitness and health in Taiwanese children and adolescents. *Eur J Clin Nutr.* (2006) 60:1367–75. doi: 10.1038/sj.ejcn.1602466
5. Chu CH, Wang JH, Jan RH, Huang CH, Cheng CF. Association between health examination items and body mass index among school children in Hualien, Taiwan. *BMC Public Health.* (2013) 13:1–7. doi: 10.1186/1471-2458-13-975
6. Davison KK, Birch LL. Childhood overweight: a contextual model and recommendations for future research. *Obes Rev.* (2001) 2:159–71. doi: 10.1046/j.1467-789x.2001.00036.x
7. Mccrindle M, Wolfinger E. *The ABC of XYZ: Understanding the Global Generations. The ABC of XYZ*. Sydney: University of New South Wales Press (2009).
8. Fu Q, Land KC. The increasing prevalence of overweight and obesity of children and youth in China, 1989–2009: an age–period–cohort analysis. *Popul Res Policy Rev.* (2015) 34:901–21. doi: 10.1007/s11113-015-9372-y
9. Hosseini M, Kelishadi R, Baikpour M, Ataei N, Qorbani M, Yousefifard M, et al. Age-period-cohort analysis of obesity and overweight in Iranian children and adolescents. *Int J Endocrinol Metab.* (2017) 15:e13561. doi: 10.5812/ijem.13561
10. Kwok MK, Tu YK, Kawachi I, Schooling CM. Age-period-cohort analysis of trends in blood pressure and body mass index in children and adolescents in Hong Kong. *J Epidemiol Community Health.* (2017) 71:1161–8. doi: 10.1136/jech-2017-209491
11. Chu CH, Chen HJ, Chang YK. Age, period and birth cohort effects on the prevalence of overweight and obesity among Taiwanese adolescents: a national population-based study. *J Public Health.* (2019) 41:90–9. doi: 10.1093/pubmed/fdy025
12. Chen FL, Lee A. Health-promoting educational settings in Taiwan: development and evaluation of the health-promoting school accreditation system. *Glob Health Promot.* (2016) 23:18–25. doi: 10.1177/1757975916638286
13. Chen W, Chang MH. New growth charts for Taiwanese children and adolescents based on World Health Organization standards and health-related physical fitness. *Pediatr Neonatol.* (2010) 51:69–79. doi: 10.1016/S1875-9572(10)60014-9
14. Hou PC, Tu SH, Liao PS, Hung YT, Chang YH. The typology of townships in Taiwan: the analysis of sampling stratification of the 2005–2006 "Taiwan Social Change Survey". *Surv Res Method Appl.* (2008) 23:7–32. doi: 10.7014/TCYCFFYYY.200804.0007
15. Yang Y, Schulhofer-Wohl S, Fu WJ, Land KC. The intrinsic estimator for age-period-cohort analysis: what it is and how to use it. *Am J Sociol.* (2008) 113:1697–736. doi: 10.1086/587154
16. Fu W. *A Practical Guide to Age-Period-Cohort Analysis: The Identification Problem and Beyond*. Boca Raton, FL: CRC Press (2018).
17. Phillips JA. A changing epidemiology of suicide? The influence of birth cohorts on suicide rates in the United States. *Soc Sci Med.* (2014) 114:151–60. doi: 10.1016/j.socscimed.2014.05.038
18. Li Z, Wang P, Gao G, Xu C, Chen X. Age-period-cohort analysis of infectious disease mortality in urban-rural China, 1990-2010. *Int J Equity Health.* (2016) 15:1–9. doi: 10.1186/s12939-016-0343-7
19. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2020). Available online at: https://www.R-project.org/ (accessed March 27, 2021).
20. Anderson PM, Butcher KF, Schanzenbach DW. Understanding recent trends in childhood obesity in the United States. *Econ Hum Biol.* (2019) 34:16–25. doi: 10.1016/j.ehb.2019.02.002
21. Aris IM, Rifas-Shiman SL, Zhang X, Yang S, Switkowski K, Fleisch AF, et al. Association of BMI with linear growth and pubertal development. *Obesity.* (2019) 27:1661–70. doi: 10.1002/oby.22592
22. Dehkhoda F, Lee CMM, Medina J, Brooks AJ. The growth hormone receptor: mechanism of receptor activation, cell signaling, physiological aspects. *Front. Endocrinol.* (2018) 9:35. doi: 10.3389/fendo.2018.00035
23. Rafael OA, Jordana HS, Divanei Z, Deyse MD, Polyana RO, Jos,é GM. Influence of muscle mass on the serum uric acid levels in children and adolescents. *Nutr Metab Cardiovasc Dis.* (2020) 30:300–05. doi: 10.1016/j.numecd.2019.08.019
24. Shirasawa T, Ochiai H, Nanri H, Nishimura R, Ohtsu T, Hoshino H, et al. Trends of underweight and overweight/obesity among japanese schoolchildren from 2003 to 2012, defined by body mass index and percentage overweight cutoffs. *J Epidemiol.* (2015) 25:482–8. doi: 10.2188/jea.JE20140144
25. Vilchis-Gil J, Galván-Portillo M, Klünder-Klünder M, Cruz M, Flores-Huerta S. Food habits, physical activities and sedentary lifestyles of eutrophic and obese school children: a case-control study. *BMC Public Health.* (2015) 15:1–8. doi: 10.1186/s12889-015-1491-1
26. Juonala M, Harcourt BE, Saner C, Sethi M, Saffery R, Magnussen CG, et al. Neighbourhood socioeconomic circumstances, adiposity and cardiometabolic risk measures in children with severe obesity. *Obes Res Clin Pract.* (2019) 13:345–51. doi: 10.1016/j.orcp.2019.05.004
27. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA.* (2014) 311:806–14. doi: 10.1001/jama.2014.732
28. Hsiao YC, Wang JH, Chu CH, Chang YH, Chen JS, Jan RH, et al. Comparison of growth velocity among school age children with different body mass index from childhood into early adolescence in Hualien county, Taiwan: a retrospective cohort study. *Front Pediatr.* (2021) 9:599730. doi: 10.3389/fped.2021.599730
29. Cuda SE, Censani M. Pediatric obesity algorithm: a practical approach to obesity diagnosis and management. *Front Pediatr.* (2019) 6:431. doi: 10.3389/fped.2018.00431
30. Imoisili OE, Goodman AB, Dooyema CA, Park S, Harrison M, Lundeen EA, et al. Referrals and management strategies for pediatric obesity—DocStyles

survey 2017. *Front Pediatr*. (2018) 6:431. doi: 10.3389/fped.2018.00367
31. World Health Organization. *Report of the Commission on Ending Childhood Obesity*. Genève: World Health Organization (2016).
32. World Health Organization. *Taking Action on Childhood Obesity*. Genève: World Health Organization (2018).
33. Martin A, Booth JN, Laird Y, Sproule J, Reilly JJ, Saunders DH. Physical activity, diet and other behavioural interventions for improving cognition and school achievement in children and adolescents with obesity or overweight. *Cochrane Database Syst Rev*. (2018) 1:CD009728. doi: 10.1002/14651858.CD009728.pub3

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Relationship Between Histological Features of Non-alcoholic Fatty Liver Disease and Ectopic Fat on Magnetic Resonance Imaging in Children and Adolescents

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Objectives: To investigate the association between ectopic fat content in the liver and pancreas, obesity-related metabolic components, and histological findings of non-alcoholic fatty liver disease (NAFLD) in children.

Methods: This cross-sectional study investigated 63 children with biopsy-proven NAFLD who underwent magnetic resonance imaging (MRI), anthropometry, laboratory tests, and body composition analysis. Clinical and metabolic parameters, MRI-measured hepatic fat fraction (HFF) and pancreatic fat fraction (PFF), and histological findings were analyzed.

Results: In a total of 63 children (48 boys, median age 12.6 years, median body mass index *z*-score 2.54), HFF was associated with histological steatosis [10.4, 23.7, and 31.1% in each steatosis grade, $P < 0.001$; Spearman's rho coefficient (rs) = 0.676; $P < 0.001$] and NAFLD activity score (rs = 0.470, $P < 0.001$), but not with lobular inflammation, hepatocyte ballooning, and hepatic fibrosis. PFF was not associated with any histological features of the liver. Waist circumference-to-height ratio and body fat percentage were associated with the steatosis grade ($P = 0.006$ and $P = 0.004$, respectively). Alanine aminotransferase was not associated with steatosis but was associated with lobular inflammation ($P = 0.008$). Lobular inflammation was also associated with high total cholesterol and low-density lipoprotein cholesterol and metabolic syndrome ($P = 0.015$, $P = 0.036$, and $P = 0.038$, respectively).

Conclusions: Hepatic steatosis on MRI was only associated with the histological steatosis grade, while elevated serum levels of liver enzymes and lipids were related to the severity of lobular inflammation. Therefore, MRI should be interpreted in conjunction with the anthropometric and laboratory findings in pediatric patients.

Keywords: obesity, intra-abdominal fat, biopsy, metabolic syndrome, child

INTRODUCTION

The prevalence of pediatric obesity has increased in recent years. Correspondingly, the incidence of non-alcoholic fatty liver disease (NAFLD) is also increasing in children since obesity is the primary etiology of NAFLD (1). NAFLD encompasses a spectrum of diseases, ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which is a progressive form of NAFLD typically associated with lobular inflammation and hepatocellular injury in addition to steatosis with or without perisinusoidal fibrosis (2, 3).

When the circulating levels of triglycerides (TG) and free fatty acid exceed the metabolic capacity of the adipose tissue, they accumulate as ectopic fat in the non-adipose tissue in patients with obesity (4, 5). Along with hepatic fat, pancreatic fat is considered an obesity-induced ectopic fat depot, which might contribute to metabolic disturbances, including diabetes mellitus (DM), hypertension (HTN), dyslipidemia, metabolic syndrome (MetS), and NAFLD (6, 7). However, little is known about the association between ectopic pancreatic fat accumulation and the histopathological severity of NAFLD, especially in children with biopsy-proven NAFLD.

According to previous studies, hepatic fibrosis at baseline is independently associated with overall mortality, liver transplantation, and liver-related events in patients with NAFLD (8, 9). However, few studies have investigated the association between the abnormalities of liver enzymes, metabolic components, and severity of histological features of NAFLD in children with biopsy-proven NAFLD.

Therefore, this study aimed to evaluate the relationship between ectopic fat measured by magnetic resonance imaging (MRI) in the liver and pancreas and the severity of each histological feature of NAFLD in children. We also aimed to investigate the relevance of histological features of the liver to anthropometry, liver enzymes, and laboratory findings of obesity-related metabolic components in pediatric patients with NAFLD.

MATERIALS AND METHODS

Study Subjects

NAFLD is a diagnosis of exclusion based on the presence of hepatic steatosis and exclusion of non-NAFLD-related causes of hepatic steatosis (2). Liver biopsy is the current standard for defining the presence and severity of NAFLD, including the presence of NASH, and ruling out alternative and/or other concurrent chronic liver diseases, which can be challenging to exclude non-invasively (2). The optimal timing of liver biopsy to confirm the diagnosis of NAFLD and follow-up on its progression has not been established (2). Although ultrasound is widely available, a normal hepatic ultrasound cannot exclude the presence of NAFLD and therefore is not useful for diagnosis or follow-up (2). When available, MRI and magnetic resonance spectroscopy (MRS) are highly accurate for identifying steatosis (2, 10–12).

The pediatric gastroenterology, hepatology, and nutrition center in our tertiary hospital has treated many children and adolescents suspected of NAFLD referred from primary clinics, secondary hospitals, or other departments of our hospital for problems such as obesity-related comorbidities and/or abnormal laboratory results, including elevation of liver enzymes. In such cases laboratory investigations and abdominal ultrasound are performed primarily to evaluate other causes of liver disease, such as masses, gall bladder disease, and changes associated with portal hypertension (2). If the patient's clinical parameters, such as elevated liver enzymes, the severity of metabolic dysregulation, and degree of obesity, indicate evidence of ongoing disease during a follow-up period of at least 6 months or more or if there is a presence of known clinical risk factors for NASH and advanced fibrosis such as panhypopituitarism or type 2 DM, liver biopsy is considered. Since 2014, we have performed liver biopsy and abdominal MRI simultaneously.

This retrospective cross-sectional observational study included 63 children and adolescents with liver biopsy-proven NAFLD who underwent both liver biopsy and abdominal MRI performed within 2 days of each other between March 2014 and February 2020. A sample size of at least 63 was estimated to have a type I error rate of 0.05, with a power of 80% (13).

In our study, six patients with NAFLD were ≥18 years of age. One patient each was 18.1, 18.2, 18.6, 18.7, 18.8, and 19.3 years old. These six subjects were enrolled in this study despite their relatively higher age because they had been followed up continuously in the "pediatric gastroenterology, hepatology, and nutrition center" of our hospital since they were diagnosed with NAFLD at an early age. When liver biopsy and abdominal MRI were performed in these subjects, they were ≥18 years old. Moreover, in the 21st edition of the Nelson textbook of Pediatrics, the definition of adolescence includes three groups based on age: early adolescence indicates the approximate age range 10–13 years, middle adolescence is 14–17 years, and late adolescence is 18–21 years (14).

All study subjects underwent anthropometric measurements, blood pressure (BP) measurements, body composition analysis, and laboratory tests. NAFLD was diagnosed as the presence of steatosis in ≥5% of the hepatocytes in the absence of other liver diseases. Secondary etiologies of hepatic steatosis, systemic diseases, and pancreatic diseases were excluded (**Figure 1**).

The study conformed to the ethical guidelines of the Declaration of Helsinki (revised in Fortaleza, Brazil, October

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; EBP, elevated blood pressure; FFM, fat-free mass; FFMI, fat-free mass index; FMI, fat mass index; FPG, fasting plasma glucose; GGT, γ-glutamyl transferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HFF, hepatic fat fraction; HOMA-IR, homeostatic model assessment of insulin resistance; HTN, hypertension; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; PDFF, proton density fat fraction; PFF, pancreatic fat fraction; PreDM, prediabetes; QUICKI, quantitative insulin sensitivity check index; rs, Spearman's rho coefficient; SBP, systolic blood pressure; TBF, total body fat; t-bil, total bilirubin; t-chol, total cholesterol; TG, triglyceride; WC, waist circumference; WHtR, waist circumference to height ratio.

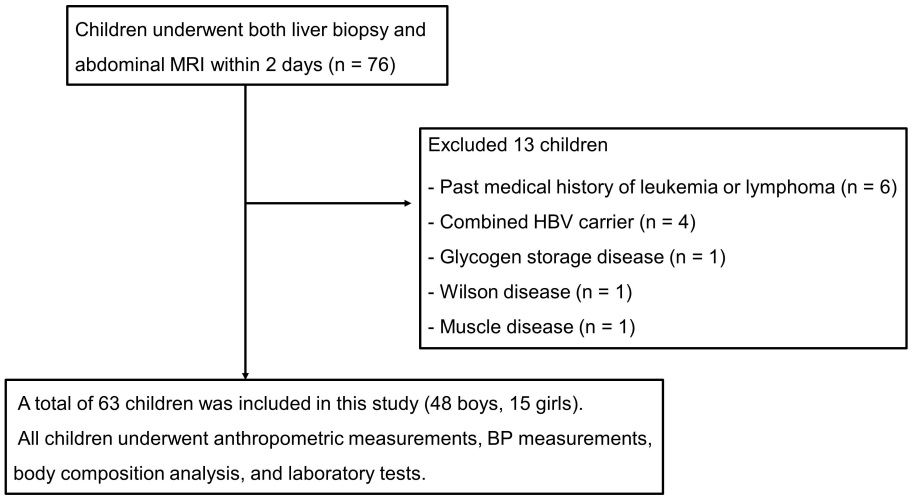

FIGURE 1 | Flow diagram showing the selection of subjects. A sample size of at least 63 was estimated to have a type I error rate of 0.05, with a power of 80%. The present study included 63 children and adolescents with liver biopsy-proven non-alcoholic fatty liver disease (NAFLD) who underwent both liver biopsy and abdominal magnetic resonance imaging (MRI) performed within 2 days from March 2014 to February 2020. NAFLD was diagnosed as the presence of steatosis in ≥5% of hepatocytes in the absence of other liver diseases. Secondary etiologies of hepatic steatosis, systemic diseases, and pancreatic diseases were excluded. HBV, hepatitis B virus; BP, blood pressure.

2013) and the recommendations of the Ethics Committee of Seoul National University Bundang Hospital. This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-2103-670-104).

Anthropometry and Blood Pressure and Body Composition Measurements

Anthropometric parameters, including height, weight, and waist circumference (WC), were measured in all children using standardized methods. Waist circumference to height ratio (WHtR), body mass index (BMI), and standard deviation score (*z*-score) were calculated. Obesity was defined as BMI ≥95th percentile, overweight as BMI between the 85th and 95th percentiles, adjusted for age and sex, according to the Korean national growth charts 2017 (15). Central obesity was defined as WC ≥90th percentile, adjusted for age and sex, according to the Korean national growth charts 2007 (16).

BP was measured during three different visits in a standardized manner (17). HTN was defined as systolic BP (SBP) or diastolic BP (DBP) ≥95th of sex-, age-, and height-specific percentiles or SBP ≥130 mm Hg or DBP ≥80 mm Hg for children aged <13 years and SBP ≥130 mm Hg or DBP ≥80 mm Hg for adolescents aged ≥13 years (17, 18). Children whose BP readings corresponded to HTN on ≥3 occasions or BP remained elevated for 1 year or more underwent 24 h ambulatory BP monitoring to confirm whether they had HTN.

Body composition was measured using bioelectrical impedance analysis (InBody J10, Biospace Co., Ltd., Seoul, Korea), and the total body fat (TBF) and fat-free mass (FFM) were recorded. The fat mass index (FMI) and fat-free mass index (FFMI) were calculated as fat mass (kg) and fat-free mass (kg), respectively, divided by the square of height (m^2).

Laboratory Tests

Blood samples were obtained after a 10 h overnight fast. We recorded the total cholesterol (t-chol), TG, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, γ-glutamyl transferase (GGT), fasting plasma glucose (FPG), insulin, and glycated hemoglobin (HbA1c) levels.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: FPG (mg/dL) × fasting insulin (μU/mL)/405 (15). The quantitative insulin sensitivity check index (QUICKI) was calculated as follows: 1/log (HOMA-IR × 405) (19).

Prediabetes and DM were defined based on the American Diabetes Association guidelines (20). Prediabetes was defined as the presence of at least one of the following: FPG 100–125 mg/dL (impaired fasting glucose), 2 h plasma glucose during oral glucose tolerance test between 140 and 199 mg/dL (impaired glucose tolerance), and an HbA1c value of 5.7–6.4% (20). DM was defined as the presence of at least one of the following: HbA1c value ≥6.5%, FPG ≥126 mg/dL, 2 h plasma glucose ≥200 mg/dL during oral glucose tolerance test, and a random plasma glucose level ≥200 mg/dL in a patient with classic symptoms of hyperglycemia (20).

Dyslipidemia was defined as the presence of at least one of the following: t-chol ≥200 mg/dL, LDL-C ≥130 mg/dL, HDL-C <40 mg/dL, TG ≥100 mg/dL (age ≤9 years) or ≥130 mg/dL (age ≥10 years) (21).

Definition of Metabolic Syndrome

MetS was defined based on a revised version of the modified National Cholesterol Education Program Adult Treatment Panel

III diagnostic criteria and was diagnosed in children who met at least three of the following five criteria: TG ≥110 mg/dL, HDL-C ≤40 mg/dL, BP ≥90th of age-, sex-, and height-specific percentiles, WC ≥90th sex-specific percentile, and FPG ≥100 mg/dL (22), not the previous criteria of FPG ≥110 mg/dL (23).

Liver Histopathology

Liver biopsy was performed by an experienced pediatric radiologist under ultrasound guidance, and the findings were interpreted by an expert liver pathologist who was blinded to the patients' clinical data.

