Follow-up Evaluation of Association between Weight Changes, Metabolic, and Hormonal Outcomes in Children – a Single-center Pilot Study

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Abstract

Introduction: Fasting blood glucose, insulin level, and lipid profile are the minimal tests according to the Romanian guidelines to evaluate obese children. Cross-sectional studies for pediatric obesity in Romania focused only on epidemiology and management.

Aim: Our study aimed to evaluate the metabolic and hormonal changes in association with follow-up bodyweight modifications.

Materials and methods: Medical charts of overweight or obese children presenting at the First Paediatric Hospital, Cluj-Napoca from January 2017 to March 2019 were retrospectively evaluated. Anthropometric measures [e.g., body mass index (BMI), and waist circumference] and blood tests such as inflammatory markers (e.g., white blood cell and neutrophil absolute/relative counts, C-reactive protein), metabolic parameters (e.g., liver enzymes, uric acid, fasting blood glucose, triglycerides, high-density lipoprotein-cholesterol), fasting blood insulin, and cortisol levels were evaluated.

Results: Twenty-two overweight or obese children (17 girls, median age of 13 years) monitored on median for 7.5 months were included in the study. Blood glucose level significantly decreased \((p=0.010)\) and fasting insulin levels increased \((p=0.051)\) at follow-up evaluation, independently of BMI-for-age z-score. Fasting insulin levels were associated with waist circumference (Spearman’s rank correlation coefficient) \(\rho=0.58, p=0.030\). BMI-for-age z-score proved to be associated with the C-reactive protein level at baseline (\(p=0.70, p=0.036, n=9\)) and high-density lipoprotein cholesterol at follow-up (\(p=0.52, p=0.033, n=17\)).

Conclusions: Present analysis found changes in fasting insulin levels in relation to the abdominal circumference and high-density lipoprotein cholesterol and C-reactive protein levels in relation to BMI-for-age z-score in obese children.

Keywords

follow-up study, pediatric obesity, practice guideline
INTRODUCTION

World Health Organization (WHO) Observatory data for 2016 estimate worldwide obesity or overweight prevalence of 18% in children aged 5 to 19 years. The prevalence of overweight and obesity in both adults and children has reached a plateaued level since 2000 in most high-income countries but has accelerated in middle and low-income countries. Three main growth chart types are used worldwide to express child feeding status. WHO criteria for overweight and obesity in children are based on age and gender-specific BMI (Body Mass Index) z-scores: a z-score ≥ 2 for underweight and z-score ≥ 3 for obesity up to the age of 5, and a z-score ≥ 1 and ≥ 2, respectively, for older children. The International Obesity Task Force (IOTF) cut-offs are based on the lambda-mu-sigma (LMS) method that links child centile curves to BMI values at 18 years. The Centers for Disease Control and Prevention (CDC) growth charts are based on American pediatric population data and are used for children aged 2 and older. In European cohorts, severe obesity is defined as a BMI-for-age z-score ≥ 3 in accordance with the WHO growth references or a cut-off point corresponding to a BMI ≥ iso-BMI 35 (BMI cut-off for obesity + 5 BMI units) according to IOTF references.

Longitudinal analyses showed that severe (or extreme) obesity in childhood increases the cardiometabolic risks in adulthood, mainly via inflammation and oxidative stress. Based on European Childhood Obesity Surveillance Initiative (COSI) and using the WHO and IOTF criteria, one out of European childhood obesity surveillance initiatives exists, including methods to screen for overweight and obesity (including obese) children was 28.3%/23%/23.2% (WHO/IOTF/CDC) and that the prevalence was stable across the years.

In Europe, population screening for childhood obesity is not recommended, and the data of effective monitoring and screening for obesity is scarce. On the other hand, the Expert Committee highlighted in 2007 the need for screening by regular medical visits in every American child starting at birth. The United States of America Preventive Services Task Force statement from 2017 concluded that screening is indicated in children aged 6 years or older and BMI-for-age and gender is the feasible evaluation tool (grade B recommendation).