All biopsy specimens were evaluated based on the NAFLD Clinical Research Network criteria (24), and the NAFLD activity score (NAS) was assessed (25). The scores for degree of steatosis, lobular inflammation, portal inflammation, and hepatocyte ballooning degeneration were calculated (steatosis 0–3, lobular inflammation 0–3, portal inflammation 0–2, hepatocyte ballooning 0–2) (24). Hepatic fibrosis was categorized into stages 0–4 (stage 0, no fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; stage 4, cirrhosis) (24). NAS was calculated using an eight-point scale as the sum of the scores for steatosis, lobular inflammation, and hepatocyte ballooning (25). The subjects were also divided into the following NAS subgroups: NAS 0–2, NAS 3–4, and NAS ≥5.

Magnetic Resonance Imaging-Based Measurement of Hepatic and Pancreatic Fat Fractions

Abdominal MRI examinations were performed using a 3.0 T MR scanner (Ingenia, Philips Healthcare, Best, Netherlands). The modified DIXON-Quant sequence was obtained during a single breath hold, which automatically reconstructed a proton density fat fraction (PDFF) map. We obtained the maps of water, fat, fat fraction, R2*, and T2* by post-processing the acquired images using the software provided by the manufacturer. All data were transferred to the IntelliSpace Portal software (version 10.0; Philips, Amsterdam, The Netherlands). Selection of the region of interest and fat fraction measurements were performed by an expert pediatric radiologist who was blinded to the patients' clinical and histopathological data. Hepatic fat fraction (HFF) measurements were performed by drawing two different regions of interest in the right and left hepatic lobes. MRI-PDFF is known to have an excellent diagnostic value for the assessment of hepatic fat content in patients with NAFLD (26). HFF ≥5.0% on MRI-PDFF is defined as a fatty liver because MRI-PDFF is highly accurate compared to the histological diagnosis of steatosis (26). Regions of interest for pancreatic fat fraction (PFF) measurements were selected in the head, body, and tail of the pancreas. The normal range of PFF to define fatty pancreas has not yet been established.

Statistical Analysis

Descriptive characteristics are presented as mean ± SD for normally distributed variables or as medians and ranges for non-normally distributed variables. Categorical measurements are expressed as absolute numbers and percentages.

Intergroup differences were evaluated using ANOVA or an independent *t*-test for parametric variables and the Kruskal-Wallis test or the Mann-Whitney *U*-test for non-parametric variables. Categorical variables were compared using the *chi*-square or Fisher's exact test. Correlations between the continuous and/or ordinal variables were tested using the Pearson's correlation matrix or the Spearman rank correlation matrix according to the property of variables.

A two-sided *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using PASW Statistics software (version 25.0; SPSS Inc., Chicago, IL, USA).

TABLE 1 | Magnetic resonance imaging based-fat fractions and histopathologic features of the liver in 63 children with non-alcoholic fatty liver disease.

Variable	Data
Magnetic resonance imaging-based fat fraction	
Hepatic fat fraction (%)	24.3 (4.7–49.9)
Pancreatic fat fraction (%)	3.8 (0.4–26.9)
Liver histopathology	
Steatosis grade (*n*, %)	
0	0
1	13 (20.6%)
2	18 (28.6%)
3	32 (50.8%)
Lobular inflammation grade (*n*, %)	
0	0
1	32 (50.8%)
2	29 (46.0%)
3	2 (3.2%)
Portal inflammation grade (*n*, %)	
0	24 (38.1%)
1	38 (60.3%)
2	1 (1.6%)
Ballooning degeneration grade (*n*, %)	
0	33 (52.4%)
1	24 (38.1%)
2	6 (9.5%)
Fibrosis stage (*n*, %)	
0	11 (17.5%)
1	41 (65.1%)
2	11 (17.5%)
3	0
4	0
NAFLD activity score (*n*, %)	
0–2	8 (12.7%)
3–4	24 (38.1%)
5–8	31 (49.2%)
NASH classification (*n*, %)	
NAFLD, non-NASH	19 (30.2%)
NASH	44 (69.8%)

Values are presented as median (range) or numbers (%). NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

TABLE 2 | Comparison of demographic, anthropometric, laboratory, and magnetic resonance imaging-measured fat fraction findings of 63 children with non-alcoholic fatty liver disease according to liver histologic steatosis grade.

Variable	Steatosis Grade 1 ($n = 13$)	Steatosis Grade 2 ($n = 18$)	Steatosis Grade 3 ($n = 32$)	Total patients ($n = 63$)	*P*-value
Sex (boys: girls)	9 (69.2%): 4 (30.8%)	14 (77.8%): 4 (22.2%)	25 (78.1%): 7 (21.9%)	48 (76.2%): 15 (23.8%)	0.857*
Age, yr	11.2 (5.6–19.3)	12.8 (10.5–18.8)	12.0 (6.1–18.6)	12.6 (5.6–19.3)	0.165
Height (cm)	155.8 (122.4–184.3)	158.1 (141.9–179.9)	150.8 (123.8–184.7)	155.8 (122.4–184.7)	0.663
Height *z*-score	1.15 (−2.90–2.82)	0.27 (−3.40–1.25)	0.88 (−1.01–2.17)	0.87 (−3.40–2.82)	0.082
Weight (kg)	63.3 (37.9–110.7)	73.4 (39.2–110.6)	63.6 (36.4–30.9)	65.3 (36.4–130.9)	0.469
Weight *z*-score	1.95 (−0.37–3.75)	1.89 (−2.80–3.68)	2.38 (0.41–5.01)	2.16 (−2.80–5.01)	0.160
WC (cm)	87.5 (75.0–105.7)	90.5 (67.0–113.4)	93.0 (75.0–120.0)	89.1 (67.0–120.0)	0.247
WHtR	0.55 (0.50–0.69)	0.57 (0.46–0.69)	0.60 (0.52–0.69)	0.59 (0.46–0.69)	0.026
BMI	25.3 (20.0–32.6)	27.9 (18.6–35.2)	27.5 (21.6–38.4)	26.7 (18.6–38.4)	0.198
BMI *z*-score	1.78 (0–4.56)	2.22 (−1.10–4.19)	2.65 (0.89–5.10)	2.54 (−1.10–5.10)	0.122
Fat mass (kg)	20.0 (12.9–39.7)	24.5 (8.2–39.7)	25.4 (12.6–54.0)	23.2 (8.2–54.0)	0.210
Fat mass (%)	32.9 (25.6–43.8)	34.7 (22.3–48.2)	39.4 (24.1–67.2)	35.8 (22.3–67.2)	0.017
FFM (kg)	41.5 (27.7–71.0)	45.5 (28.6–74.2)	34.5 (23.5–79.7)	41.0 (23.5–79.7)	0.695
FFM (%)	67.1 (56.3–74.4)	65.3 (51.8–77.7)	60.6 (32.8–75.8)	64.1 (32.8–77.7)	0.019
FMI	8.2 (5.8–12.7)	8.8 (3.9–17.1)	10.4 (7.1–18.8)	9.9 (3.9–18.8)	0.018
FFMI	17.3 (13.2–20.9)	18.5 (13.6–22.9)	15.8 (9.2–23.4)	17.1 (9.2–23.4)	0.778
mSBP (mmHg)	114.0 (100–144)	121 (100–148)	117.0 (106–159)	118.0 (100–159)	0.271
mDBP (mmHg)	62.0 (52–75)	67.0 (52–84)	63.5 (53–90)	63.0 (52–90)	0.285
AST (IU/L)	49.0 (17–161)	52.0 (27–196)	61.0 (23–226)	54.0 (17–226)	0.429
ALT (IU/L)	88.0 (21–331)	120.5 (20–287)	134.5 (48–366)	122.0 (20–366)	0.301
AST/ALT	0.53 (0.40–1.26)	0.51 (0.31–1.52)	0.47 (0.28–1.26)	0.48 (0.28–1.52)	0.133
t-bil (mg/dL)	0.6 (0.3–0.9)	0.7 (0.2–1.3)	0.5 (0.3–1.1)	0.6 (0.2–1.3)	0.991
GGT (IU/L)	28.0 (12–117)	36.5 (19–133)	43.0 (17–184)	38.0 (12–184)	0.186
t-chol (mg/dL)	172.0 (105–277)	176.0 (122–258)	187.5 (122–275)	180.0 (105–277)	0.458
TG (mg/dL)	130.0 (56–364)	99.0 (48–357)	122.5 (54–280)	121.0 (48–364)	0.851
HDL-C (mg/dL)	45.0 (28–68)	46.0 (37–67)	45.5 (28–61)	45.0 (28–68)	0.948
LDL-C (mg/dL)	96.0 (55–168)	105.5 (66–146)	110.5 (61–175)	108.0 (55–175)	0.412
FPG (mg/dL)	90.0 (71–113)	102.5 (76–270)	90.5 (71–179)	93.0 (71–270)	0.005
Insulin (mIU/L)	19.9 (8.9–75.6)	21.7 (11.1–47.9)	20.9 (4.6–75.4)	20.9 (4.6–75.6)	0.894
HbA1c (%)	5.5 (5.0–7.3)	5.9 (5.0–11.0)	5.3 (5.1–13.5)	5.4 (5.0–13.5)	0.248
HOMA-IR	4.2 (2.1–17.0)	6.0 (2.1–14.0)	4.7 (1.0–14.5)	5.1 (1.0–17.0)	0.295
QUICKI	0.31 (0.26–0.34)	0.30 (0.27–0.34)	0.31 (0.27–0.39)	0.30 (0.26–0.39)	0.295
MRI HFF (%)	10.4 (4.7–21.0)	23.7 (9.2–33.0)	31.1 (11.6–49.9)	24.3 (4.7–49.9)	<0.001
MRI PFF (%)	2.7 (1.2–11.9)	4.4 (0.4–26.9)	3.6 (1.4–15.8)	3.8 (0.4–26.9)	0.241
BMI: Normal/Overweight/Obesity (*n*, %)	1 (7.7%)/5 (38.5%)/7 (53.8%)	3 (16.7%)/5 (27.8%)/10 (55.6%)	1 (3.1%)/3 (9.4%)/28 (87.5%)	5 (7.9%)/13 (20.6%)/45 (71.4%)	0.032* 0.029†
Central obesity (*n*, %)	11 (84.6%)	13 (72.2%)	30 (93.8%)	54 (85.7%)	0.115*
BP category: Normal/EBP + HTN (*n*, %)	9 (69.2%)/4 (30.8%)	9 (50%)/9 (50%)	20 (62.5%)/12 (37.5%)	38 (60.3%)/25 (39.7%)	0.523‡
Dyslipidemia (*n*, %)	7 (53.8%)	12 (66.7%)	23 (71.9%)	42 (66.7%)	0.509‡
DM category: Normal/PreDM + DM (*n*, %)	9 (69.2%)/4 (30.8%)	6 (33.3%)/12 (66.7%)	25 (78.1%)/7 (21.9%)	40 (63.5%)/23 (36.5%)	0.006‡
MetS (*n*, %)	3 (23.1%)	8 (44.4%)	14 (43.8%)	25 (39.7%)	0.389‡

*P-value was calculated by Kruskal-Wallis test. *P-value was calculated by Fisher's exact test. †P-value was calculated for linear trend of obesity proportion. ‡P-value was calculated by Chi-square test. Values are presented as median (range) or numbers (%).*

WC, waist circumference; WHtR, waist circumference-to-height ratio; BMI, body mass index; FFM, fat-free mass; FMI, fat mass index = fat mass(kg)/height(m)^2; FFMI, fat-free mass index = FFM(kg)/height(m)^2; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; t-bil, total bilirubin; GGT, γ-glutamyl transferase; t-chol, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; MRI, magnetic resonance imaging; HFF, hepatic fat fraction; PFF, pancreatic fat fraction; BP, blood pressure; EBP, elevated BP; HTN, hypertension; DM, diabetes mellitus; PreDM, prediabetes; MetS, metabolic syndrome.

RESULTS

Patient Characteristics

We investigated 63 children with biopsy-proven NAFLD (48 boys, 15 girls; median age 12.6 years, ranging 5.6–19.3 years). The MRI-measured ectopic fat content and histological findings of the liver are shown in **Table 1**. Most subjects were overweight or obese (median BMI *z*-score 2.54, range −1.10–5.10). Thirteen (20.6%) children were overweight, 45 (71.4%) children were obese, and only 5 (7.9%) children were within the normal range (BMI <85th percentile) (**Table 2**). Central obesity was confirmed in 54 (85.7%) children. Elevated BP was noted in 9 (14.3%) children, and HTN was diagnosed in 16 (25.4%) children. Prediabetes was diagnosed in 14 (22.2%), and DM in 9 (14.3%) children. Dyslipidemia was observed in 42 (66.7%) and MetS was diagnosed in 25 (39.7%) children (**Table 2**).

Comparison Between MRI PDFF-Measured HFF and PFF According to Histological Features of NAFLD

When HFF and PFF were compared according to the severity of each histological feature, HFF significantly increased with increase in the grade of hepatic steatosis (10.4, 23.7, and 31.1% in steatosis grade 1, 2, and 3, respectively, $P < 0.001$) (**Figure 2**; **Table 2**). HFF correlated with the grade of steatosis [Spearman's rho coefficient (rs) = 0.676, $P < 0.001$]. However, HFF was not significantly different with different grades of other histological features of NAFLD, such as lobular inflammation, ballooning degeneration, and fibrosis (**Figure 2**; **Tables 3**–**5**). Furthermore, HFF did not correlate with other histological features of NAFLD, except for hepatic steatosis.

HFF was significantly different among the three groups divided by NAS (10.1% in NAS 0–2, 24.3% in NAS 3–4, and 27.0%

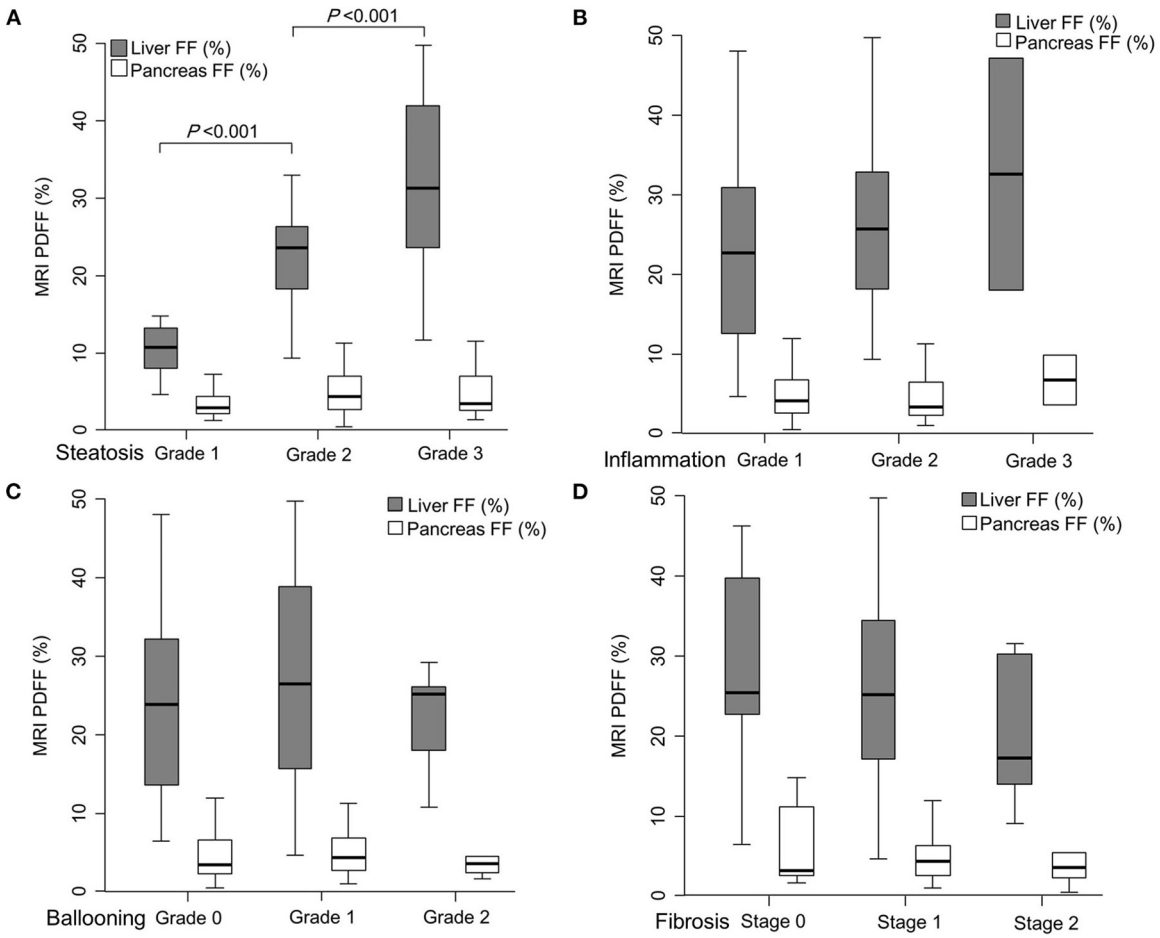

FIGURE 2 | Comparison of magnetic resonance imaging (MRI) proton density fat fraction (PDFF)-measured hepatic fat fraction (HFF) and pancreatic fat fraction (PFF) according to each histological feature of non-alcoholic fatty liver disease (NAFLD); steatosis grade **(A)**, lobular inflammation grade **(B)**, ballooning degeneration grade **(C)**, and hepatic fibrosis stage **(D)**. HFF significantly increased with the rise of hepatic steatosis grade (10.4, 23.7, and 31.1% in each steatosis grade, $P < 0.001$). However, HFF was not significantly different when observing other histological features of NAFLD, such as lobular inflammation, ballooning degeneration, and hepatic fibrosis. PFF was not related to any liver histologic features of NAFLD.

TABLE 3 | Comparison of demographic, anthropometric, laboratory, and magnetic resonance imaging-measured fat fraction findings of 63 children with non-alcoholic fatty liver disease by liver histologic lobular inflammation grade.