No consensus on targeted prevention in pediatric obesity management exists, including methods to screen for comorbidities, risk factors, and behaviour. Management of children with weight problems includes evaluation of family and personal history with an emphasis on health risk factors and physical examination. The American Medical Association Expert Committee recommends laboratory testing according to the BMI percentiles and risk factors, namely fasting lipid profile and measurement of transaminase levels, fasting blood glucose for overweight – BMI between the 85th and 94th percentiles – or when risk factors are present, respectively additional testing of blood urea nitrogen and creatinine levels in case of obesity (BMI ≥ 95th percentile). The latest International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus guidelines recommend screening for type 2 diabetes in every child with a BMI-for-age ≥ 85th percentile who presents risk factors after the puberty onset (or after the age of 10) (A-level of recommendation according to SEARCH and TODAY studies).

Barlow and Expert Committee recommend the laboratory testing for children above the 85th percentile, mainly to detect abnormal cholesterol levels, NAFLD (non-alcoholic fatty liver disease), and type 2 diabetes mellitus. The main laboratory testing includes fasting glucose, fasting lipid profile, and liver enzymes (alanine transaminase (ALT) and aspartate aminotransferase (AST)). Liver ultrasound, uric acid, and fasting serum insulin may be useful when the medical doctor considers the possibility of obesity-related comorbidities. Abnormal values should be repeated every two years for underweight children under the age of 10 if risk factors are present and in all obese children. Baseline evaluation of vitamin D and fasting insulin dosage to determine insulin resistance may be considered as they can be related to adipose tissue excess.

Gungor et al. identified eight main groups of obesity-related comorbidities (endocrine, cardiovascular, gastrointestinal, pulmonary, orthopedic, neurologic, dermatologic, and psychosocial comorbidities). The Children’s Hospital Association Statement named the comorbidities that should be managed in overweight and obese children: impaired fasting glucose and/or impaired glucose tolerance, non-alcoholic fatty liver disease, or steatohepatitis and dyslipidemia. Also, underlying genetic causes should be considered in a child under the age of five years with severe obesity and endocrinopathies should be taken into consideration when a decreased linear growth is observed. Starting from puberty, urine microalbumin/creatinine ratio should be considered to detect impaired renal function (due to hypertension or diabetes).

The Romanian Ministry of Health Guideline for Pediatric Obesity stated a minimal evaluation including arterial pressure measurement, fasting blood glycemia and lipid profile (total cholesterol, HDL-cholesterol and triglycerides) and optional investigations based on clinical indications (oral glucose tolerance test (OGTT), fasting blood insulin, cortisol, androgens, liver enzymes, and abdominal ultrasound, echocardiography and psychological exam). The international...
nal and national recommendations for laboratory analysis in pediatric obesity are summarized in Table 1.

The targeted prevention in obese children has the aim of weight stabilization and comorbidities management. The intervention process comprises 4-5 stages, starting with brief counseling (prevention plus stage), followed by stages that require more time and resources: structured weight management, comprehensive multidisciplinary intervention, and tertiary care intervention.17

The majority of preventive interventions are based on behaviour-based interventions rather than environment/community-based interventions and proved to be inadequate and insufficient.26 Behavioral weight management programs have been used successfully within the pediatric population.27 Family-based weight management programs are considered the most successful.28 Lifestyle-based weight-loss interventions of a minimum of 26 hours are likely to help reduce excess weight in children and adolescents.29 The school-based programs were identified as the most effective intervention programs among children aged 6-12 years from high-income countries, especially if associated with a home-based intervention.30 American Medical Association statements outline the importance of at least 60-minute moderate to vigorous daily physical activity as an A-level of evidence recommendation, along with a decrease of sedentary time and sweeteners consumption.20 A randomized-control trial based on a parent-support program for pediatric obesity is in progress since July 2019 in Romania, Spain, and Sweden.31 Motivational interviewing has been shown to be effective in pediatric obesity management.32