Variable	Lobular inflammation Grade 1 ($n = 32$)	Lobular inflammation Grade 2 ($n = 29$)	Lobular inflammation Grade 3 ($n = 2$)	*P*-value
Sex (boys: girls)	25 (78.1%): 7 (21.9%)	22 (75.9%): 7 (24.1%)	1 (50.0%): 1 (50.0%)	0.638*
Age, yr	12.3 (5.6–19.3)	12.7 (7.5–18.8)	13.1 (10.3–15.9)	0.708
Height (cm)	152.7 (122.4–184.5)	158.2 (134.5–184.7)	153.0 (142.5–163.4)	0.692
Height *z*-score	0.94 (−3.40–2.82)	0.86 (−1.78–2.17)	0.51 (0.37–0.65)	0.631
Weight (kg)	63.2 (36.4–117.5)	73.9 (41.2–130.9)	69.1 (58.3–79.9)	0.239
Weight *z*-score	2.01 (−2.80–4.19)	2.34 (0.69–5.01)	2.60 (2.37–2.82)	0.531
WC (cm)	87.3 (67.0–111.5)	93.0 (75.0–120.0)	93.5 (93.0–94.0)	0.141
WHtR	0.57 (0.46–0.69)	0.61 (0.51–0.69)	0.62 (0.57–0.66)	0.139
BMI	25.4 (18.6–34.5)	28.6 (21.6–38.4)	29.3 (28.7–29.9)	0.064
BMI *z*-score	2.27 (−1.10–4.56)	2.69 (0.68–5.10)	3.03 (3.0–3.05)	0.156
Fat mass (kg)	21.5 (8.2–54.0)	26.5 (13.5–52.4)	29.1 (25.8–32.3)	0.103
Fat mass (%)	35.0 (22.3–67.2)	36.5 (25.6–48.2)	43.1 (39.3–46.8)	0.101
FFM (kg)	40.0 (23.5–79.7)	42.6 (27.2–76.3)	39.6 (29.3–49.8)	0.678
FFM (%)	65.0 (32.8–77.7)	63.5 (51.8–74.4)	56.9 (53.2–60.7)	0.098
FMI	8.8 (3.9–18.8)	11.0 (6.2–17.1)	12.4 (12.1–12.7)	0.042
FFMI	16.9 (9.2–12.4)	18.0 (13.2–22.9)	16.5 (14.4–18.7)	0.492
mSBP (mmHg)	116.5 (100–150)	120.0 (106–159)	120.0 (114–126)	0.331
mDBP (mmHg)	63.0 (52–75)	66.0 (52–90)	60.5 (60–61)	0.234
AST (IU/L)	47.5 (17–161)	61.0 (30–226)	184.0 (178–190)	0.004
ALT (IU/L)	93.5 (20–331)	139.0 (31–342)	337.0 (308–366)	0.010
AST/ALT	0.47 (0.28–1.45)	0.49 (0.31–1.52)	0.55 (0.49–0.62)	0.695
Total bilirubin (mg/dL)	0.6 (0.3–0.9)	0.6 (0.2–1.3)	0.6 (0.5–0.6)	0.789
GGT (IU/L)	33.0 (12–158)	40.0 (19–184)	89.5 (71–108)	0.103
Total cholesterol (mg/dL)	170.5 (105–277)	197.0 (122–258)	246.5 (244–249)	0.021
Triglyceride (mg/dL)	114.0 (48–364)	125.0 (54–293)	180 (118–242)	0.637
HDL-C (mg/dL)	45.0 (28–58)	46.0 (28–68)	49.0 (45–53)	0.717
LDL-C (mg/dL)	97.5 (55–175)	117.0 (61–164)	152.0 (150–154)	0.036
FPG (mg/dL)	92.0 (71–179)	95.0 (71–270)	126.5 (105–148)	0.189
Insulin (mIU/L)	20.5 (4.6–75.6)	22.4 (9.1–75.4)	17.5 (6.4–28.5)	0.661
HbA1c (%)	5.3 (5.0–13.5)	5.4 (5.1–11.0)	8.4 (6.3–10.5)	0.060
HOMA-IR	4.7 (1.0–17.0)	5.9 (2.0–14.5)	4.9 (2.3–7.4)	0.719
QUICKI	0.31 (0.26–0.39)	0.30 (0.27–0.34)	0.31 (0.29–0.34)	0.719
MRI HFF (%)	23.0 (4.7–48.0)	25.7 (9.2–49.9)	32.6 (18.0–47.2)	0.346
MRI PFF (%)	4.1 (0.4–14.8)	3.3 (1.0–26.9)	6.7 (3.6–9.9)	0.668
BMI: Normal/Overweight/Obesity (*n*, %)	4 (12.5%)/7 (21.9%)/21 (65.6%)	1 (3.4%)/6 (20.7%)/22 (75.9%)	0 (0%)/0 (0%)/2 (100%)	0.670*
Central obesity (*n*, %)	26 (81.3%)	26 (89.7%)	2 (100%)	0.617*
Normal BP/EBP + HTN (*n*, %)	23 (71.9%)/9 (28.1%)	14 (48.3%)/15 (51.7%)	1 (50.0%)/1 (50.0%)	0.088*
Dyslipidemia (*n*, %)	18 (56.3%)	22 (75.9%)	2 (100%)	0.231*
Normal/PreDM + DM (*n*, %)	23 (71.9%)/9 (28.1%)	17 (58.6%)/12 (41.4%)	0 (0%)/2 (100%)	0.078*
MetS (*n*, %)	9 (28.1%)	14 (48.3%)	2 (100%)	0.050* 0.038†

*P-value was calculated by Kruskal-Wallis test. *P-value was analyzed by Fisher's exact test. †P-value was calculated for linear trend of metabolic syndrome proportion. Values are presented as median (range) or numbers (%).*

WC, waist circumference; WHtR, waist circumference-to-height ratio; BMI, body mass index; FFM, fat free mass; FMI, fat mass index = fat mass(kg)/height(m)^2; FFMI, fat free mass index = FFM(kg)/height(m)^2; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; MRI, magnetic resonance imaging; HFF, hepatic fat fraction; PFF, pancreatic fat fraction; BP, blood pressure; EBP, elevated BP; HTN, hypertension; DM, diabetes mellitus; PreDM, prediabetes; MetS, metabolic syndrome.

TABLE 4 | Comparison of demographic, anthropometric, laboratory, and magnetic resonance imaging-measured fat fraction findings of 63 children with non-alcoholic fatty liver disease by liver histologic ballooning degeneration grade.

Variable	Hepatocyte ballooning Grade 1 ($n = 33$)	Hepatocyte ballooning Grade 2 ($n = 24$)	Hepatocyte ballooning Grade 3 ($n = 6$)	*P*-value
Sex (boys: girls)	24 (72.7%): 9 (27.3%)	21 (87.5%): 3 (12.5%)	3 (50.0%): 3 (50.0%)	0.099*
Age, yr	12.4 (5.6–19.3)	12.4 (8.7–17.8)	14.7 (7.7–16.5)	0.882
Height (cm)	153.7(122.4–184.5)	157.1 (134.5–184.7)	156.1 (136.2–168.9)	0.759
Height *z*-score	0.98 (−3.40–2.17)	0.75 (−1.38–2.82)	0.50 (−1.78–1.93)	0.522
Weight (kg)	64.6 (36.4–117.5)	67.3 (46.3–130.9)	67.9 (43.6–79.9)	0.481
Weight *z*-score	2.40 (−2.80–4.19)	2.00 (0.99–5.01)	1.71 (0.69–3.0)	0.621
WC (cm)	87.0 (67.0–113.0)	92.0 (79.0–120.0)	89.5 (75.0–93.5)	0.341
WHtR	0.59 (0.46–0.69)	0.62 (0.51–0.69)	0.58 (0.52–0.65)	0.257
BMI	26.7 (18.6–34.5)	27.4 (21.9–38.4)	26.5 (21.6–31.0)	0.501
BMI *z*-score	2.46 (−1.10–4.56)	2.60 (0.68–5.10)	2.49 (1.34–3.23)	0.764
Fat mass (kg)	22.7 (8.2–54.0)	23.7 (15.9–52.4)	25.4 (13.8–32.3)	0.317
Fat mass (%)	34.8 (22.3–67.2)	40.4 (26.3–48.2)	36.6 (31.4–45.1)	0.100
FFM (kg)	22.7 (8.2–54.0)	23.7 (15.9–52.4)	25.4 (13.8–32.3)	0.317
FFM (%)	65.3 (32.8–77.7)	59.5 (51.8–73.7)	63.4 (54.8–68.7)	0.101
FMI	8.9 (3.9–18.8)	10.5 (6.2–17.1)	10.5 (6.8–12.1)	0.196
FFMI	16.6 (9.2–23.4)	17.5 (14.4–22.6)	17.9 (13.2–20.0)	0.914
mSBP (mmHg)	118.0 (100–150)	118.5 (102–159)	117.0 (111–133)	0.882
mDBP (mmHg)	63.0 (52–81)	64.0 (52–90)	63.5 (55–75)	0.371
AST (IU/L)	51.0 (17–196)	59.5 (21–216)	88.0 (25–226)	0.334
ALT (IU/L)	115.0 (20–287)	125.0 (21–342)	183.0 (48–366)	0.247
AST/ALT	0.47 (0.29–1.45)	0.49 (0.28–1.52)	0.52 (0.42–0.77)	0.755
Total bilirubin (mg/dL)	0.6 (0.3–1.3)	0.6 (0.2–1.1)	0.5 (0.3–1.1)	0.922
GGT (IU/L)	35.0 (12–158)	44.0 (12–117)	34.0 (20–184)	0.657
Total cholesterol (mg/dL)	169.0 (105–277)	198.0 (120–275)	192.5 (175–244)	0.082
Triglyceride (mg/dL)	130.0 (48–364)	98.5 (64–280)	101.5 (64–144)	0.278
HDL-C (mg/dL)	46.0 (36–68)	45.0 (28–61)	48.5 (37–53)	0.861
LDL-C (mg/dL)	99.0 (55–168)	119.5 (61–175)	119.5 (99–150)	0.060
FPG (mg/dL)	91.0 (76–189)	95.5 (71–270)	98.5 (77–148)	0.954
Insulin (mIU/L)	22.0 (4.6–75.6)	22.6 (9.5–75.4)	17.2 (6.4–19.3)	0.097
HbA1c (%)	5.5 (5.0–8.0)	5.3 (5.1–13.5)	5.3 (5.1–10.5)	0.826
HOMA-IR	4.8 (1.0–17.0)	5.9 (2.1–14.5)	3.4 (2.3–4.7)	0.085
QUICKI	0.30 (0.26–0.39)	0.30 (0.27–0.34)	0.32 (0.31–0.34)	0.085
MRI HFF (%)	23.9 (6.4–48.1)	26.5 (4.7–49.9)	25.2 (10.7–29.2)	0.734
MRI PFF (%)	3.4 (0.4–26.9)	4.4 (1.0–15.8)	3.6 (1.7–10.4)	0.644
BMI: Normal/Overweight/Obesity (*n*, %)	4 (12.1%)/6 (18.2%)/23 (69.7%)	1 (4.2%)/5 (20.8%)/18 (75.0%)	0 (0%)/2 (33.3%)/4 (66.7%)	0.763*
Central obesity (*n*, %)	26 (78.8%)	23 (95.8%)	5 (83.3%)	0.145*
Normal BP/EBP + HTN (*n*, %)	21 (63.6%)/12 (36.4%)	14 (58.3%)/10 (41.7%)	3 (50.0%)/3 (50.0%)	0.804*
Dyslipidemia (*n*, %)	23 (69.7%)	16 (66.7%)	3 (50.0%)	0.679*
Normal/PreDM + DM (*n*, %)	20 (60.6%)/13 (39.4%)	15 (62.5%)/9 (37.5%)	5 (83.3%)/1 (16.7%)	0.689*
MetS (*n*, %)	13 (39.4%)	9 (37.5%)	3 (50.0%)	0.564*

*P-value was calculated by Kruskal-Wallis test. *P-value was analyzed by Fisher's exact test. Values are presented as median (range) or numbers (%).*
WC, waist circumference; WHtR, waist circumference-to-height ratio; BMI, body mass index; FFM, fat free mass; FMI, fat mass index = fat mass(kg)/height(m)^2; FFMI, fat free mass index = FFM(kg)/height(m)^2; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; MRI, magnetic resonance imaging; HFF, hepatic fat fraction; PFF, pancreatic fat fraction; BP, blood pressure; EBP, elevated BP; HTN, hypertension; DM, diabetes mellitus; PreDM, prediabetes; MetS, metabolic syndrome.

in NAS ≥5, $P = 0.002$). HFF correlated with NAS (rs = 0.470, $P < 0.001$).

However, PFF did not significantly differ according to the histological features of NAFLD (**Tables 2–5**). PFF was also not correlated with any histological characteristic of NAFLD. Furthermore, PFF was not correlated with HFF (Pearson's correlation coefficient = 0.170, $P = 0.191$).

TABLE 5 | Comparison of demographic, anthropometric, laboratory, and magnetic resonance imaging-measured fat fraction findings of 63 children with non-alcoholic fatty liver disease by liver histologic fibrosis stage.

Variable	Hepatic fibrosis Stage 0 ($n = 11$)	Hepatic fibrosis Stage 1 ($n = 41$)	Hepatic fibrosis Stage 2 ($n = 11$)	*P*-value
Sex (boys: girls)	10 (90.9%): 1 (9.1%)	32 (78.0%): 9 (22.0%)	6 (54.5%): 5 (45.5%)	0.172*
Age, yr	12.2 (5.6–17.4)	12.6 (6.1–19.3)	12.6 (9.9–18.1)	0.907
Height (cm)	153.7 (122.4–183.0)	152.6 (123.8–184.7)	156.5 (144.3–184.5)	0.824
Height *z*-score	1.02 (−0.57–1.95)	0.85 (−3.40–2.17)	0.89 (−2.90–2.82)	0.686
Weight (kg)	63.0 (37.9–112.7)	69.3 (36.4–130.9)	64.6 (45.2–117.5)	0.989
Weight *z*-score	2.43 (0.41–4.19)	2.16 (−2.80–5.01)	1.86 (−0.32–4.18)	0.519
WC (cm)	87.0 (81.0–113.2)	90.0 (67.0–120.0)	88.3 (80.0–110.0)	0.774
WHtR	0.60 (0.52–0.69)	0.60 (0.46–0.69)	0.56 (0.53–0.66)	0.338
BMI	26.7 (22.6–38.3)	27.5 (18.6–38.4)	26.1 (21.2–34.5)	0.633
BMI *z*-score	2.31 (0.89–4.93)	2.62 (−1.10–5.10)	1.63 (0.38–4.07)	0.441
Fat mass (kg)	22.8 (17.5–46.0)	24.4 (8.2–54.0)	20.6 (14.5–35.3)	0.533
Fat mass (%)	36.3 (29.8–41.5)	36.1 (22.3–67.2)	33.1 (22.5–45.1)	0.487
FFM (kg)	46.4 (27.8–74.7)	40.3 (23.5–76.3)	46.0 (29.4–79.7)	0.583
FFM (%)	63.8 (58.5–70.2)	63.9 (32.8–77.7)	67.0 (54.8–77.5)	0.475
FMI	9.4 (7.9–16.0)	10.3 (3.9–18.8)	8.2 (5.5–13.2)	0.324
FFMI	17.8 (13.2–22.6)	16.5 (9.2–22.9)	18.0 (13.2–23.4)	0.820
mSBP (mmHg)	115.0 (100–159)	120.0 (100–148)	114.0 (105–136)	0.304
mDBP (mmHg)	63.0 (53–90)	66.0 (52–84)	61.0 (52–74)	0.251
AST (IU/L)	46.0 (29–74)	57.0 (17–216)	61.0 (27–226)	0.390
ALT (IU/L)	98.0 (23–187)	126.0 (21–342)	122.0 (20–366)	0.414
AST/ALT	0.44 (0.37–1.26)	0.48 (0.28–1.52)	0.52 (0.29–1.45)	0.815
Total bilirubin (mg/dL)	0.5 (0.3–1.1)	0.6 (0.2–1.3)	0.6 (0.3–1.1)	0.816
GGT (IU/L)	29.0 (12–101)	38.0 (12–133)	50.0 (21–184)	0.151
Total cholesterol (mg/dL)	169.0 (139–230)	177.0 (105–275)	201.0 (122–277)	0.581
Triglyceride (mg/dL)	101.0 (73–251)	122.0 (54–357)	130.0 (48–364)	0.643
HDL-C (mg/dL)	44.0 (36–58)	46.0 (28–68)	41.0 (28–53)	0.415
LDL-C (mg/dL)	94.0 (79–147)	106.0 (55–175)	120.0 (61–168)	0.623
FPG (mg/dL)	90.0 (84–119)	97.0 (78–270)	91.0 (71–153)	0.250
Insulin (mIU/L)	20.6 (4.6–51.2)	22.4 (8.9–75.6)	16.9 (6.4–37.1)	0.421
HbA1c (%)	5.1 (5.0–5.7)	5.4 (5.1–13.5)	5.5 (5.0–10.5)	0.140
HOMA-IR	4.5 (1.0–12.8)	6.0 (2.0–17.0)	4.5 (2.1–7.9)	0.182
QUICKI	0.31 (0.27–0.39)	0.30 (0.26–0.34)	0.31 (0.29–0.34)	0.182
MRI HFF (%)	25.4 (6.4–46.2)	25.2 (4.7–49.9)	17.2 (9.0–31.5)	0.369
MRI PFF (%)	3.2 (1.6–14.8)	4.3 (1.0–26.9)	3.6 (0.4–15.8)	0.909
BMI: Normal/Overweight/Obesity (*n*, %)	1 (9.1%)/1 (9.1%)/9 (81.8%)	3 (7.3%)/7 (17.1%)/31 (75.6%)	1 (9.1%)/5 (45.5%)/5 (45.5%)	0.219*
Central obesity (*n*, %)	9 (81.8%)	36 (87.8%)	9 (81.8%)	0.655*
Normal BP/EBP + HTN (*n*, %)	7 (63.6%)/4 (36.4%)	23 (56.1%)/18 (43.9%)	8 (72.7%)/3 (27.3%)	0.654*
Dyslipidemia (*n*, %)	6 (54.5%)	27 (65.9%)	9 (81.8%)	0.393*
Normal/PreDM + DM (*n*, %)	7 (63.6%)/4 (36.4%)	25 (61.0%)/16 (39.0%)	8 (72.7%)/3 (27.3%)	0.927*
MetS (*n*, %)	4 (36.4%)	15 (36.6%)	6 (54.5%)	0.564*

*P-value was calculated by Kruskal-Wallis test. *P-value was analyzed by Fisher's exact test. Values are presented as median (range) or numbers (%).*
WC, waist circumference; WHtR, waist circumference-to-height ratio; BMI, body mass index; FFM, fat free mass; FMI, fat mass index = fat mass(kg)/height(m)^2; FFMI, fat free mass index = FFM(kg)/height(m)^2; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; MRI, magnetic resonance imaging; HFF, hepatic fat fraction; PFF, pancreatic fat fraction; BP, blood pressure; EBP, elevated BP; HTN, hypertension; DM, diabetes mellitus; PreDM, prediabetes; MetS, metabolic syndrome.

Association Between Liver Histological Features and Liver Enzymes

Liver enzymes, such as AST, ALT, and GGT were not significantly different among the subgroups based on the grades of hepatic steatosis, ballooning degeneration, portal inflammation, and stage of hepatic fibrosis (**Tables 2**, **4**–**6**).

However, AST and ALT significantly increased according to the grade of lobular inflammation (47.5

TABLE 6 | Comparison of demographic, anthropometric, laboratory, and magnetic resonance imaging-measured fat fraction findings of 63 children with non-alcoholic fatty liver disease by liver histologic portal inflammation grade.

Variable	Portal inflammation Grade 0 ($n = 24$)	Portal inflammation Grade 1–2 ($n = 39$)	*P*-value
Sex (boys: girls)	19 (78.2%): 5 (20.8%)	29 (74.4%): 10 (25.6%)	0.663*
Age, yr	13.8 (5.6–19.3)	12.2 (6.1–18.2)	0.176
Height (cm)	156.1 (122.4–184.7)	155.8 (123.8–184.5)	0.318
Height *z*-score	1.0 (−1.78–2.17)	0.85 (−3.40–2.82)	0.697
Weight (kg)	75.8 (37.9–130.9)	64.6 (36.4–117.5)	0.123
Weight *z*-score	2.78 (−0.37–5.01)	1.96 (−2.80–4.18)	0.066
WC (cm)	99.9 (75.0–120.0)	88.0 (67.0–113.2)	0.015
WHtR	0.61 (0.50–0.69)	0.57 (0.46–0.67)	0.004
BMI	30.0 (20.0–38.4)	26.2 (18.6–38.3)	0.058
BMI *z*-score	3.36 (0–5.10)	2.40 (−1.10–4.93)	0.022
Fat mass (kg)	27.1 (12.9–52.4)	22.4 (8.2–54.0)	0.036
Fat mass (%)	37.5 (25.6–48.2)	34.9 (22.3–67.2)	0.165
FFM (kg)	42.7 (27.8–76.3)	40.0 (23.5–79.7)	0.179
FFM (%)	62.5 (51.8–74.4)	65.1 (32.8–77.7)	0.172
FMI	11.0 (5.8–17.1)	8.7 (3.9–18.8)	0.033
FFMI	18.2 (13.2–22.9)	16.3 (9.2–23.4)	0.172
mSBP (mmHg)	118.0 (100–150)	117.0 (100–159)	0.210
mDBP (mmHg)	65.0 (52–84)	63.0 (52–90)	0.205
AST (IU/L)	62.0 (25–226)	51.0 (17–190)	0.361
ALT (IU/L)	127.0 (20–342)	115.0 (21–366)	0.666
AST/ALT	0.48 (0.31–1.45)	0.49 (0.28–1.52)	0.246
Total bilirubin (mg/dL)	0.6 (0.2–1.3)	0.5 (0.3–1.1)	0.224
GGT (IU/L)	39.0 (12–184)	37.0 (12–158)	0.739
Total cholesterol (mg/dL)	179.0 (122–221)	180.0 (105–277)	0.488
Triglyceride (mg/dL)	129.0 (48–280)	118.0 (56–364)	0.462
HDL-C (mg/dL)	45.5 (28–68)	45.0 (28–67)	0.228
LDL-C (mg/dL)	113.0 (66–147)	106.0 (55–175)	0.506
FPG (mg/dL)	90.0 (71–270)	94.0 (71–179)	0.266
Insulin (mIU/L)	21.0 (4.6–75.4)	20.9 (6.4–75.6)	0.837
HbA1c (%)	5.4 (5.0–11.0)	5.3 (5.1–13.5)	0.964
HOMA-IR	4.9 (1.0–14.5)	5.1 (2.0–17.0)	0.932
QUICKI	0.30 (0.27–0.39)	0.30 (0.26–0.34)	0.932
MRI HFF (%)	23.4 (6.4–48.1)	26.3 (4.7–49.9)	0.192
MRI PFF (%)	4.0 (1.0–26.9)	3.8 (0.4–15.8)	0.887
BMI: Normal/Overweight/Obesity (*n*, %)	2 (8.3%)/3 (12.5%)/19 (79.2%)	3 (7.7%)/10 (25.6%)/26 (66.7%)	0.470†
Central obesity (*n*, %)	22 (91.7%)	32 (82.1%)	0.462†
Normal BP/EBP + HTN (*n*, %)	13 (54.2%)/11 (45.8%)	25 (64.1%)/14 (35.9%)	0.434*
Dyslipidemia (*n*, %)	16 (66.7%)	25 (64.1%)	0.836*
Normal/PreDM + DM (*n*, %)	16 (66.7%)/8 (33.3%)	24 (61.5%)/15 (38.5%)	0.681*
MetS (*n*, %)	12 (50.0%)	13 (33.3%)	0.189*

*P-value was calculated by Mann Whitney U-test. *P-value was calculated by Chi-square test. † P-value was calculated by Fisher's exact test. Values are presented as median (range) or numbers (%).*

WC, waist circumference; WHtR, waist circumference-to-height ratio; BMI, body mass index; FFM, fat free mass; FMI, fat mass index = fat mass(kg)/height(m)^2; FFMI, fat free mass index = FFM(kg)/height(m)^2; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; MRI, magnetic resonance imaging; HFF, hepatic fat fraction; PFF, pancreatic fat fraction; BP, blood pressure; EBP, elevated BP; HTN, hypertension; DM, diabetes mellitus; PreDM, prediabetes; MetS, metabolic syndrome.

in grade 1, 61.0 in grade 2, and 184.0 IU/L in grade 3, respectively, for AST, $P = 0.004$; 93.5, 139.0, and 337.0 IU/L, respectively, for ALT, $P = 0.010$) (**Table 3**). AST and ALT levels were significantly correlated with lobular inflammation (rs = 0.398, $P = 0.001$, and rs = 0.333, $P = 0.008$, respectively).

AST and ALT were also correlated with NAS (rs = 0.302, P = 0.016, and rs = 0.303, P = 0.016, respectively).

Comparison Between Histological Features of the Liver and Measurements of Body Composition

As the grade of hepatic steatosis increased, the TBF percentage increased significantly (32.9% in grade 1, 34.7% in grade 2, and 39.4% in grade 3, P = 0.017), and correspondingly, the FFM percentage significantly decreased (67.1, 65.3, and 60.6%, respectively, P = 0.019) (**Table 2**). TBF percentage was positively correlated with the grade of steatosis (rs = 0.363, P = 0.004), and the FFM percentage was negatively correlated with the grade of steatosis (rs = −0.359, P = 0.005). Likewise, FMI was significantly increased with the grade of hepatic steatosis (8.2 in grade 1, 8.8 in grade 2, and 10.4 in grade 3, P = 0.018) (**Table 2**) and FMI was positively correlated with the grade of steatosis (rs = 0.366, P = 0.004).

Furthermore, FMI significantly increased according to the grade of lobular inflammation (8.8 in grade 1, 11.0 in grade 2, and 12.4 in grade 3, P = 0.042), and FMI was positively correlated with the grade of lobular inflammation (rs = 0.309, P = 0.016).

Association Between Liver Histological Features and Metabolic Components

As the grade of hepatic steatosis increased, the proportion of obesity significantly increased (53.8% in grade 1, 55.6% in grade 2, and 87.5% in grade 3; P = 0.032, P for linear trend = 0.029) (**Table 2**). WHtR also significantly increased as the grade of steatosis increased (0.55, 0.57, and 0.60, respectively, P = 0.026) (**Table 2**), and it was correlated with the steatosis grade (rs = 0.342, P = 0.006).

As the grade of lobular inflammation increased, t-chol and LDL-C significantly increased (170.5, 197.0, and 246.5 mg/dL, P = 0.021 for t-chol; 97.5, 117.0, and 152.0 mg/dL, P = 0.036 for LDL-C) (**Table 3**). Both t-chol and LDL-C levels correlated with lobular inflammation (rs = 0.304, P = 0.015, and rs = 0.264, P = 0.036, respectively).

In addition, the difference in the proportion of subjects with MetS was marginally significant with the grade of lobular inflammation (28.1, 48.3, and 100%, respectively, P = 0.050, P for linear trend = 0.038) (**Table 3**).

However, t-chol, LDL-C, and MetS were not significantly affected by portal inflammation (**Table 6**). Furthermore, other metabolic parameters showed no significant difference according to the grade of ballooning degeneration and the stage of hepatic fibrosis (**Tables 4**, **5**).

DISCUSSION

To our knowledge, the present study is the first to evaluate the clinical findings, metabolic parameters, and MRI-measured fractions of ectopic fat using the histopathological findings of the liver in children with NAFLD. We highlight the following findings. MRI-measured HFF was associated with histological steatosis but was not associated with lobular inflammation, hepatocyte ballooning, and hepatic fibrosis in children with NAFLD. MRI-measured PFF was not associated with any histological features of the liver. Although some obesity-related factors were associated with the grade of steatosis, liver enzymes (AST and ALT) were associated with lobular inflammation. Lobular inflammation was also associated with elevated serum levels of t-chol and LDL-C in children with NAFLD, while BMI z-score, TBF percentage, and MRI-measured HFF and PFF showed no significant differences based on the grade of lobular inflammation.

In our study, MRI-measured HFF was correlated only with the grade of hepatic steatosis among the histological criteria of NAFLD. This suggests that the severity of hepatic steatosis is not necessarily proportional to the severity of hepatic inflammation. A recent meta-analysis showed that MRI-PDFF of the liver had an excellent diagnostic value for the assessment of hepatic fat content and the classification of histologic steatosis in patients with NAFLD (26). In particular, it is known that the correlation between MRI-estimated hepatic fat and histologically-determined steatosis is better in patients with no or mild hepatic fibrosis than in patients with moderate or severe fibrosis because of the reduction in the number of hepatocytes due to replacement by fibrosis (27, 28). In line with our findings, Bril et al. (29) showed that histological severity of NAFLD, including inflammation, ballooning, and fibrosis, was not associated with the amount of intrahepatic fat content measured by MRS. They suggested that histological activity appears to have an early threshold of about 6 ± 2% and is not significantly influenced by the increasing amounts of intrahepatic TG accumulation beyond this threshold (29). Another study on adults with NAFLD showed that MRI-PDFF of the liver positively correlated with the grade of histologic steatosis and NAS and did not correlate with the grade of ballooning or lobular inflammation, which is similar to the results of our study (30). However, this study on adults showed that MRI-PDFF of the liver negatively correlated with the stage of fibrosis (30), while our study in pediatric NAFLD patients revealed no correlation between HFF and stage of fibrosis. Another study on adult patients with NAFLD showed that MRI-PDFF findings of the liver showed no statistically significant difference between fibrosis stages 0–2 and stages 3–4 (31). In another study, repeat MRI-PDFF was performed to assess hepatic steatosis, and magnetic resonance elastography (MRE) was performed to assess hepatic fibrosis; both MRI examinations were performed simultaneously in children with NAFLD over a mean follow-up of 27 months (32). The study showed that there was no significant correlation between the changes in MRI-measured HFF and those in MRE-measured hepatic stiffness (32). Some interventional studies on adult patients with NAFLD have shown significant improvement in the histologic grade of steatosis or on MRI-PDFF of the liver without any changes in inflammation, ballooning, or fibrosis (33, 34). However, only a few studies have shown that hepatic steatosis is associated with other histological features of NAFLD. Idilman et al. (35) showed a correlation between the histological grade of steatosis and the grade of necroinflammation. Ajmera et al. (36) showed that a higher

liver MRI-PDFF at baseline was associated with the progression of fibrosis after a median follow-up of 1.75 years in adults with NAFLD.

Our study revealed that PFF was neither correlated with MRI-measured HFF nor the histological steatosis grade of NAFLD, suggesting that the pathophysiology contributing to pancreatic fat might be different from that of hepatic fat, although both represent ectopic fat accumulation in patients with obesity. Based on previous studies, the association between pancreatic and hepatic steatosis remains inconsistent in patients with NAFLD. To date, there have been only a few studies on adults and no study based on the histopathological findings in pediatric patients. Patel et al. (37) showed that MRI-determined PFF significantly increased with increasing grade of histological steatosis in adults with NAFLD. In contrast, Idilman et al. (35) showed no correlation between liver MRI-PDFF or histological steatosis grade and pancreatic MRI-PDFF in adults with NAFLD.

Regarding the relations between liver enzymes and histological features of the liver or MRI findings, the results from our study revealed that serum AST and ALT levels significantly increased with increasing lobular inflammation but not with histological steatosis or MRI-measured HFF. Since elevated liver enzymes represent hepatic inflammation, this result also verifies that hepatic inflammation is not caused by hepatic steatosis alone. The association between liver enzymes and histological features in NAFLD remains unclear. Idilman et al. (35) showed that in adults with biopsy-proven NAFLD, there was a positive correlation between histological steatosis and laboratory markers, including AST, ALT, and total bilirubin, as well as between histological steatosis and the grade of necroinflammation on liver histopathology. Meanwhile, Bril et al. (29) showed that regardless of the lack of association between MRS-measured intrahepatic fat and the severity of liver histology, measured by inflammation, ballooning, and fibrosis, the increase in intrahepatic fat content was associated with a linear increase in ALT and AST levels. Permutt et al. (38) showed that adult NAFLD patients with steatosis grade 1 might display characteristics of advanced liver disease (higher average AST/ALT ratio, GGT, and higher stage of fibrosis and hepatocellular ballooning), rather than those with grade 3 steatosis, highlighting that a low HFF does not necessarily indicate mild NAFLD. Schwimmer et al. (39) reported a weak but significant correlation between ALT and MRE-measured liver stiffness as a biomarker of hepatic fibrosis in children with biopsy-proven NAFLD. However, Mouzaki et al. (32) found no correlation between changes in MRE-measured liver stiffness and ALT changes, and only a trend toward a weak correlation between changes in ALT and changes in MRI-PDFF measured-HFF. In a previous study on pediatric patients, the entire spectrum of histologic features of NAFLD, including advanced fibrosis, was observed even in children with normal liver enzymes (40).

In our study, as the grade of lobular inflammation increased, t-chol and LDL-C significantly increased, although obesity-related factors, such as BMI *z*-score, TBF percentage, and MRI-measured ectopic fat, were not significantly different among lobular inflammation grades. MRI-measured HFF or the histological grade of steatosis was not correlated with any components of the lipid profile. We suggest that hepatic inflammation in NAFLD might have an adverse effect on lipid metabolism. Bril et al. (29) showed that neither plasma TG nor HDL-C levels were correlated with MRS-measured intrahepatic fat content beyond an intrahepatic fat content of >8%. Idilman et al. (35) showed a negative correlation between histological steatosis or MRI-measured HFF and HDL-C, which could be due to a significant correlation between the histologic steatosis and necroinflammation. Mouzaki et al. (32) showed that changes in MRI-measured HFF were inversely correlated with changes in serum HDL-C levels; however, changes in MRE-measured liver stiffness as a surrogate biomarker of hepatic fibrosis did not correlate with any changes in the lipid profiles.

Our study included 18 (28.6%) subjects with non-obese NAFLD; of these, 13 (20.6%) were overweight (the 85th ≤ BMI <95th percentiles), and 5 (7.9%) were within the normal range (BMI <85th percentile). A systematic meta-analysis showed the prevalence of NAFLD in children and adolescents with normal weight to be 2.3% (95% CI, 1.5–3.6), in subjects with overweight to be 12.5% (95% CI, 9.2–16.7), and that in subjects with obesity to be 36.1% (95% CI, 24.6–49.4) in studies conducted among the general population (41). Non-obese NAFLD who may be overweight (BMI 85th−95th percentile adjusted for age and sex) tend to be younger, male, and have lower BP, FPG, HbA1c levels, and genetic polymorphisms (42). Until now, there are no published studies on the histological features of pediatric non-obese NAFLD (42). In one study wherein the main objective was to assess the hepatic iron content in children with biopsy-proven NAFLD, normal weight children with NAFLD did not show significant differences in laboratory parameters and liver histology compared to overweight or obese children with NAFLD (42, 43). In our study, the comparison between the non-obese ($n = 18$) and obese groups ($n = 45$) with NAFLD did not show significant differences in liver enzymes, metabolic parameters (**Supplementary Table 1**), and liver histological features except for hepatic steatosis (**Supplementary Table 2**). The number of non-obese patients with NAFLD might have been insufficient to analyze the differences between the two groups. More studies are required to identify the differences between non-obese NAFLD and obese NAFLD.

The present study has some limitations. First, children showing severe histological findings are relatively uncommon. Among 63 children, two had grade 3 lobular inflammation; only one had grade 2 portal inflammation, and there were no children with stage 3–4 fibrosis. This might be because children with NAFLD tend to have a relatively shorter duration of the disease than adults with NAFLD; thus, pediatric NAFLD with severe histological findings is generally infrequent. Second, we did not perform MRE to compare the severity of fibrosis using MRI modalities with histologic findings of fibrosis. Identification of fibrosis in children with NAFLD is important because fibrosis is more likely to progress to cirrhosis, hepatocellular carcinoma, and liver-related mortality (2, 9, 44). To date, MRE has predominantly been performed in adults. However, surrogate quantitative imaging biomarkers, including MRE, are necessary for indirect assessment of hepatic fibrosis because repeated liver

biopsy over a short-term period is impractical due to its invasive nature, the potential for sampling error, and reluctance to biopsy (2). A few studies conducted in children have assessed hepatic fibrosis using MRE (32, 39, 45–47). Further validation studies using MRE are warranted to determine the optimal cut-off points and the ability to assess fibrosis in children with NAFLD longitudinally (2).

Third, the retrospective cross-sectional design and relatively small sample size used in this study warrant further large-scale prospective longitudinal studies in the future to assess the association between MRI-measured ectopic fat content, histological findings, and metabolic changes, especially in pediatric patients with NAFLD.

In conclusion, the present study showed that hepatic steatosis on MRI was only associated with the grade of liver steatosis on histopathology. The increase in hepatic steatosis (MRI-measured HFF or histologic steatosis grade) was not correlated with other histological features of NAFLD, including lobular inflammation, hepatocyte ballooning, and hepatic fibrosis. In addition, MRI-measured pancreatic fat as additional obesity-related ectopic fat was not related to any histologic features of the liver. Instead, elevated serum liver enzymes (AST and ALT) and some lipid profiles (t-chol, LDL-C) were associated with lobular inflammation. This study shows the importance of interpreting MRI in conjunction with anthropometric and laboratory findings of obesity and obesity-related complications in assessing pediatric patients suspected of NAFLD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-2103-670-104). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the institutional requirements.

AUTHOR CONTRIBUTIONS

EL conducting the study, collecting and interpreting data, and drafting the manuscript. HY planning the study, interpreting data, and drafting the manuscript. JK conducting the study, interpreting data, and drafting the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.685795/full#supplementary-material

REFERENCES

1. Yang HR, Kim HR, Kim MJ, Ko JS, Seo JK. Noninvasive parameters and hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *World J Gastroenterol.* (2012) 18:1525–30. doi: 10.3748/wjg.v18.i13.1525
2. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr.* (2017) 64:319–34. doi: 10.1097/MPG.0000000000001482
3. Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. *Semin Liver Dis.* (2012) 32:3–13. doi: 10.1055/s-0032-1306421
4. Romana BS, Chela H, Dailey FE, Dailey FE, Nassir F, Tahan V. Non-alcoholic fatty pancreas disease (NAFPD): a silent spectator or the fifth component of metabolic syndrome? A literature review. *Endocr Metab Immune Disord Drug Targets.* (2018) 18:547–54. doi: 10.2174/1871530318666180328111302
5. Yu TY, Wang CY. Impact of non-alcoholic fatty pancreas disease on glucose metabolism. *J Diabetes Investig.* (2017) 8:735–47. doi: 10.1111/jdi.12665
6. Majumder S, Philip NA, Takahashi N, Levy MJ, Singh VP, Chari ST. Fatty pancreas: should we be concerned? *Pancreas.* (2017) 46:1251–8. doi: 10.1097/MPA.0000000000000941
7. Singh RG, Yoon HD, Wu LM, Lu J, Plank LD, Petrov MS. Ectopic fat accumulation in the pancreas and its clinical relevance: a systematic review, meta-analysis, and meta-regression. *Metabolism.* (2017) 69:1–13. doi: 10.1016/j.metabol.2016.12.012
8. Ko JS. New perspectives in pediatric nonalcoholic fatty liver disease: epidemiology, genetics, diagnosis, and natural history. *Pediatr Gastroenterol Hepatol Nutr.* (2019) 22:501–10. doi: 10.5223/pghn.2019.22.6.501
9. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2015) 149:389–97.e10. doi: 10.1053/j.gastro.2015.04.043
10. Schwimmer JB, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, et al. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *Hepatology.* (2015) 61:1887–95. doi: 10.1002/hep.27666
11. Lee MJ, Bagci P, Kong J, Vos MB, Sharma P, Kalb B, et al. Liver steatosis assessment: correlations among pathology, radiology, clinical data and automated image analysis software. *Pathol Res Pract.* (2013) 209:371–9. doi: 10.1016/j.prp.2013.04.001
12. Tang A, Desai A, Hamilton G, Wolfson T, Gamst A, Lam J, et al. Accuracy of MR imaging-estimated proton denstiy fat fraction for classification of dichotomized histologic steatosis grades in nonalcoholic fatty liver disease. *Radiology.* (2015) 274:416–25. doi: 10.1148/radiol.14140754
13. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* (2007) 39:175–91. doi: 10.3758/bf03193146
14. Holland-Hall CM. Chapter 132. Adolescent physical and social development. In: Robert MK, and Joseph St. G, editors. *Nelson Textbook of Pediatrics.* 21st ed. Philadelphia, PA: Elsevier Press (2019). p. 1014–20.