Table 1. Laboratory tests for screening for main obesity-related comorbidities

| Blood marker                      | Comorbidity                                      | International guidelines17,20,33 | National guidelines25 |
|-----------------------------------|--------------------------------------------------|---------------------------------|-----------------------|
| ALT and AST (transaminases)       | Non-alcoholic fatty liver disease, steatohepatitis| strong recommendation*         | optional**            |
| Fasting blood glucose (±HbA1c, OGTT, if indicated) | Impaired glucose metabolism to type 2 diabetes   | strong recommendation*         | minimal***            |
| Fasting insulin                   | Insulin resistance                               | strong recommendation*         | optional              |
| Triglycerides                     | Dyslipidemia                                     | strong recommendation*         | minimal               |
| Cholesterol (HDL, LDL, total)     | Cardiovascular risk                              | strong recommendation*         | minimal               |
| Uric acid                         | Subclinical inflammation                         | no suggestion                  | no suggestion         |
| CRP/fibrinogen/hs-CRP             | Subclinical inflammation                         | no suggestion                  | no suggestion         |
| Vitamin D                         | Vitamin D deficiency                             | no suggestion                  | no suggestion         |
| Free and total testosterone and SHBG | PCOS                                             | strong recommendation*         | optional              |

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; HbA1c: glycated hemoglobin; OGTT: oral glucose tolerance test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; (hs-)CRP: (high-sensitivity) C-reactive protein; SHBG: sex hormone-binding globulin, PCOS: polycystic ovary syndrome.

*according to GRADE (Grading of Recommendations, Assessment, Development and Evaluation group); ** to be performed according to medical advice; ***to be evaluated in every patient with a bodyweight problem.

Previous cross-sectional studies have shown that metabolic and hormonal changes accompany weight excess, but no longitudinal study has evaluated this relationship as provided by Romanian screening for obesity-related comorbidities guidelines. In this context, we aimed to evaluate the pattern of evolution of clinical and biochemical markers in obese and overweight children, as reflected in the screening for obesity-related comorbidities protocol.

MATERIALS AND METHODS

A longitudinal study with retrospective data collection was conducted between January 2017 and March 2019. Medical charts of children aged from 2 to 17 years presenting with obesity or overweight to the Clinical Emergency Paediatric Hospital Cluj-Napoca, First Paediatric Clinic, Romania were included. Patients evaluated at least twice and a minimum of 4-month follow-up without any comorbidity at the baseline evaluation were included. The median of medical follow-up time duration from the date of diagnosis was one year. C-reactive protein level ≥ 10 mg/dl indicates an infectious or inflammatory disease, and this was an exclusion criterion for our study.34

Trained professionals performed the anthropometric measurements. The height was measured by stadiometer (0.1 cm accuracy) and weight by mechanical beam scale (0.1 kg accuracy) after collection of the blood samples, before lunch.

We evaluated the change in body mass index (WHO z-score, percentile), blood pressure (systolic and diastolic
blood pressure) and change in inflammatory markers (white blood cell and neutrophil absolute counts, C-reactive protein), metabolic outcomes (aspartate aminotransferase, AST, alanine aminotransferase, ALT, uric acid, fasting blood glucose, and lipids panel (triglycerides, high-density lipoprotein (HDL)-cholesterol), fasting blood insulin and cortisol levels. The management plan consisted of a 20-minute evaluation of the eating behaviour and oral or written recommendations given by the clinician.

Blood parameters measurements were performed according to standard guidelines for each technique. The differentiated blood cell count was performed via cytometry, impedance, and colorimetry on Mindray 6800 hematology analyzer (Mindray, Shenzhen, China). Biochemical parameters were measured via spectrophotometry on KONELAB 60i P2 (Thermo, Vantaa, Finland), and hormonal measurements were performed on chemiluminescence on Mindray CL-1200i analyzer (Mindray, Shenzhen, China). For each patient, the measurements were interpreted as elevated, normal or decreased according to the American Academy of Pediatrics (AAP) Pediatric Hypertension Guidelines from 2017.

The WHO criteria were used for defining underweight, obesity, and severe obesity. We performed measurements according to WHO criteria, as it is most accurate compared to the IOTF (has the limits of a maximum percentile) and CDC (is based on American population ranges).