15. Kim JH, Yun S, Hwang SS, Shim JO, Chae HW, Lee YJ, et al. The 2017 Korean national growth charts for children and adolescents: development, improvement, and prospects. *Korean J Pediatr.* (2018) 61:135–49. doi: 10.3345/kjp.2018.61.5.135
16. National Institute of Health. *2007 Korean National Growth Charts [in Korean]*. (2008). Available online at: http://www.nih.go.kr/board/board.es?mid=a40801000000&bid=0050&act=view&list_no=1235 (accessed March 10, 2021).
17. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll A, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics.* (2017) 140:e20171904. doi: 10.1542/peds.2017-1904
18. Weaver DJ Jr. Pediatric hypertension: review of updated guidelines. *Pediatr Rev.* (2019) 40:354–8. doi: 10.1542/pir.2018-0014
19. Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. *Diabetes Care.* (2004) 27:314–9. doi: 10.2337/diacare.27.2.314
20. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2013) 36(Suppl. 1):S67–74. doi: 10.2337/dc13-S067
21. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics.* (2011) 128(Suppl. 5):S213–56. doi: 10.1542/peds.2009-2107C
22. Ford ES, Ajani UA, Mokdad AH, National Health and Nutrition Examination. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. *Diabetes Care.* (2005) 28:878–81. doi: 10.2337/diacare.28.4.878
23. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third national health and nutrition examination survey, 1988–1994. *Arch Pediatr Adolesc Med.* (2003) 157:821–7. doi: 10.1001/archpedi.157.8.821
24. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* (2005) 41:1313–21. doi: 10.1002/hep.20701
25. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, NASH Clinical Research Network. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology.* (2011) 53:810–20. doi: 10.1002/hep.24127
26. Gu J, Liu S, Du S, Zhang Q, Xiao J, Dong Q, et al. Diagnostic value of MRI-PDFF for hepatic steatosis in patients with non-alcoholic fatty liver disease: a meta-analysis. *Eur Radiol.* (2019) 29:3564–73. doi: 10.1007/s00330-019-06072-4
27. Idilman IS, Keskin O, Celik A, Savas B, Elhan AH, Idilman R, et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. *Acta Radiol.* (2016) 57:271–8. doi: 10.1177/0284185115580488
28. McPherson S, Jonsson JR, Cowin GJ, O'Rourke P, Clouston AD, Volp A, et al. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. *J Hepatol.* (2009) 51:389–97. doi: 10.1016/j.jhep.2009.04.012
29. Bril F, Barb D, Portillo-Sanchez P, Biernacki D, Lomonaco R, Suman A, et al. Metabolic and histological implications of intrahepatic triglyceride content in nonalcoholic fatty liver disease. *Hepatology.* (2017) 65:1132–44. doi: 10.1002/hep.28985
30. Wildman-Tobriner B, Middleton MM, Moylan CA, Rossi S, Flores O, Chang ZA, et al. Association between magnetic resonance imaging-proton density fat fraction and liver histology features in patients with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis. *Gastroenterology.* (2018) 155:1428–35.e2. doi: 10.1053/j.gastro.2018.07.018
31. Lin SC, Heba E, Bettencourt R, Lin GY, Valasek MA, Lunde O, et al. Assessment of treatment response in non-alcoholic steatohepatitis using advanced magnetic resonance imaging. *Aliment Pharmacol Ther.* (2017) 45:844–54. doi: 10.1111/apt.13951
32. Mouzaki M, Trout AT, Arce-Clachar AC, Bramlage K, Kuhnell P, Dillman JR, et al. Assessment of nonalcoholic fatty liver disease progression in children using magnetic resonance imaging. *J Pediatr.* (2018) 201:86–92. doi: 10.1016/j.jpeds.2018.05.024
33. Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, et al. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled fatty liver improvement with rosiglitazone therapy (FLIRT) trial. *Gastroenterology.* (2008) 135:100–10. doi: 10.1053/j.gastro.2008.03.078
34. Noureddin M, Lam J, Peterson MR, Middleton M, Hamilton G, Le TA, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. *Hepatology.* (2013) 58:1930–40. doi: 10.1002/hep.26455
35. Idilman IS, Tuzun A, Savas B, Elhan AH, Celik A, Idilman R, et al. Quantification of liver, pancreas, kidney, and vertebral body MRI-PDFF in non-alcoholic fatty liver disease. *Abdom Imaging.* (2015) 40:1512–9. doi: 10.1007/s00261-015-0385-0
36. Ajmera V, Park CC, Caussy C, Singh S, Hernandez C, Bettencourt R, et al. Magnetic resonance imaging proton density fat fraction associates with progression of fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2018) 155:307–10.e2. doi: 10.1053/j.gastro.2018.04.014
37. Patel NS, Peterson MR, Brenner DA, Heba E, Sirlin C, Loomba R. Association between novel MRI-estimated pancreatic fat and liver histology-determined steatosis and fibrosis in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* (2013) 37:630–9. doi: 10.1111/apt.12237
38. Permutt Z, Le TA, Peterson MR, Seki E, Brenner DA, Sirlin C, et al. Correlation between liver histology and novel magnetic resonance imaging in adult patients with non-alcoholic fatty liver disease - MRI accurately quantifies hepatic steatosis in NAFLD. *Aliment Pharmacol Ther.* (2012) 36:22–9. doi: 10.1111/j.1365-2036.2012.05121.x
39. Schwimmer JB, Behling C, Angeles JE, Paiz M, Durelle J, Africa J, et al. Magnetic resonance elastography measured shear stiffness as a biomarker of fibrosis in pediatric nonalcoholic fatty liver disease. *Hepatology.* (2017) 66:1474–85. doi: 10.1002/hep.29241
40. HH AK, Henderson J, Vanhoesen K, Ghishan F, Bhattacharyya A. Nonalcoholic fatty liver disease in children: a single center experience. *Clin Gastroenterol Hepatol.* (2008) 6:799–802. doi: 10.1016/j.cgh.2008.03.001
41. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PLoS ONE.* (2015) 10:e0140908. doi: 10.1371/journal.pone.0140908
42. Wang AY, Dhaliwal J, Mouzaki M. Lean non-alcoholic fatty liver disease. *Clin Nutr.* (2019) 38:975–81. doi: 10.1016/j.clnu.2018.08.008
43. Manco M, Alisi A, Real JF, Equitani F, DeVito R, Valenti L, et al. Early interplay of intra-hepatic iron and insulin resistance in children with non-alcoholic fatty liver disease. *J Hepatol.* (2011) 55:647–53. doi: 10.1016/j.jhep.2010.12.007
44. Loomba R, Chalasani N. The hierarchial model of NAFLD: prognostic significance of histologic features in NASH. *Gastroenterology.* (2015) 149:278–81. doi: 10.1053/j.gastro.2015.06.016
45. Xanthakos SA, Podberesky DJ, Serai SD, Miles L, King EC, Balistreri WF, et al. Use of magnetic resonance elastography to assess hepatic fibrosis in children with chronic liver disease. *J Pediatr.* (2014) 164:186–8. doi: 10.1016/j.jpeds.2013.07.050
46. Etchell E, Juge L, Hatt A, Sinkus R, Bilston LE. Liver stiffness values are lower in pediatric subjects than in adults and increase with age: a multifrequency MR elastography study. *Radiology.* (2017) 283:222–30. doi: 10.1148/radiol.2016160252
47. Goyal NP, Sawh MC, Ugalde-Nicalo P, Angeles JE, Proudfoot JA, Newton KP, et al. Evaluation of quantitative imaging biomarkers for early-phase clinical trials of steatohepatitis in adolescents. *J Pediatr Gastroenterol Nutr.* (2020) 70:99–105. doi: 10.1097/MPG.0000000000002535

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Possible Hepatoprotective Effect of Tocotrienol-Rich Fraction Vitamin E in Non-alcoholic Fatty Liver Disease in Obese Children and Adolescents

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Obesity has become a worldwide health concern among the pediatric population. The prevalence of non-alcoholic fatty liver disease (NAFLD) is growing rapidly, alongside the high prevalence of obesity. NAFLD refers to a multifactorial disorder that includes simple steatosis to non-alcoholic steatohepatitis (NASH) with or devoid of fibrosis. NAFLD is regarded as a systemic disorder that influences glucose, lipid, and energy metabolism with hepatic manifestations. A sedentary lifestyle and poor choice of food remain the major contributors to the disease. Prompt and timely diagnosis of NAFLD among overweight children is crucial to prevent the progression of the condition. Yet, there has been no approved pharmacological treatment for NAFLD in adults or children. As indicated by clinical evidence, lifestyle modification plays a vital role as a primary form of therapy for managing and treating NAFLD. Emphasis is on the significance of caloric restriction, particularly macronutrients (fats, carbohydrates, and proteins) in altering the disease consequences. A growing number of studies are now focusing on establishing a link between vitamins and NAFLD. Different types of vitamin supplements have been shown to be effective in treating NAFLD. In this review, we elaborate on the potential role of vitamin E with a high content of tocotrienol as a therapeutic alternative in treating NAFLD in obese children.

Keywords: non-alcoholic fatty liver disease, vitamin E, tocotrienol, obesity, children, overweight

INTRODUCTION

The primary reason for chronic liver disease among children can be attributed to non-alcoholic fatty liver disease (1). NAFLD emerges as a common condition, usually accompanied by obesity, with males having a higher risk of developing the disease (2). NAFLD is described as hepatic fat infiltration of more than 5% of liver weight with no underlying liver diseases. It constitutes a series of conditions, ranging from intrahepatic fat accumulation to necrotic fibrosis and inflammation (3). The disease is generally asymptomatic, yet if left untreated, it will eventually lead to liver cirrhosis and, in some cases, hepatocellular carcinoma (4). In children, NAFLD is linked with metabolic

impairments, which considerably increases the risk of metabolic and cardiovascular complications. Obesity is a central player in the development of metabolic syndrome, which is also highly related to NAFLD progression. Research has noted that many non-communicable ailments such as type II diabetes mellitus (T2DM), cardiovascular diseases, and cancers are also associated with obesity, which could further endorse the burden of illnesses and death rates (5). Obesity occurring in childhood has short-term and long-term effects on physical health in a child as well as in adult life. According to the previous research, obese and overweight children were more likely to stay obese in adulthood (6). Obesity occurring in childhood is an intricate issue, and it is the outcome of interactions between many aspects. These aspects can be split into two classes: changeable aspects (e.g., physical activity, socioeconomic status, diet, and parental elements) and unchangeable factors, including genetics, ethnicity, intrauterine components (7). For instance, Partap et al. suggested that obese and overweight children in Malaysia were highly associated with parental obesity where children of one or both obese parents had a 2-fold risk of being obese (8).

According to the National Health and Morbidity Survey (NHMS) 2015, the prevalence of obesity among children aged 10–14 years in Malaysia was 14.4% (9). While in the "MyBreakfast" study, the prevalence of overweight and obesity among children aged 6–12 years was 14.7% in Malaysia (10). Likewise, the NHMS conducted in three separate years 2006, 2011, and 2015, documented an uprising in the prevalence of obesity and overweight among Malaysian youths of <18 years old at 5.4, 5.7, and 11.9%, respectively (9, 11, 12). The rise in the rate of overweight or obese children could increase NAFLD prevalence among pediatrics. Risk factors, including, including insulin resistance, high body mass index (BMI), and genetic predisposition of NAFLD, should alert the parents or guardians to screen for NAFLD among children (13). Based on the NAFLD guidelines by the North American Society Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), overweight or obese child above 8 years old with chronically elevated alanine aminotransferase (ALT) and identified risk factors should be screened for NAFLD. The NASPGHAN suggested intervening against the disease at a younger age, especially those with a greater risk of certain subpopulations, including ethnicity and presence of metabolic syndromes (14). To date, there is no pharmacological treatment for NAFLD, both in adults and children. The first-line therapeutic approach for NAFLD among children is lifestyle changes, including diet and exercise (15). A weight loss of 5–10% of the original body weight is recommended to treat NAFLD (16). Interestingly, Cheng et al. showed a pronounced reduction in hepatic fat content in pre-diabetic adult patients with NAFLD who received a combination of fiber-enriched diet and aerobic exercise training compared to patients receiving either increased fiber-intake or exercise alone (17). A Mediterranean diet is a promising approach to the treatment of NAFLD. A significant decrease in fat deposition in the liver was observed among overweight adult patients with NAFLD after 6 months of intervention (18). Likewise, Corte et al. showed a lowered risk of non-alcoholic steatohepatitis (NASH) and diabetes in obese pediatric patients who adhered to the Mediterranean diet compared to children and adolescents who did not (19). In a randomized controlled trial of 40 adolescent boys, Schwimmer et al. reported a significant decrease in hepatic steatosis and ALT level in the intervention group receiving a diet of free sugar intake to <3% of daily calories. The finding suggested the provision of a diet low in free sugar for a better disease outcome (20). Besides, epidemiological studies demonstrated that children and adolescents with NAFLD consumed a higher amount of fructose intake than healthy controls. Therefore, reduced consumption of high-sugar beverages could prevent NAFLD (21).

Until today, there is no FDA approved drug in managing NAFLD among children. The safer choice of treatment to tackle this disease was probiotics or antioxidant agents (22). The rationale of using an antioxidant agent such as vitamin E was based upon the fact that oxidative stress is one of the possible underlying mechanisms that have a role in the progression of liver steatosis to fibrosis. The imbalance of antioxidants and production of reactive oxygen species (ROS) occurred during fatty acid β-oxidation and oxidative phosphorylation which lead to hepatic damage and injury (23). Moreover, an excessive amount of ROS could trigger the production of pro-inflammatory cytokines hence exacerbating the disease (24). This review summarizes the insights on NAFLD occurring among children and the use of vitamin E, a well-established fat-soluble antioxidant, in the treatment for obese pediatric NAFLD (25).

PEDIATRIC NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Epidemiology

The prevalence of NAFLD was closely related to the increased prevalence of obesity in adults and children (26). The occurrence of obese and overweight children has attained epidemic levels in several emerging nations, including Malaysia. Overweight and obese children had a higher risk of NAFLD, with an estimated prevalence of 26.0% in children with obesity (27). In a recent meta-analysis, the global prevalence of NAFLD among adults was estimated to be at 25.24% (4). The prevalence of NAFLD in Asia among adults increased significantly from 25.28% in 1999–2005 to 28.46% in 2006–2011, and 33.9% in 2012–2017 with higher prevalence observed among obese to non-obese populations in most Asian countries (28). Anderson et al. performed a meta-analysis on NAFLD among youths and estimated the prevalence of NAFLD in children from the general population was at 7.6% (95% CI: 5.5–10.3%), while from 56 independent studies in child obesity clinics, the prevalence was much higher at 34.2% (95% CI: 27.8–41.2%) (2). It is projected for the next 10 years; the prevalence of pediatric NAFLD would emerge as the most widespread reason for liver failure and a warning for liver transplantation in kids and adolescents (2, 29).

The prevalence of NAFLD increases with age, with a greater percentage of NAFLD among adolescents (15–19 years old) at 17.3% as compared to toddlers (2–4 years old) at 0.7% (30). NAFLD increased occurrence in adolescents could be attributed to several changes occurring during childhood to adulthood,

including variation in diet, hormone levels, and metabolism (31). The prevalence rate of NAFLD was 13.4% (278/2,080) in boys, which was significantly higher than girls (2.8%, 57/2,061) (32). A probable hypothesis was that gender disparity in NAFLD is potentially due to differences in sex hormones and that females are more resistant to NAFLD progression than males due to the presence of estrogen, which potentially has a liver-protective effect (33). However, the role of gender in influencing the prevalence of NAFLD remains a question. Other factors that contribute to pediatric NAFLD were the sedentary lifestyle of children and their unhealthy food choices (34). In the United States, the prevalence of NAFLD, was also affected by ethnicity based on a meta-analysis study of 34 studies, in which a higher risk of NASH was observed in Hispanics compared to Caucasians and African Americans (35). Likewise, among adolescents with NAFLD, a higher prevalence was recorded among Hispanics (59.6%) compared to Caucasians (42.9%) and African Americans (15.7%) (36). In Malaysia, of 469 adult subjects analyzed, a higher prevalence of NAFLD was observed among Indian (33.3%) and Malay (25.5%) males compared to Chinese males (6.8%) (37). The ethnic differences among NAFLD patients could probably be explained by an increased insulin resistance where Asian Indians were reported to have the least insulin sensitivity compared to Chinese or Malay males (38). In a cross-sectional study on the impact of insulin resistance and hypertriglyceridemia, non-Hispanic Whites and African Americans displayed greater insulin sensitivity compared to Hispanic White, East Asian, and South Asian (39). Pediatric NAFLD had a strong association with central or generalized obesity and metabolic syndromes, including insulin resistance and dyslipidaemia (14). In children and adolescents with biopsy-proven NAFLD, a higher prevalence of abnormal glucose tolerance was observed than children without NAFLD. Ting et al. reported a significant association between metabolic syndrome and advanced liver fibrosis in 57 children visiting pediatric obesity and diabetic clinics (40). The severe form of NAFLD, NASH, was detected more among children with abnormal glucose tolerance (41). Mohamed et al. reported that 62% of obese children with NAFLD had metabolic syndrome (42).

Genetic predisposition is another factor that could affect the prevalence and severity of both adult and pediatric NAFLD. In Korea, an additive effect of single nucleotide polymorphisms (SNPs) was noted in the patatin-like phospholipase domain containing three genes (*PNPLA3*) and transmembrane six superfamily 2 (*TM6SF2*) that affect the severity of liver damage as reported in adult patients with NAFLD (43). In a PANIC study, overweight Caucasian children (6–8 years) with *PNPLA3*, I148M polymorphisms were associated with an increased ALT, indicating early onset of NAFLD (44). The *PNPLA3* polymorphisms among obese young individuals (below 20 years) with metabolic syndrome were shown to have a significant increase in their ALT levels (45). A meta-analysis study of pediatric and adolescents suggested that carriers of the G allele of *PNPLA3* have a more severe type NASH together with a high level of liver fat content, ALT, aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) (46). In an association study investigating the relationship between *PNPLA3* and hepatic fat accumulation among children, Stanislawski et al. found hepatic fat accumulation is highly associated with *PNPLA3* among Hispanic compared to non-Hispanic (47). On the other hand, Grandone et al. reported that obese children carrying *TM6FS2* E167K were more predisposed to NAFLD (48).

Pathogenesis

The underlying mechanism in the pathogenesis of NAFLD is complex and involves many factors. Initially, the "two-hit theory" emerged where an accumulation of lipids due to a sedentary lifestyle, high fat diet, insulin resistance, and obesity act as the first hit. This was followed by the second hit theory of fatty acid accumulation that induced inflammatory cascades resulting in fibrosis (49). Nonetheless, a more recent "multiple-hit theory" better describes the intricacy of the disease where multiple parallel factors are involved in NAFLD progression. The "multiple-hits" hypothesis primarily elaborates on the NAFLD pathogenesis, which includes genetic factors, insulin resistance (IR), hyperlipidaemia, and obesity as the main culprits in the development of NASH (50). At the onset of NAFLD, it is characterized by insulin resistance and triglycerides (TGs) accumulation in the liver termed as steatosis. Factors, including hypercaloric diets, epigenetics, genetic susceptibility, and a sedentary lifestyle, significantly promoted NAFLD pathogenesis. Besides liver damage, this condition could lead to other related comorbid diseases. Nevertheless, the relationship and interactions between these mechanisms are not yet well understood (51). The fatty liver condition is non-pathogenic for most patients and is reversible through appropriate interventions, while 30% of them have the probability of progressing to NASH (52). The differences in storing fat capability in different adipose tissue compartments differed significantly between children with and without NAFLD (53). These dissimilarities were visible after 3 years of age but not at birth, indicating that the initial 3 years of life might signify a vital window, in which different interactions between environmental, genetic, epigenetic, and metabolic aspects lead to the prospective risk of primary NAFLD (54) (**Figure 1**) (29). Yet, Wesolowski et al. suggested the offspring of obese mothers may have increased risk for obesity and NAFLD at birth, in which the intrauterine aspects played a role in the rapid progression of NAFLD to NASH in children (55). In terms of metabolic aspect, insulin sensitivity is considered one of the hallmarks of NAFLD, is strongly associated with obesity. The impaired ability of insulin to suppress endogenous glucose and free fatty acids (FFAs) production is indicative hepatic insulin resistance (56). It was also suggested that IR was the result of an accumulation of excess fat that cannot be stored in the adipose tissue, which overflows into the visceral compartment and accumulates in organs such as the liver (57). On the other hand, secondary NAFLD may develop due a myriad of other causes including endocrine diseases such as primary hypothyroidism, hypogonadism, polycystic ovary syndrome (PCOS) and growth hormone deficiency which resulted in obesity (58).

Fatty liver is the build-up of TGs in hepatocytes due to a disparity between energy metabolism and energy consumption (59). Dyslipidaemia, which is a component of metabolic

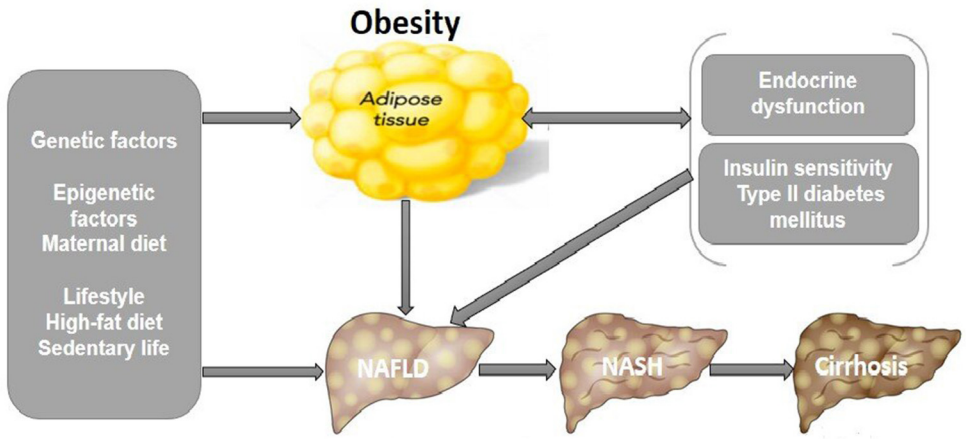

FIGURE 1 | Pathophysiological mechanisms that link pediatric NAFLD and obesity. Unhealthy lifestyles, genetic and epigenetic factors are responsible for NAFLD and its progression to NASH and hepatic cirrhosis, increasing the risk of mortality. Proposed pathogenic mechanisms include endocrine dysfunction, hepatic insulin resistance from T2DM, and obesity increases lipid accumulation and inflammation in the liver leading to NAFLD. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, type II diabetes mellitus (29).

syndrome, is also one of the hallmarks of pediatric NAFLD. It is characterized by the accumulation of TGs and low high-density lipoprotein cholesterol (HDL-C). Reduced fatty acid β-oxidation in lipid degrading organelles (e.g., mitochondria and peroxisomes) and a decreased lipid excretion of very-low-density lipoproteins (VLDL) were other factors that contribute to the accumulation of hepatic fat (60). In turn, the adipose tissue production contributes to the increased in FFAs turnover. In a retrospective study of 309 children diagnosed with NAFLD, elevated levels of TGs, non-HDL-C and low-density lipoprotein cholesterol (LDL-C) were highly prevalent (60, 61). Local data documented that high TG was a predictor for NAFLD among overweight and obese children attending the Pediatric Obesity Clinic at the University Malaya Medical Center (42). In addition, increased TG synthesis could also be contributed by *de novo* lipogenesis in the liver. For instance, the conversion of fructose to fat in the liver further drives the accumulation of liver fat (62). Schwarz et al. reported that short-term isocaloric fructose restriction in children with obesity and metabolic syndrome resulted in significant decreased liver fat and *de novo* lipogenesis as well as improved insulin kinetics (63). Other factors contributing to NASH progression include lipotoxicity-induced inflammation and oxidative stress (59, 64). The ectopic fat accumulation may induce excessive release of pro-inflammatory cytokines such as TNF-α and IL-6, thus worsening the disease condition. Adipocytokines (or adipokines), secreted by the adipose tissues, were shown to be highly involved in various processes such as inflammation, immunity, insulin sensitivity, simple liver steatosis, and NASH (65). Studies have shown serum levels of adiponectin were altered in patients with NAFLD. A prospective case-control study suggested elevated serum chemerin and decreased serum adiponectin were highly associated with an increased NAFLD likelihood in non-diabetic obese children (66). In a prospective study of 3 years among adults without fatty liver, similar observation of decreased adiponectin level was observed among subjects who developed NAFLD (67). These novel adipokines (e.g., omentin and chemerin) have been suggested as potential markers of ectopic lipid accumulation in the liver and NAFLD pathogenesis (68). Pediatric NAFLD progression is illustrated through a complicated sequence of interactions between resident hepatic and recruited cells. The activation of Kupffer cells (liver macrophages) and hepatic stellate cells (collagen-producing cells) influenced NAFLD's progression to NASH. The activation of Kupffer cells led to the production of inflammatory cytokines as a direct outcome of apoptosis, hepatic steatosis, hepatocyte injury, and indirect reaction to hepatic damage (69, 70). Dysregulation of pro-inflammatory adipokines and cytokines were found to be globally prevalent in NAFLD patients. Mitochondrial, endoplasmic reticulum, cytokine-mediated oxidative stress, and hepatocytic apoptosis were the causes of NASH (71, 72).

Obesity can also be defined as low-grade inflammation in adipose tissue, which resulted in IR and other metabolic complications associated with obesity (73). Inflamed adipose tissue was associated with abnormal productions of inflammatory cytokines and activation of inflammatory signaling in adipocytes (74). Furthermore, extracellular and intracellular stress induced by the accumulation of lipids, glucose, and cytokines and steatosis could trigger the hepatic endoplasmic reticulum (ER) stress response. The progression of steatosis to NASH could be due to the hepatic ER stress response's activation, triggering the cell death and inflammation, hence promoting metabolic disorders (75). However, there are limited studies on the association of inflammatory markers with NAFLD. **Figure 2** illustrates the mechanisms involving multiple factors implicated in the pathogenesis of NAFLD to NASH. (50).

Diagnosis

Pediatric NAFLD continues to be an underdiagnosed condition due to the dearth of symptoms' recognition and

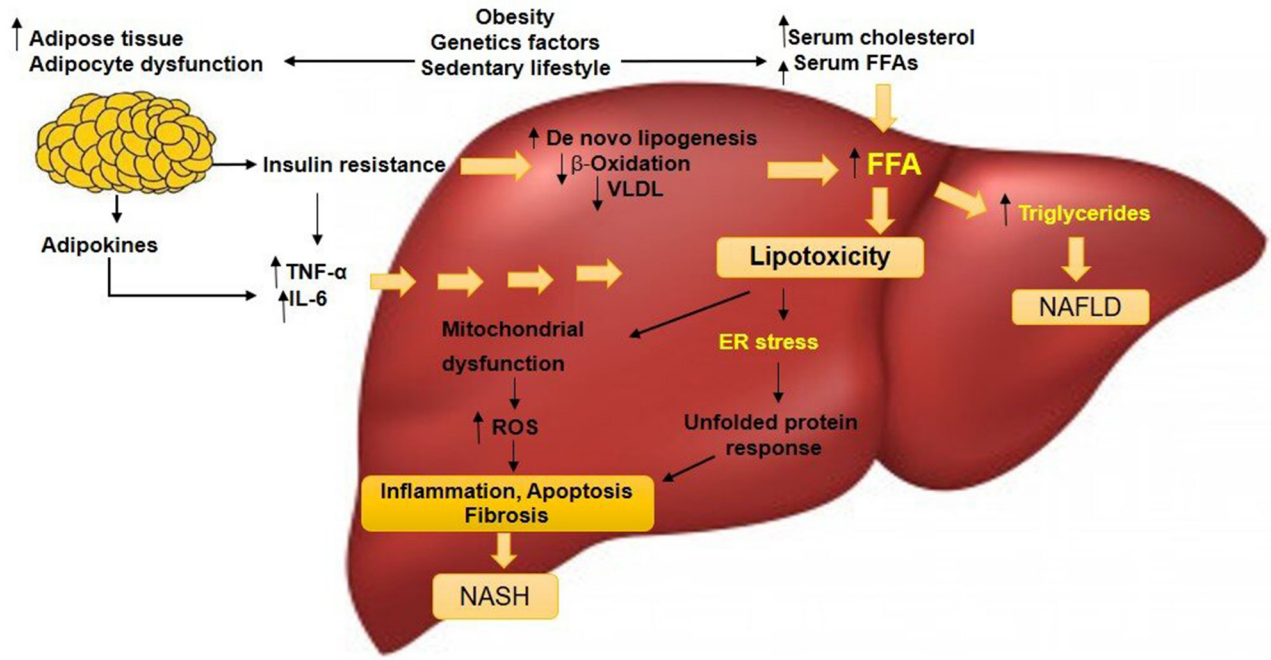

FIGURE 2 | A simplified version of the pathogenesis of pediatric NAFLD. Factors including obesity, sedentary lifestyle, and genetic factors could increase serum levels of FFAs and cholesterol, stimulate adipocyte proliferation and dysfunction. Insulin resistance will develop and this condition acts on adipose tissue that deteriorates the adipocyte dysfunction. Lipolysis is induced, adipokines and pro-inflammatory cytokines such as TNF-α and IL-6 in the liver are released. Insulin resistance amplifies *de novo* lipogenesis and reduces β-oxidation and VLDL level, generating excessive FFAs. The increased hepatic FFAs leads to two situations: (1) synthesis and accumulation of TGs leading to persistent NAFLD and (2) increased 'toxic' levels of FFAs and free cholesterol, causing mitochondrial dysfunction, evidenced by an elevated ROS and ER stress. Hepatic inflammation occurs that progress to NASH. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; FFAs, free fatty acids; VLDL, very-low-density lipoproteins; ROS, reactive oxygen species; ER, endoplasmic reticulum; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6. (50).

under-appreciation of its related complications. There is no specific screening strategy for NAFLD in children, even though there are clinical and pathophysiological variations between adult and pediatric NAFLD. Most diagnostic algorithms and risk prediction scores, including NAFLD activity scores, are used for adults but are limited for children; therefore, they could not be relied on (76, 77). In place of a procedure is excluded, NAFLD diagnosis needs to be actively considered in all obese or overweight children more than 10 years old, specifically in the contextual of acanthosis nigricans, hypertension, and evidence of hepatomegaly as clinical evidence of metabolic syndromes (29, 77, 78). Liver biopsy is the current benchmark approach for NAFLD diagnosis, and it is the sole mode of distinguishing between NASH and simple steatosis. It also could exclude other causes of elevated transaminases and hepatic infiltration due to fats such as autoimmune hepatitis and metabolic liver diseases (79). Nevertheless, liver biopsy is an invasive process with several drawbacks, such as high cost and risks, and hence it is often not carried out as a preliminary step for diagnosis (79). Therefore, a non-invasive evaluation in imaging tests, biochemical parameters, and serum biomarkers is used to assert the diagnosis of fatty liver disease among children, particularly in a typical patient with metabolic syndromes (80). Liver function and abdominal ultrasonography tests are the choices for diagnosing NAFLD among youth despite their narrow sensitivities (29, 80). As suggested by the American Academy of Pediatrics, regular semi-annual screenings for serum AST and ALT must be conducted for every obese child above 10 years of age, a BMI score ranged from the 85th percentile to the 94th percentile, and who possesses related metabolic risk factors (14). The European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggested that all overweight and obese children above 3 years old should be screened for NAFLD by performing abdominal ultrasound and liver function tests. Ezaizi et al. suggested using a combination of elevated ALT and fatty infiltration on ultrasound to increase the detection rate and to avoid missed detection of suspected NAFLD children at risk (81). Ultrasonography is a safe and reliable tool for the diagnosis of NAFLD. Nonetheless, the hepatic ultrasound is insensitive when the fatty liver is moderate to severe as frequent mild level of hepatic steatosis was reported in advanced pediatric NASH. Therefore, liver biopsy is usually necessary for accurate pediatric NAFLD diagnoses. Histopathological and radiological findings are essential and must be interpreted cautiously, while serum ALT and AST levels are standard procedures in most pediatric NAFLD cases, regardless of the severity of the disease (29).

BENEFICIAL EFFECTS OF VITAMIN E (TOCOTRIENOL) IN NON-ALCOHOLIC FATTY LIVER DISEASE

Presently, no therapeutic strategy was approved for NAFLD, both in adults and pediatrics. Lifestyle modification via diet and exercise was shown to benefit patients with NAFLD (82). Consequently, most clinical efforts are focused on treating the elements of metabolic syndrome, specifically obesity, hypertension, diabetes, and dyslipidaemia. Other interventions focused on specific pathways potentially implicated in NAFLD pathogenesis, including insulin resistance, apoptosis, pro-inflammatory cytokines, oxidative stress, angiotensin pathways, and bacterial overgrowth (83). In metabolic syndrome, the excess amount of FFA intake in the liver induces oxidative stress hence creating ROS that can cause liver damage. Thus, an antioxidant is an appropriate approach for adjunct therapy to lifestyle modification in treating NAFLD. Furthermore, Stenzel et al. analyzed the serum antioxidant level in metabolically healthy and unhealthy obese adolescents. They found a strong negative association between vitamin E and the waist circumference of unhealthy obese adolescents. In addition, lower frequency of NAFLD was documented among metabolically healthy obese subjects (84). The National Institute for Health and Care Excellence (NICE) and the American Association for the Study of Liver Diseases (AASLD) in their guidelines proposed using vitamin E in NAFLD patients, but the AASLD only approved vitamin E as a pharmacological treatment among adults without diabetes and with biopsy-proven NASH (85). Clinical trials testing the efficacy of vitamin E in both adults and pediatric NAFLD reported contradictory findings. In a meta-analysis by Amanullah et al. it was reported that adjuvant vitamin E therapy improved biochemical and histological characteristics in adult patients with NAFLD (86). However, in pediatric patients, the efficacy of vitamin E was moderate when compared to the placebo. An earlier studies that looked at the effect of vitamin E in children with NAFLD found that antioxidant therapy of α-tocopherol 600 IU/day and ascorbic acid 500 mg/day did not have significant effect on the liver function and glucose control (87). The authors suggested that diet and physical exercise are necessary for improving liver function and glucose metabolism in children with NAFLD. Notably, in a randomized, double-blind placebo-controlled trial between 80 adolescents with biopsy-proven NAFLD receiving either placebo or vitamin E and hydroxytyrosol (HXT), the results showed improved insulin resistance, steatosis, and oxidative stress parameters in children with NAFLD in the intervention arm (88). The use of vitamin E in combination with other nutraceuticals as a treatment in pediatric NAFLD showed promising results. About 70 children with NAFLD receiving vitamin E (10 mg) and hydroxytyrosol (7.5 mg) for a duration of 4 months showed significant reduction in TG level and improved liver steatosis (89). Zöhrer et al. treated 60 children (4–16 years) with liver biopsy-proven NASH with a combination of 250 mg of docosahexaenoic acid (DHA), 39 UI of vitamin E, and 201 mg of choline (DHA-CHO-VE) for 12 months. Significant improvement of liver steatosis was observed in the treated group accompanied by reduced ALT and glucose levels (90). Administration of vitamin E (400 IU/day) plus spironolactone (25 mg once daily) for 52 weeks showed a significant decrease in liver fat score and homeostasis model of assessment-insulin resistance (HOMA-IR) in women with NAFLD (91).

Vitamin E is a primary fat-soluble antioxidant and comprises of a family of organic compounds including two isoforms, tocotrienol and tocopherol. Both tocopherol and tocotrienol appear in four isomers, α (alpha), β (beta), δ (delta), and γ (gamma). Unlike tocopherol, tocotrienol is an unsaturated form that features an isoprenoid side chain, making it easier to absorb and penetrate better into tissues with saturated fatty layers, including the liver and the brain (92). Tocotrienol was preferably issued to the liver, and α-tocotrienol is 40–60 times stronger than α-tocopherol in countering lipid peroxidation of liver microsomes (93). On the other hand, tocopherol is naturally lipophilic, protecting the polyunsaturated fatty acid compounds from peroxidation reactions, including lipoproteins, cellular membranes, and fat deposits (92). Interestingly, comparative studies on both vitamin E types showed that tocotrienol was far more potent than tocopherol in mitigating oxidative stress and inflammation (94, 95). Tocotrienol, as a free radical scavenger, can mitigate oxidative stress in metabolic disorders and protecting cellular functions (96). The δ-tocotrienol featured better bioavailability when supplemented at higher doses in healthy subjects, as reported by Qureshi et al. (97). Since most of the orally treated tocotrienol were metabolized inside the liver, these tests' outcomes suggested that tocotrienol supplements improved the obesity-stricken liver function and the energy spending of the entire body through improved oxidation of hepatic fatty acids (73).

In vitro Evidence of Hepatoprotective Effect of Tocotrienol

Tocotrienol has been shown to have hypolipidemic properties in cells. The hypolipidemic property of tocotrienol is mediated through reduction of HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), a key enzyme for the cholesterol biosynthesis (98, 99). *In vitro* research revealed that α-tocotrienol considerably decreased the total cholesterol (TC) compared to untreated SH-SY5Y cells and cells treated with α-tocopherol (100). A study using HepG2 cells treated with δ- and γ-tocotrienol indicated suppression of genes involved in lipid homeostasis including HMCR and APOB100 hence reducing TGs, TCs, and VLDL biosynthesis (101). Tocotrienol also has a powerful effect on decreasing adiposity as well as fat cell formation. It was postulated that γ-tocotrienol could decrease adipogenesis as the process shared the same pathway as in tumorigenesis. Fat cell development (adipogenesis) shares numerous similar signaling pathways with tumorigenesis; including the STAT, Wnt, mTOR signaling, and Akt autophagy pathways (98). Adipogenesis is defined as the differentiation of preadipocytes into mature adipocytes and increased accumulation and synthesis of intracellular TGs and lipid droplets (102). Zhao et al. employed hASCs as a human adipocyte model to identify the inhibitory effects

of γ-tocotrienol on adipogenesis (74). The study found that γ-tocotrienol prevented adipogenesis in differentiating hASCs in an isomer- and dose-dependent manner. In several adipocyte cell lines, γ- and δ-tocotrienol were reported to exert anti-obesity characteristics through Bax-mediated mitochondrial or AMPK signaling pathways (103, 104). Based on the study of γ-tocotrienol effect on prostate cancer cells, activation of 5′ AMP-activated protein kinase (AMPK) signaling is vital for anti-adipogenic activity induced by γ-tocotrienol to elicit a cytotoxic effect on cancer cells (105). Nevertheless, inhibition of AMPK did not change the γ-tocotrienol-induced autophagy, implying that autophagy might not necessarily be directly related to the anti-adipogenic activity induced by γ-tocotrienol (73). These results showed that AMPK is a significant target for tocotrienol induced anti-adipogenesis in which the anti-adipogenic activity could be exhibited by its anti-proliferative capabilities. The AMPK is a modulator for lipid and cholesterol pathways thus, an excellent therapeutic target for treating NAFLD. On the other hand, Torabi et al. reported that *d*-δ-tocotrienol inhibited the differentiation of murine 3T3-F442A preadipocytes through the downregulation of peroxisome proliferator-activated receptor γ (PPARγ), a key regulator of adipocyte differentiation (99). Apart from anti-adipogenic property, tocotrienol also possesses anti-inflammatory property. In 2016, Kim et al. reported inhibition of nod-like receptor 3 (NLRP3) inflammasome in iJ774 macrophages by γ-tocotrienol thus attenuated adipose tissue inflammation and insulin resistance (106). Shen et al. reported δ-tocotrienol isolated from rice bran significantly inhibited inflammation via mitogen-activated protein kinase (MAPK) and PPAR signaling in lipopolysaccharide (LPS)-stimulated macrophages via inhibition of TNF-α, IFN-γ, IL-1β, and IL-6 (107, 108). The LPS stimulates the release of inflammatory cytokines, including IL-6, IL-1β, and TNF-α (109). The activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and MAPK signaling were attenuated significantly in γ-tocotrienol-treated human adipocytes (107). Additionally, the δ-tocotrienol inhibited MAPK activation, which is essential for initiation of inflammation process, by inhibiting phosphorylation of p38, c-June N-terminal kinase (JNK), and extracellular signal-regulated kinase (110) in LPS-induced hASCs (107).Similar anti-inflammatory activity was exerted by δ-tocotrienol on TNF-α stimulated NF-κB activation in RAW 264.7 macrophages in a dose-dependent manner (111). In human gastric cells, γ-tocotrienol exhibited anti-cancer property via regulation of NF-κB signaling pathway. The aversion of NF-κB activation, an important pro-inflammatory marker involved in cell proliferation and apoptosis, subsequently halted tissue inflammation (112, 113).