AnthroPlus application v1.0.4 was used to compute the z-scores and percentiles for BMI-for-age, height-for-age, and weight-for-age. According to 2006 WHO report, the definition of the z-score is "the deviation of an individual's value from the median value of a reference population, divided by the standard deviation of the reference population (or transformed to normal distribution)".36

A BMI-for-age z-score ≥ 1 is interpreted as overweight, ≥ 2 as obesity, and a BMI-for-age z-score ≥ 3 as severe obesity. The z-score associated percentiles are not available for values higher than 3 (equivalent to 99.865th percentile) as they are invariant to changes over this value. Arterial blood pressure values were interpreted as normal or elevated at baseline and follow-up according to cut-offs reported in the on-line calculator37 adjusted to meet the 2017 AAP Pediatric Hypertension Guidelines.38

Ethical approval was obtained from the "Iuliu Hațieganu" University of Medicine and Pharmacy Ethics Committee (approval no. 179/30.05.2019) and from the ethics committee of the Clinical Emergency Paediatric Hospital Cluj-Napoca (approval No. 71/20.06.2019).

Statistical analysis was done using the Statistica program (v.13.5, StatSoft, OK, USA) at a significance level of 5%. Data were reported as number whenever quantitative, respectively as the median and interquartile range (IQR defined as the first quartile to the third quartile) and [minimum to maximum] whenever quantitative. The association between studied parameters was tested with Spearman’s rank correlation coefficient (ρ). The association of obese/overweight at baseline and follow-up was evaluated with the McNemar test. Wilcoxon test was used to compare baseline with follow-up values of studied parameters, and the Mann-Whitney test was used to compare boys with girls.

RESULTS

Twenty-two children with a median age of 13 years (range from 2 to 17 years, IQR = 7.25 to 15) years were evaluated. The evaluated sample comprised 17 girls. The follow-up evaluation ranged from 4 to 21 months, with a median of 7.5 months (IQR = 6.25 to 9.75 months). The main characteristics of the evaluated children and the follow-up vs. baseline differences are presented in Table 2.

Uric acid had significantly higher values for boys as compared to girls both at baseline [median (IQR) 7.1 mg/dl (6.1 to 7.3) for boys and 5.3 mg/dl (4.9 to 6.2) for girls; Mann-Whitney test Z = 2.27, p = 0.023] and follow-up [median (IQR) 7.9 mg/dl (7.1 to 8.5) for boys and 5.3 mg/dl (4.8 to 6.5) for girls; Mann-Whitney test Z = 2.37, p = 0.018]. No other significant differences were observed between boys and girls (P-values>0.06).

Normal arterial blood pressure was observed in only 9 children at baseline (9/22), and five children had elevated values corresponding to stage 1 and two to stage 2. Baseline absolute neutrophil count was elevated in only one child at baseline (1/18). One child had elevated values of AST (1/20) and two children of ALT (2/22). Triglycerides had elevated levels in two children at baseline (2/20) and HDL-cholesterol was within normal ranges in only one child at baseline (1/11) with all other cases as borderline (10/11). At baseline, the uric acid was elevated in only one child (1/21), C-reactive protein was high in two children (2/9), hyperinsulinemia in one (1/11). Blood cortisol levels at baseline were within normal ranges to all children.

At follow-up, normal arterial pressure was observed in 13 children (13/19), elevated value corresponding to stage 1 in 4/19, and to stage 2 in 2/19; leucocyte count (18/18), absolute neutrophils count (17/17), AST (20/20), blood glucose (22/22), and blood cortisol levels (18/18) were within normal ranges. One child was with elevated ALT values (1/21), two children with elevated triglycerides (2/22), two with elevated uric acid values (2/21), three with elevated PCR (3/10), five with hyperinsulinemia (5/18). HDL-cholesterol values at follow-up were in the majority of cases at borderline (13/17) with three cases as lower values (3/17) and only one case with normal value (1/17).

Eighteen children were with obesity (≥ 2 WHO BMI-for-age z-score, ten of them with severe obesity) and four were overweight (≥1 WHO BMI-for-age z-score) at the baseline evaluation. The follow-up status was changed for two girls, one obese girl becoming overweight (16 years) and another obese one becoming severely obese (2 years).

In one patient, the BMI-for-age z-score at follow-up remained unchanged. A decreasing trend of the BMI-for-age z-score was observed without reaching statistical significance (McNemar test: p>0.999). Twelve children had a...
smaller follow-up BMI-for-age z-score as compared with the baseline value (Fig. 1a). The highest increase in the follow-up BMI-for-age z-score was observed in the youngest children, in two girls, the one 2 years old and the other 4 years old (Fig. 1b).