In vivo Evidence of Hepatoprotective Effect of Tocotrienol

Tocotrienol has a beneficial influence on reducing body fat or mass, plasma concentration of free fatty acid, cholesterol, and improving insulin tolerance in animal models and humans (73). Excessive amount of adipocytes secrete excess amount of adipokines and cytokines resulted in dysregulated lipid metabolism and then obesity therefore, fat cell apoptosis is necessary for inhibition of adipogenesis (73). Tocotrienol demonstrated an anti-adipogenic activity by causing adipocyte cell death (112). *In vivo* analysis of TRF supplementation with royal jelly in calorie restricted diet-fed rats indicated significant weight reduction and reduced expression of inflammatory cytokines (i.e. TNF-a and MCP1) as compared to TRF or royal jelly intervention alone (114). Kim et al. reported supplementation of γ-tocotrienol in Western-diet fed mice induced a profound decrease in TC and LDL levels apart from TG reduction by 50% (64). Furthermore, rice-bran tocotrienol inhibited adipogenesis in Western-diet fed mice via the upregulation of lipid metabolism genes, carnitine palmitoyltransferase 1A (*CPT1A*), and cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*) (115). High-purity tocotrienols also possessed antiatherogenic property, where apolipoprotein E knockout mice fed with cholesterol-containing diet had reduced atherosclerotic lesion formation in the aortic root when compared to α-tocopherol treatment possibly through upregulation of *Slc27a1* gene (116). Since NAFLD and IR are highly associated with inflammation, many studies have shown tocotrienol as a potent anti-inflammatory inhibitor in animal models. In HF-fed mice supplemented with δ-tocotrienol at 400 mg/kg or 1600 mg/kg for 14 weeks, reduced expression of *tumor necrosis factor-alpha* (TNF-α) mRNA, a pro-inflammatory marker was observed (117). The authors suggested that the observed reduction in hepatic steatosis and serum TG levels when compared to HF-fed mice was due to reduced inflammation both in the liver and adipose tissue (117). Furthermore, treating T2DM in db/db mice with γ-tocotrienol delayed the progression of T2DM by suppressing the activation of NLRP3 inflammasome and associated inflammatory cytokines (106). Shen et al. observed better glucose homeostasis in HF-diet induce T2DM mice when treated with annatto-extracted tocotrienol by alleviating inflammatory response (118). Likewise, Kim et al. reported the supplementation of γ-tocotrienol in Western-diet fed mice reduced the expression level of pro-inflammatory mRNAs, including monocyte chemoattractant protein 1 (*Mcp1*), *Nlrp3, Tnfα, Il-1β,* and *cdc11* (119).

Hepatoprotective Effect of Tocotrienol in Clinical Trials

Tocotrienol has anti-sclerotic and hypo-cholesterol impacts on humans (73). The tocotrienol-rich fraction (TRF) derived from palm oil was found to reduce total serum cholesterol, apolipoprotein B, LDL-cholesterol, and TG levels in patients with non-familial hypercholesterolemia (120). In a cross-sectional study ($n = 200$) of subjects with or without NAFLD, Ezaizi et al. reported a significant association between high-sensitivity C-reactive protein (hs-CRP) with NAFLD in Asian Indians (81). The hs-CRP is known to be associated with inflammation in the liver. Pervez et al. also reported decreased level of hs-CRP in addition to HOMA-IR, ALT and AST level in patients with NAFLD treated with δ-tocotrienol compared to placebo (121). Recently, Gao et al. reported the involvement of CYP4A11

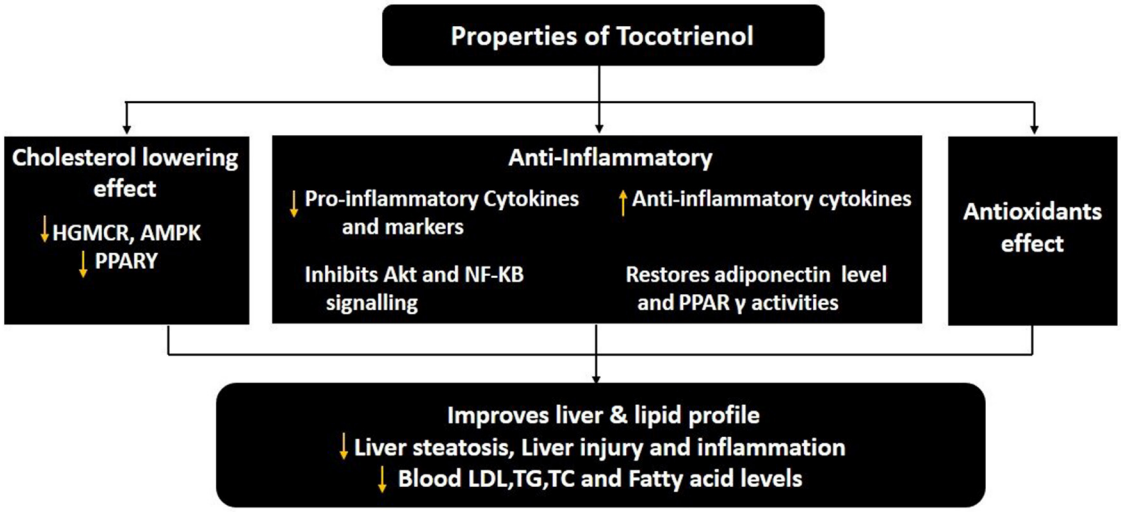

FIGURE 3 | The pharmacological properties of tocotrienol for the treatment of pediatric NAFLD. ↑, increase or upregulate; ↓, decrease or downregulate; PPARγ, peroxisome proliferator-activated receptor γ; NF-κB, nuclear factor-κB; LDL, low-density lipoprotein; TG, triglyceride; TC, total cholesterol, ((125)).

in the development of NAFLD through ROS-induced lipid peroxidation and inflammation (122). Since CYP4A11 belongs to the CYP4 enzyme family involved in the metabolism of medium- and long-chain fatty acids, it is crucial in NAFLD pathogenesis. The patients with NAFLD were shown to have an increased amount of CYP4A11, possibly induced by a high-fat diet. The CYP4A11 increased ROS production during fatty acid oxidation, thus promoting oxidative stress. In a randomized controlled trial of 54 patients with diabetic nephropathy, the group receiving tocotrienol-rich vitamin E at 400 mg/day for 12 weeks had a significant reduction in serum creatinine levels and increased eGFR, hallmarks of inflammation, compared to placebo (123). To date, limited studies are available that documented the effect of tocotrienol on pediatric NAFLD. In a TONIC clinical trial for pediatric NAFLD, Lavine et al. (2011) found no significant difference in patients receiving either Vitamin E, metformin or placebo in terms of ALT level and histological features. Thus, designing clinical trials to test the effect of tocotrienol in the pediatric population should be properly examined due to the differences in several aspects that differentiate adult and pediatric NAFLD (124). **Figure 3** summarizes the properties of tocotrienol as an adjunct therapy for the treatment of pediatric NAFLD.

CONCLUSION

Despite high occurrence and rigorous research, NAFLD treatment remains an unfulfilled medical requirement. Presently, no specific pharmacological therapy was authorized for NAFLD. Nonetheless, patients are encouraged to improve their lifestyles through physical activities, diet, and managing related comorbidity (i.e., metabolic syndrome and obesity elements), which are supposed to reduce cardiovascular-related, hepatic morbidity in NASH patients. Nevertheless, patients often found that participating in physical activity and changing dietary habits are tough to achieve and maintain. Therefore, the characteristics of tocotrienol, which are easily found and exceptional acceptability to people, have made it a practical treatment choice for NAFLD patients. NAFLD clinical experiments showed a moderate improvement in histology and liver biochemistry induced by tocotrienol treatment. However, further monotherapy clinical trials and pharmacological assessments are still required to explain the fundamental molecular mechanisms of treatment. Also, possible unpleasant consequences of tocotrienol to identify the best daily intake needed for pediatric NAFLD. In conclusion, tocotrienol is recommended as a functional diet to be used in combination with existing diabetes and obesity regulation strategies for the treatment of pediatric NAFLD.

AUTHOR CONTRIBUTIONS

NM and ZA contributed to the conception of the idea for the manuscript. FA-B and AI performed literature search and drafted the manuscript. RR, NM, ZA, and KM critically revised the work. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Marietti M, Bugianesi E. Obesity: Childhood obesity: time bomb for future burden of chronic liver disease. *Nat Rev Gastroenterol Hepatol*. (2016) 13:506. doi: 10.1038/nrgastro.2016.120
2. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PloS ONE*. (2015) 10:e0140908. doi: 10.1371/journal.pone.0140908
3. Sherif ZA. The rise in the prevalence of nonalcoholic fatty liver disease and hepatocellular carcinoma. *Nonalcoholic Fatty Liver Disease-An Update*. (2019) 1–22. doi: 10.5772/intechopen.85780
4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. (2016) 64:73–84. doi: 10.1002/hep.28431
5. Koene RJ, Prizment AE, Blaes A, Konety SH. Shared risk factors in cardiovascular disease and cancer. *Circulation*. (2016) 133:1104–14. doi: 10.1161/CIRCULATIONAHA.115.020406
6. Umer A, Kelley GA, Cottrell LE, Giacobbi P, Innes KE, Lilly CL. Childhood obesity and adult cardiovascular disease risk factors: a systematic review with meta-analysis. *BMC Public Health*. (2017) 17:683. doi: 10.1186/s12889-017-4691-z
7. Lanigan J, Tee L, Brandreth R. Childhood obesity. *Medicine*. (2019) 47:190–4. doi: 10.1016/j.mpmed.2018.12.007
8. Partap U, Young EH, Allotey P, Sandhu MS, Reidpath DD. Anthropometric and cardiometabolic risk factors in parents and child obesity in Segamat, Malaysia. *Int J Epidemiol*. (2017) 46:1523–32. doi: 10.1093/ije/dyx114
9. Institute for Public Health (IPH). *National Health and Morbidity Survey 2015 (NHMS 2015). Non-Communicable Diseases, Risk Factors & Other Health Problems Kuala Lumpur. Kuala Lumpur:Ministry of Health Malaysia (2015)*.
10. Mohd Nasir MT, Nurliyana AR, Norimah AK, Jan Mohamed HJB, Tan SY, Appukutty M, et al. Consumption of ready-to-eat cereals (RTEC) among Malaysian children and association with socio-demographics and nutrient intakes–findings from the MyBreakfast study. *Food Nutr Res*. (2017) 61:1304692. doi: 10.1080/16546628.2017.1304692
11. Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, et al. NAFLD and cardiovascular diseases: A clinical review. *Clin Res Cardiol*. (2020) 110:921–37. doi: 10.1007/s00392-020-01709-7
12. Institute for Public Health (IPH). *National Health and Morbidity Survey 2011 (NHMS 2011) Vol II: Non-Communicable Diseases Kuala Lumpur. Kuala Lumpur:Ministry of Health Malaysia (2011)*.
13. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J. Hepatol*. (2017) 9:715. doi: 10.4254/wjh.v9.i16.715
14. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr*. (2017) 64:319. doi: 10.1097/MPG.0000000000001482
15. El-Agroudy NN, Kurzbach A, Rodionov RN, O'Sullivan J, Roden M, Birkenfeld AL, et al. Are lifestyle therapies effective for nafld treatment? *Trends Endrocrinol. Metab*. (2019) 30:701–9. doi: 10.1016/j.tem.2019.07.013
16. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. (2018) 67:32–57. doi: 10.1002/hep.29367
17. Cheng S, Ge J, Zhao C, Le S, Yang Y, Ke D, et al. Effect of aerobic exercise and diet on liver fat in pre-diabetic patients with non-alcoholic-fatty-liver-disease: a randomized controlled trial. *Sci Rep*. (2017) 7:1–11. doi: 10.1038/s41598-017-16159-x
18. Trovato FM, Catalano D, Martines GF, Pace P, Trovato GM. Mediterranean diet and non-alcoholic fatty liver disease: the need of extended and comprehensive interventions. *Clin Nutr*. (2015) 34:86–8. doi: 10.1016/j.clnu.2014.01.018
19. Della Corte C, Mosca A, Vania A, Alterio A, Iasevoli S, Nobili V. Good adherence to the Mediterranean diet reduces the risk for NASH and diabetes in pediatric patients with obesity: the results of an Italian Study. *Nutr*. (2017) 39:8–14. doi: 10.1016/j.nut.2017.02.008
20. Schwimmer JB, Ugalde-Nicalo P, Welsh JA, Angeles JE, Cordero M, Harlow KE, et al. Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: a randomized clinical trial. *Jama*. (2019) 321:256–65. doi: 10.1001/jama.2018.20579
21. Hamza RT, Ahmed AY, Rezk DG, Hamed AI. Dietary fructose intake in obese children and adolescents: relation to procollagen type III N-terminal peptide (P3NP) and non-alcoholic fatty liver disease. *Ann Pediatr Endocrinol Metab*. (2016) 29:1345–52. doi: 10.1515/jpem-2016-0015
22. Perumpail BJ, Li AA, John N, Sallam S, Shah ND, Kwong W, et al. The Role of Vitamin E in the Treatment of NAFLD. *Diseases*. (2018) 6:86. doi: 10.3390/diseases6040086
23. Takahashi Y, Sugimoto K, Inui H, Fukusato T. Current pharmacological therapies for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J. Gastroenterol*. (2015) 21:3777. doi: 10.3748/wjg.v21.i13.3777
24. Del Campo JA, Gallego P, Grande L. Role of Inflammatory Response in Liver Diseases: Therapeutic Strategies. *World J Hepatol*. (2018) 10:1. doi: 10.4254/wjh.v10.i1.1
25. Eder R, Mappala H. *IDDF2019-ABS-0194* The role of tocotrienols in the treatment of non-alcoholic steatohepatitis-a meta-analysis. *BMJ Publishing Group*. (2019) 68(Suppl.1):A1–166. doi: 10.1136/gutjnl-2019-IDDFAbstracts.280
26. Fan J-G, Kim S-U, Wong VW-S. New trends on obesity and NAFLD in Asia. *J Hepatol*. (2017) 67:862–73. doi: 10.1016/j.jhep.2017.06.003
27. Elizabeth LY, Golshan S, Harlow KE, Angeles JE, Durelle J, Goyal NP, et al. Prevalence of nonalcoholic fatty liver disease in children with obesity. *J Pediatr*. (2019) 207:64–70. doi: 10.1016/j.jpeds.2018.11.021
28. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. (2019) 4:389–98. doi: 10.1016/S2468-1253(19)30039-1
29. Temple JL, Cordero P, Li J, Nguyen V, Oben JA. A guide to non-alcoholic fatty liver disease in childhood and adolescence. *Int. J. Mol. Sci*. (2016) 17:947. doi: 10.3390/ijms17060947
30. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. (2006) 118:1388–93. doi: 10.1542/peds.2006-1212
31. De Nooijer A, Vreugdenhil A, Karnebeek K, van Hasselt PM, Fuchs SA. A narrative review of factors associated with the development and progression of non-alcoholic fatty liver disease. *GastroHep*. (2019) 1:180–91. doi: 10.1002/ygh2.354
32. Rong Y, Chun-Yan N, Hong-Xin Z, Lu Y, Wen W, Yu T. Association of adolescent obesity with nonalcoholic fatty liver disease and related risk factors in Xi'an, China. *Ann Hepatol*. (2018) 17:85–91. doi: 10.5604/01.3001.0010.7538
33. Lee C, Kim J, Jung Y. Potential Therapeutic Application of Estrogen in Gender Disparity of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. *Cells*. (2019) 8:1259. doi: 10.3390/cells8101259
34. Nier A, Brandt A, Conzelmann IB, Özel Y, Bergheim I. Non-alcoholic fatty liver disease in overweight children: role of fructose intake and dietary pattern. *Nutrients*. (2018) 10:1329. doi: 10.3390/nu10091329
35. Rich NE, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, et al. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2018) 16:198-210. doi: 10.1016/j.cgh.2017.09.041
36. Trico D, Caprio S, Umano GR, Galderisi A, Mata MM, Nouws J, et al. Ethnicity and common genetic variants modulate nonalcoholic fatty liver disease (NAFLD) metabolic phenotype in obese youth. *Diabetes*. (2018) 67(suppl.1). doi: 10.2337/db18-313-OR
37. Chan W-K, Bahar N, Razlan H, Vijayananthan A, Sithaneshwar P, Goh K-L. Non-alcoholic fatty liver disease in a young multiracial Asian population: a worrying ethnic predilection in Malay and Indian males. *Hepatol Int*. (2014) 8:121-7. doi: 10.1007/s12072-013-9510-8
38. Tan V, Lee Y, Venkataraman K, Khoo E, Tai E, Chong Y, et al. Ethnic differences in insulin sensitivity and beta-cell function among Asian men. *Nutr Diabetes*. (2015) 5:e173. doi: 10.1038/nutd.2015.24

39. Raygor V, Abbasi F, Lazzeroni LC, Kim S, Ingelsson E, Reaven GM, et al. Impact of race/ethnicity on insulin resistance and hypertriglyceridaemia. *Diabetes Vasc Dis Res*. (2019) 16:153–9. doi: 10.1177/1479164118813890
40. Ting YW, Wong SW, Anuar Zaini A, Mohamed R, Jalaludin MY. Metabolic syndrome is associated with advanced liver fibrosis among pediatric patients with non-alcoholic fatty liver disease. *Front Pediat*. (2019) 7:491. doi: 10.3389/fped.2019.00491
41. Nobili V, Mantovani A, Cianfarani S, Alisi A, Mosca A, Sartorelli MR, et al. Prevalence of prediabetes and diabetes in children and adolescents with biopsy-proven non-alcoholic fatty liver disease. *J Hepatol*. (2019) 71:802–10. doi: 10.1016/j.jhep.2019.06.023
42. Mohamed RZ, Jalaludin MY, Zaini AA. Predictors of non-alcoholic fatty liver disease (NAFLD) among children with obesity. *J Pediatric Endocrinol Metab*. (2020) 33:247–53. doi: 10.1515/jpem-2019-0403
43. Koo BK, Joo SK, Kim D, Bae JM, Park JH, Kim JH, et al. Additive effects of PNPLA3 and TM6SF2 on the histological severity of non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*. (2018) 33:1277–85. doi: 10.1111/jgh.14056
44. Viitasalo A, Pihlajamaki J, Lindi V, Atalay M, Kaminska D, Joro R, et al. Associations of I148M variant in PNPLA3 gene with plasma ALT levels during 2-year follow-up in normal weight and overweight children: the PANIC Study. *Pediatric Obesity*. (2015) 10:84–90. doi: 10.1111/ijpo.234
45. Mangge H, Baumgartner B, Zelzer S, Prüller F, Schnedl W, Reininghaus E, et al. Patatin-like phospholipase 3 (rs738409) gene polymorphism is associated with increased liver enzymes in obese adolescents and metabolic syndrome in all ages. *Aliment Pharmacol Ther*. (2015) 42:99–105. doi: 10.1111/apt.13232
46. Li J, Hua W, Ji C, Rui J, Zhao Y, Xie C, et al. Effect of the patatin-like phospholipase domain containing 3 gene (PNPLA3) I148M polymorphism on the risk and severity of nonalcoholic fatty liver disease and metabolic syndromes: A meta-analysis of paediatric and adolescent individuals. *Pediatric Obesity*. (2020) 15:e12615. doi: 10.1111/ijpo.12615
47. Stanislawski MA, Shaw J, Litkowski E, Lange EM, Perng W, Dabelea D, et al. Genetic risk for hepatic fat among an ethnically diverse cohort of youth: the exploring perinatal outcomes among children study. *J. Pediatr*. (2020) 220:146–53 e2. doi: 10.1016/j.jpeds.2020.01.031
48. Grandone A, Cozzolino D, Marzuillo P, Cirillo G, Di Sessa A, Ruggiero L, et al. TM6SF2 G lu167 L ys polymorphism is associated with low levels of LDL-cholesterol and increased liver injury in obese children. *Pediatric Obesity*. (2016) 11:115–9. doi: 10.1111/ijpo.12032
49. Fang Y-L, Chen H, Wang C-L, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model". *World J. Gastroenterol*. (2018) 24:2974. doi: 10.3748/wjg.v24.i27.2974
50. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism*. (2016) 65:1038–48. doi: 10.1016/j.metabol.2015.12.012
51. Goyal NP, Schwimmer JB. The progression and natural history of pediatric nonalcoholic fatty liver disease. *Clin Liver Dis*. (2016) 20:325–38. doi: 10.1016/j.cld.2015.10.003
52. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. (2018) 15:11. doi: 10.1038/nrgastro.2017.109
53. Sheldon RD, Kanosky KM, Wells KD, Miles L, Perfield JW, Xanthakos S, et al. Transcriptomic differences in intra-abdominal adipose tissue in extremely obese adolescents with different stages of NAFLD. *Physiol Genomics*. (2016) 48:897–911. doi: 10.1152/physiolgenomics.00020.2016
54. Brumbaugh DE, Friedman JE. Developmental origins of nonalcoholic fatty liver disease. *Pediatr Res*. (2014) 75:140–7. doi: 10.1038/pr.2013.193
55. Wesolowski SR, El Kasmi KC, Jonscher KR, Friedman JE. Developmental origins of NAFLD: a womb with a clue. *Nat Rev Gastroenterol Hepatol*. (2017) 14:81. doi: 10.1038/nrgastro.2016.160
56. Bugianesi E, Moscatiello S, Ciaravella M, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des*. (2010) 16:1941–51. doi: 10.2174/138161210791208875
57. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med*. (2014) 371:1131–41. doi: 10.1056/NEJMra1011035
58. Lonardo A, Mantovani A, Lugari S, Targher G. Nafld in some common endocrine diseases: prevalence, pathophysiology, and principles of diagnosis and management. *Int J Mol Sci*. (2019) 20:2841. doi: 10.3390/ijms20112841
59. Manne V, Handa P, Kowdley KV. Pathophysiology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Clin Liver Dis*. (2018) 22:23–37. doi: 10.1016/j.cld.2017.08.007
60. Knebel B, Fahlbusch P, Dille M, Wahlers N, Hartwig S, Jacob S, et al. Fatty liver due to increased de novo lipogenesis: alterations in the hepatic peroxisomal proteome. *Front Cell Dev Biol*. (2019) 7:248. doi: 10.3389/fcell.2019.00248
61. Dowla S, Aslibekyan S, Goss A, Fontaine K, Ashraf AP. Dyslipidemia is associated with pediatric nonalcoholic fatty liver disease. *J Clin Lipidol*. (2018) 12:981–7. doi: 10.1016/j.jacl.2018.03.089
62. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*. (2014) 510:84–91. doi: 10.1038/nature13478
63. Schwarz J-M, Noworolski SM, Erkin-Cakmak A, Korn NJ, Wen MJ, Tai VW, et al. Effects of dietary fructose restriction on liver fat, de novo lipogenesis, and insulin kinetics in children with obesity. *Gastroenterology*. (2017) 153:743–52. doi: 10.1053/j.gastro.2017.05.043
64. Kim KH, Lee M-S. Pathogenesis of nonalcoholic steatohepatitis and hormone-based therapeutic approaches. *Front Endocrinol*. (2018) 9:485. doi: 10.3389/fendo.2018.00485
65. Flisiak-Jackiewicz M, Lebensztejn DM. Update on pathogenesis, diagnostics and therapy of nonalcoholic fatty liver disease in children. *Clin Exp Hepatol*. (2019) 5:11. doi: 10.5114/ceh.2019.83152
66. Mohamed AA, Sabry S, Abdallah AM, Abd Elazeem NA, Refaey D, Algebaly HAF, et al. Circulating adipokines in children with nonalcoholic fatty liver disease: possible noninvasive diagnostic markers. *Ann Gastroenterol*. (2017) 30:457. doi: 10.20524/aog.2017.0148
67. Zhang H, Niu Y, Gu H, Lu S, Zhang W, Li X, et al. Low serum adiponectin is a predictor of progressing to nonalcoholic fatty liver disease. *J Clin Lab Analy*. (2019) 33:e22709. doi: 10.1002/jcla.22709
68. Clemente MG, Mandato C, Poeta M, Vajro P. Pediatric non-alcoholic fatty liver disease: recent solutions, unresolved issues, and future research directions. *World J Gastroenterol*. (2016) 22:8078. doi: 10.3748/wjg.v22.i36.8078
69. Lanthier N. Targeting Kupffer cells in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why and how? *World J Hepatol*. (2015) 7:2184. doi: 10.4254/wjh.v7.i19.2184
70. Marcher A-B, Bendixen SM, Terkelsen MK, Hohmann SS, Hansen MH, Larsen BD, et al. Transcriptional regulation of hepatic stellate cell activation in NASH. *Sci Rep*. (2019) 9:1–13. doi: 10.1038/s41598-019-39112-6
71. Mann JP, Raponi M, Nobili V. Clinical implications of understanding the association between oxidative stress and pediatric NAFLD. *Expert Rev Gastroenterol Hepatol*. (2017) 11:371–82. doi: 10.1080/17474124.2017.1291340
72. Boutari C, Perakakis N, Mantzoros CS. Association of adipokines with development and progression of nonalcoholic fatty liver disease. *Endocrinol Metab*. (2018) 33:33–43. doi: 10.3803/EnM.2018.33.1.33
73. Zhao L, Fang X, Marshall MR, Chung S. Regulation of obesity and metabolic complications by gamma and delta tocotrienols. *Molecules*. (2016) 21:344. doi: 10.3390/molecules21030344
74. Zhao L, Yagiz Y, Xu C, Lu J, Chung S, Marshall MR. Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammation. *Food Funct*. (2015) 6:2293–302. doi: 10.1039/C5FO00261C
75. Suzuki T, Gao J, Ishigaki Y, Kondo K, Sawada S, Izumi T, et al. ER stress protein CHOP mediates insulin resistance by modulating adipose tissue macrophage polarity. *Cell Reports*. (2017) 18:2045–57. doi: 10.1016/j.celrep.2017.01.076
76. Mansoor S, Yerian L, Kohli R, Xanthakos S, Angulo P, Ling S, et al. The evaluation of hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *Dig Dis Sci*. (2015) 60:1440–7. doi: 10.1007/s10620-014-3494-7
77. Shah J, Okubote T, Alkhouri N. Overview of updated practice guidelines for pediatric nonalcoholic fatty liver disease. *J Gastroenterol Hepatol*. (2018) 14:407.
78. Bush H, Golabi P, Younossi ZM. Pediatric non-alcoholic fatty liver disease. *Children*. (2017) 4:48. doi: 10.3390/children4060048

79. Brunt EM. Nonalcoholic fatty liver disease and the ongoing role of liver biopsy evaluation. *Hepatol Commun.* (2017) 1:370–8. doi: 10.1002/hep4.1055
80. Di Sessa A, Cirillo G, Guarino S, Marzuillo P, del Giudice EM. Pediatric non-alcoholic fatty liver disease: current perspectives on diagnosis and management. Pediatric Health Med Ther. (2019) 10:89. doi: 10.2147/PHMT.S188989
81. Ezaizi Y, Kabbany MN, Selvakumar PKC, Sarmini MT, Singh A, Lopez R, et al. Comparison between non-alcoholic fatty liver disease screening guidelines in children and adolescents. *JHEP Rep.* (2019) 1:259–64. doi: 10.1016/j.jhepr.2019.06.005
82. Akshintala D, Chugh R, Amer F, Cusi K. Nonalcoholic Fatty Liver Disease: The Overlooked Complication of Type 2 Diabetes. *Endotext*. MDText. com, Inc. (2019).
83. Jeznach-Steinhagen A, Ostrowska J, Czerwonogrodzka-Senczyna A, Boniecka I, Shahnazaryan U, Kuryłowicz A. Dietary and pharmacological treatment of nonalcoholic fatty liver disease. *Medicina*. (2019) 55:166. doi: 10.3390/medicina55050166
84. Stenzel AP, Carvalho R, Jesus P, Bull A, Pereira S, Saboya C, et al. Serum antioxidant associations with metabolic characteristics in metabolically healthy and unhealthy adolescents with severe obesity: an observational study. *Nutrients*. (2018) 10:150. doi: 10.3390/nu10020150
85. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J Gastroenterol.* (2018) 24:3361. doi: 10.3748/wjg.v24.i30.3361
86. Amanullah I, Khan YH, Anwar I, Gulzar A, Mallhi TH, Raja AA. Effect of vitamin E in non-alcoholic fatty liver disease: a systematic review and meta-analysis of randomised controlled trials. *Postgrad Med J.* (2019) 95:601–11. doi: 10.1136/postgradmedj-2018-136364
87. Nobili V, Manco M, Devito R, Ciampalini P, Piemonte F, Marcellini M. Effect of vitamin E on aminotransferase levels and insulin resistance in children with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. (2006) 24:1553–61. doi: 10.1111/j.1365-2036.2006.03161.x
88. Nobili V, Alisi A, Mosca A, Crudele A, Zaffina S, Denaro M, et al. The antioxidant effects of hydroxytyrosol and vitamin E on pediatric nonalcoholic fatty liver disease, in a clinical trial: a new treatment? *Antioxid Redox Signal.* (2019) 31:127–33. doi: 10.1089/ars.2018.7704
89. Mosca A, Crudele A, Smeriglio A, Braghini MR, Panera N, Comparcola D, et al. Antioxidant activity of Hydroxytyrosol and Vitamin E reduces systemic inflammation in children with paediatric NAFLD. *Digestive and Liver Disease*. (in press). doi: 10.1016/j.dld.2020.09.021
90. Zöhrer E, Alisi A, Jahnel Jr, Moscla A, Della Corte C, Crudele A, et al. Efficacy of docosahexaenoic acid-choline-vitamin E (DHA-CHO-VE) in paediatric NASH: a randomized controlled clinical trial. *Appl Physiol Nutr Metab*. (2017) 42:948–54. doi: 10.1139/apnm-2016-0689
91. Polyzos SA, Kountouras J, Mantzoros CS, Polymerou V, Katsinelos P. Effects of combined low-dose spironolactone plus vitamin E vs vitamin E monotherapy on insulin resistance, non-invasive indices of steatosis and fibrosis, and adipokine levels in non-alcoholic fatty liver disease: a randomized controlled trial. *Diabetes, Obesity and Metabolism.* (2017) 19:1805–9. doi: 10.1111/dom.12989
92. Ahsan H, Ahad A, Siddiqui WA. A review of characterization of tocotrienols from plant oils and foods. *J Chem Biol.* (2015) 8:45–59. doi: 10.1007/s12154-014-0127-8
93. Abdullah A, Atia A. The role of tocotrienols in liver health and disease. *Curr Top Pharmacol. (2018)* 22:135–42
94. Ahsan H, Ahad A, Iqbal J, Siddiqui WA. Pharmacological potential of tocotrienols: a review. *Nutr Metab.* (2014) 11:52. doi: 10.1186/1743-7075-11-52
95. Shahidi F, De Camargo AC. Tocopherols and tocotrienols in common and emerging dietary sources: Occurrence, applications, and health benefits. *Int J Mol Sci.* (2016) 17:1745. doi: 10.3390/ijms17101745
96. Ali SF, Nguyen JC, Jenkins TA, Woodman OL. Tocotrienol-rich tocomin attenuates oxidative stress and improves endothelium-dependent relaxation in aortae from rats fed a high-fat western diet. *Front Cardiovasc Med.* (2016) 3:39. doi: 10.3389/fcvm.2016.00039
97. Qureshi AA, Khan DA, Silswal N, Saleem S, Qureshi N. Evaluation of pharmacokinetics, and bioavailability of higher doses of tocotrienols in healthy fed humans. *Exp Clin Cardiol.* (2016) 7. doi: 10.4172/2155-9880.1000434
98. Ramanathan N, Tan E, Loh LJ, Soh BS, Yap WN. Tocotrienol is a cardioprotective agent against ageing-associated cardiovascular disease and its associated morbidities. *Nutr Metab.* (2018) 15:6. doi: 10.1186/s12986-018-0244-4
99. Torabi S, Yeganehjoo H, Shen C-L, Mo H. Peroxisome proliferator–activated receptor γ down-regulation mediates the inhibitory effect of d-δ-tocotrienol on the differentiation of murine 3T3-F442A preadipocytes. *Nutr Res.* (2016) 36:1345–52. doi: 10.1016/j.nutres.2016.11.001
100. Grimm MO, Regner L, Mett J, Stahlmann CP, Schorr P, Nelke C, et al. Tocotrienol affects oxidative stress, cholesterol homeostasis and the amyloidogenic pathway in neuroblastoma cells: consequences for Alzheimer's disease. *Int J Mol Sci.* (2016) 17:1809. doi: 10.3390/ijms17111809
101. Zaiden N, WN Y, Ong S, VH T, Nesaretnam K, Shiba S. Gamma delta tocotrienols reduce hepatic triglyceride synthesis and VLDL secretion. *J Atherosclerosis Thrombosis.* (2010) 1008090267. doi: 10.5551/jat.4911
102. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol.* (2019) 20:242–58. doi: 10.1038/s41580-018-0093-z
103. Burdeos GC, Nakagawa K, Abe T, Kimura F, Miyazawa T. Tocotrienol modulates crucial lipid metabolism-related genes in differentiated 3T3-L1 preadipocytes. *Food & Function*. (2014) 5:2221–7. doi: 10.1039/C4FO00463A
104. Wu S-J, Huang G-Y, Ng L-T. γ-Tocotrienol induced cell cycle arrest and apoptosis via activating the Bax-mediated mitochondrial and AMPK signaling pathways in 3T3-L1 adipocytes. *Food Chem Toxicol.* (2013) 59:501–13. doi: 10.1016/j.fct.2013.06.011
105. Tang KD, Liu J, Russell PJ, Clements JA, Ling M-T. Gamma-Tocotrienol Induces Apoptosis in Prostate Cancer Cells by Targeting the Ang-1/Tie-2 Signalling Pathway. *Int J Mol Sci*. (2019) 20:1164. doi: 10.3390/ijms20051164
106. Kim Y, Wang W, Okla M, Kang I, Moreau R, Chung S. Suppression of NLRP3 inflammasome by γ-tocotrienol ameliorates type 2 diabetes. *J Lipid Res*. (2016) 57:66–76. doi: 10.1194/jlr.M062828
107. Zhao L, Kang I, Fang X, Wang W, Lee M, Hollins RR, et al. Gamma-tocotrienol attenuates high-fat diet-induced obesity and insulin resistance by inhibiting adipose inflammation and M1 macrophage recruitment. *Int J Obesity.* (2015) 39:438–46. doi: 10.1038/ijo.2014.124
108. Shen J, Yang T, Xu Y, Luo Y, Zhong X, Shi L, et al. δ-Tocotrienol, isolated from rice bran, exerts an anti-inflammatory effect via MAPKs and PPARs signaling pathways in lipopolysaccharide-stimulated macrophages. *Int J Mol Sci.* (2018) 19(10):3022. doi: 10.3390/ijms19103022
109. Ceccarelli S, Panera N, Mina M, Gnani D, De Stefanis C, Crudele A, et al. LPS-induced TNF-α factor mediates pro-inflammatory and pro-fibrogenic pattern in non-alcoholic fatty liver disease. *Oncotarget.* (2015) 6:41434. doi: 10.18632/oncotarget.5163
110. Maes M, Leonard B, Fernandez A, Kubera M, Nowak G, Veerhuis R, et al. (Neuro)inflammation and neuroprogression as new pathways and drug targets in depression: from antioxidants to kinase inhibitors. *Prog Neuropsychopharmacol Biol Psychiatry*. (2011) 35:659–63. doi: 10.1016/j.pnpbp.2011.02.019
111. Yang C, Jiang Q. Vitamin E δ-tocotrienol inhibits TNF-α-stimulated NF-κB activation by up-regulation of anti-inflammatory A20 via modulation of sphingolipid including elevation of intracellular dihydroceramides. *J Nutr Biochem.* (2019) 64:101–9. doi: 10.1016/j.jnutbio.2018.10.013
112. Pang K-L, Chin K-Y. The role of tocotrienol in protecting against metabolic diseases. *Molecules.* (2019) 24:923. doi: 10.3390/molecules24050923
113. Sun W-G, Song R-P, Wang Y, Zhang Y-H, Wang H-X, Ge S, et al. γ-Tocotrienol-Inhibited Cell Proliferation of Human Gastric Cancer by Regulation of Nuclear Factor-κB Activity. *J Agric Food Chem*. (2018) 67:441–51. doi: 10.1021/acs.jafc.8b05832
114. Mesri Alamdari N, Irandoost P, Roshanravan N, Vafa M, Asghari Jafarabadi M, Alipour S, et al. Royal Jelly and Tocotrienol Rich Fraction in obesity treatment of calorie-restricted obese rats: a focus on white fat browning properties and thermogenic capacity. *Nutr Metab*. (2020) 17:42. doi: 10.1186/s12986-020-00458-8
115. Shibata A, Kawakami Y, Kimura T, Miyazawa T, Nakagawa K. α-tocopherol attenuates the triglyceride-and cholesterol-lowering effects of rice bran tocotrienol in rats fed a western diet. *J Agric Food Chem.* (2016) 64:5361–6. doi: 10.1021/acs.jafc.6b02228

116. Shibata A, Kobayashi T, Asai A, Eitsuka T, Oikawa S, Miyazawa T, et al. High purity tocotrienols attenuate atherosclerotic lesion formation in apoE-KO mice. *J Nutr Biochem*. (2017) 48:44–50. doi: 10.1016/j.jnutbio.2017.06.009
117. Allen L, Ramalingam L, Menikdiwela K, Scoggin S, Shen C-L, Tomison MD, et al. Effects of delta-tocotrienol on obesity-related adipocyte hypertrophy, inflammation and hepatic steatosis in high-fat-fed mice. *J Nutr Biochem*. (2017) 48:128–37. doi: 10.1016/j.jnutbio.2017.07.003
118. Shen C-L, Kaur G, Wanders D, Sharma S, Tomison MD, Ramalingam L, et al. Annatto-extracted tocotrienols improve glucose homeostasis and bone properties in high-fat diet-induced type 2 diabetic mice by decreasing the inflammatory response. *Sci Rep*. (2018) 8:1–10. doi: 10.1038/s41598-018-29063-9
119. Kim Y, Natarajan SK, Chung S. Gamma-tocotrienol attenuates the hepatic inflammation and fibrosis by suppressing endoplasmic reticulum stress in mice. *Mol Nutr Food Res*. (2018) 62:1800519. doi: 10.1002/mnfr.201800519
120. Muid S, Hamid Z, Nawawi H. Tocotrienol rich fraction supplement reduces oxidative stress in non familial hypercholesterolaemia: beyond the lipid lowering capability. *Int Food Res J*. (2018) 25.
121. Pervez MA, Khan DA, Slehria AUR, Ijaz A. Delta-tocotrienol supplementation improves biochemical markers of hepatocellular injury and steatosis in patients with nonalcoholic fatty liver disease: a randomized, placebo-controlled trial. *Complementary Therapies in Medicine*. (2020) 52:102494. doi: 10.1016/j.ctim.2020.102494
122. Gao H, Cao Y, Xia H, Zhu X, Jin Y. CYP4A11 is involved in the development of nonalcoholic fatty liver disease via ROS-induced lipid peroxidation and inflammation. *Int J Mol Med*. (2020) 45:1121–9. doi: 10.3892/ijmm.2020.4479
123. Tan GCJ, Tan SMQ, Phang SCW, Ng YT, Ng EY, Ahmad B, et al. Tocotrienol-rich vitamin E improves diabetic nephropathy and persists 6–9 months after washout: a phase IIa randomized controlled trial. *Ther Adv Endocrinol Metab*. (2019) 10:2042018819895462. doi: 10.1177/2042018819895462
124. Alkhouri N, Kohli R, Feldstein AE. Designing Clinical Trials in Pediatric Nonalcoholic Steatohepatitis: Tips for Patient Selection and Appropriate Endpoints. *Hepatol Commun*. (2019) 3:1563–70. doi: 10.1002/hep4.1449
125. Wong SK, Kamisah Y, Mohamed N, Muhammad N, Masbah N, Fahami NAM, et al. Potential Role of Tocotrienols on Non-Communicable Diseases: A Review of Current Evidence. *Nutrients*. (2020) 12:259. doi: 10.3390/nu12010259

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