The changes in the BMI-for-age z-scores proved not associated with follow-up duration \((p=0.23, p=0.294)\). The highest difference of BMI-for-age z-score was found in patients with a follow-up duration of 8 months (Fig. 2).

The BMI-for-age z-score difference correlated negatively with baseline NEU \((p=-0.56, p=0.016, n=18)\) and positively with baseline C-reactive protein \((p=0.78, p=0.013, n=9)\), and follow-up insulinemia \((p=0.57, p=0.017, n=17)\). The baseline BMI-for-age z-score correlated positively with baseline C-reactive protein \((p=0.70, p=0.036, n=9)\) and the follow-up BMI-for-age z-score correlated negatively with the follow-up HDL-cholesterol \((p=-0.52, p=0.033, n=17)\). Abdominal circumference positively correlated with insulinemia at follow-up \((p=0.59, p=0.032, n=13)\).
DISCUSSION

The evaluation of changes in anthropometric measurements, and metabolic and hormonal on pediatric patients with body-weight problems showed modifications of fasting blood insulin and glucose profiles. The significant change of fasting insulin level led to a decrease in glycemia that remained within normal ranges in all patients. Almost 30% of the patients developed euglycemic hyperinsulinemia at the follow-up evaluation (Table 2). Insulin directly influences the lipid accumulation via lipolysis inhibition, and studies on animal models showed that high insulin level precede weight gain and is an independent predictor of type 2 diabetes.39,40 Thus, fasting hyperinsulinemia may precede the changes in weight and could be an early indicator of and carbohydrate metabolism dysregulation in obese children.41 For metabolic dysfunction to occur in obesity hyperinsulinemia must be followed by insulin resistance. The role and mechanisms of insulin resistance syndrome in obese children have been extensively studied.42,43 Adipose tissue excess is characterized by subclinical inflammation, which leads to a decrease in insulin sensitivity. The pro-inflammatory macrophages M1 produce chemokines (e.g., interleukin-1β and α-tumor necrosis factor) that contribute to insulin resistance in adipocytes and other cells.44 We assume that glucose level is largely influenced by insulin level in our analysis, but other factors such as physical exercise load, calorie intake and stress may also cause blood glucose variation.45

We also observed that fasting insulin level changed independently of BMI-for-age z-score variation, but it was significantly correlated with waist circumference (p=0.58, p=0.0304). Pancreatic β-cell dysfunction may be triggered by visceral adiposity (VAT) excess.46 Waist circumference (WC) is a good indicator of VAT which is associated with cardiometabolic risk in adults and children.47 Moreover, WC was also shown to be a predictor of insulin resistance in obese children.48 The high variability of BMI-for-age z-score, as shown in Fig. 2, may be due to the heterogeneity of the group in terms of age, the follow-up duration, and the small sample size. When the group was divided according to the pattern of bodyweight changes, we found the highest variability in the youngest children (Fig. 1).

Physiological insulin resistance develops at puberty49, and blood insulin levels cut-offs should be adapted to pubertal stages.50 Data regarding pubertal staging in our patients was not available and our findings may interfere with pubertal development.

The national recommendations suggest that blood insulin measurement should be done in obese children. International guidelines recommend against blood insulin testing for several reasons: the evidence that it is not a reliable indicator of insulin sensitivity, the lack of cut-off values in pediatric obese patients, and the lack of clinical value for comorbidities onset (type 2 diabetes mellitus).33 As far as the lipid profile is concerned, HDL-cholesterol was the sole lipid profile parameter that was associated with bodyweight at the follow-up evaluation in our sample. A possible explanation for the interaction between HDL-cholesterol and BMI-for-age z-score is that HDL-cholesterol decrease is part of the metabolic syndrome cluster in obese children. Low levels of HDL-cholesterol are known to increase cardiovascular risk because it limits the reverse transport of cholesterol to the liver.51

Other studies also reported associations between changes in metabolic parameters such as triglycerides and high-density lipoproteins and arterial pressure values.33,52 Obesity is characterized by a low-grade inflammatory status, which leads to obesity-related comorbidities.53 C-reactive protein level was positively correlated with body...
weight at the baseline evaluation, and this result may indicate its role in obesity-driven inflammation. Apart from classic markers, a-tumour necrosis factor, interleukin-6, and C-reactive protein, novel proteins are studied for indicating the inflammatory status in obese patients.54,55

A strength of our study is that it is the first analysis of the metabolic and clinical changes as present in obesity-related comorbidities guideline in a Romanian center. In the Romanian pediatric population, cross-sectional epidemiological studies, as part of international projects such as JANPA (Joint Action on Nutrition and Physical Activity)56 and COSI 201778 focused on prevalence and risk factors in obese children have been conducted. Longitudinal studies depicted trends in eating behaviours and socioeconomic factors that influence the obesity prevalence57 and interventions for weight management in adolescents.31

The major limitations of our study are the small sample size, the retrospective data collection, the patients’ age heterogeneity, and the relatively short follow-up duration. The retrospective data collection also contributes to the small sample because not all evaluated data were available in the medical charts. Furthermore, lack of information regarding the physical exercise load, calorie intake, and eating behavior could interfere with the observed results. Regarding the age heterogeneity, information regarding pubertal staging was not available, and our findings may also interfere with pubertal development. As a consequence, the results reflect strictly the evaluated sample and should not be generalized. A prospective design is needed on a larger sample size, and with a longer follow-up duration, considering the possible implications in pre-pubertal and pubertal children as well as their particularities, inclusive possible genetic implications would be necessary to evaluate obesity in children properly.

CONCLUSIONS

The present longitudinal study showed that hyperinsulinaemia with euglycemia occurs independently of BMI-for-age z-score, but abdominal circumference may indicate the early metabolic changes in obese children. Our analysis, based on the national guideline for the management of obesity-related comorbidities in children, also identifies interactions of HDL-cholesterol and C-reactive protein levels with BMI-for-age z-score. Future longitudinal studies, including Romanian multicenter studies, are needed to identify important early subclinical changes in obese children.

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Последующая оценка связи между изменением веса, метаболическими и гормональными исходами у детей – одноцентровое пилотное исследование

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Резюме

Введение: Уровень глюкозы в крови натощак, уровень инсулина и липидный профиль являются минимальными тестами в соответствии с румынскими рекомендациями по оценке детского ожирения. Секционные исследования детского ожирения в Румынии сосредоточены исключительно на эпидемиологии и борьбе с ним.

Цель: Наше исследование было направлено на оценку метаболических и гормональных изменений, связанных с контролируемыми изменениями массы тела.

Материалы и методы: Были ретроспективно оценены медицинские карты детей с ожирением или избыточным весом, которые посетили Первую педиатрическую больницу в Клуж-Напока с января 2017 года по март 2019 года. Были оценены антропометрические показатели (например, индекс массы тела (ИМТ) и окружность талии) и анализы крови, такие как маркеры воспаления (например, абсолютное / относительное количество лейкоцитов и нейтрофилов, С-реактивный белок), параметры метаболизма (например, ферменты печени, мочевая кислота, глюкоза крови натощак, триглицериды, липопротеин-белок высокой плотности), уровень инсулина и кортизола в крови натощак.

Результаты: В исследование были включены 22 ребёнка с ожирением или избыточной массой тела (17 девочек, средний возраст 13 лет), наблюдавшиеся в среднем в течение 7,5 месяцев. Уровни глюкозы в крови значительно снизились (p=0.010), а уровень инсулина натощак увеличился (p=0.051) в контрольной оценке, независимо от стандартного показателя ИМТ для возраста. Уровни инсулина натощак были связаны с окружностью талии (коэффициент ранговой корреляции Спирмена (p=0.58, p=0.030). Было обнаружено, что стандартный показатель ИМТ для возраста связан с исходными уровнями С-реактивного белка (p=0.70, p=0.036, n=9) и холестерина ЛПВП в контроле (p=0.52, p=0.033, n=17).

Заключение: Настоящий анализ выявил изменения в уровнях инсулина натощак в зависимости от окружности живота и уровней холестерина ЛПВП и С-реактивного белка по отношению к стандартному показателю ИМТ для возраста у детей с ожирением.

Ключевые слова
последующее исследование, детское ожирение, практические рекомендации