Association between obesity phenotypes in adolescents and adult metabolic syndrome: Tehran Lipid and Glucose Study

Golaleh Asghari (PhD)[a], Farhad Hosseinpanah (MD)[b*], Sara Serahati (MSc)[c], Shadi Haghi (MD)[b], Fereidoun Azizi (MD)[d]

Affiliation:

[a]Nutrition and Endocrine Research Center, Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[b]Obesity Research Center, Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[c]School of Public Health, University of Saskatchewan, Saskatoon, SK S7N 2Z4, Canada

[d]Endocrine Research Center, Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Contact info:**

Farhad Hosseinpanah

Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran

P.O. Box: 19395-476

Phone: +98-21-22432500

Cell: +98-912-343 1927

Fax: +98-21-22416264

E-mail address: fhospanah@endocrine.ac.ir

10.1017/S0007114519002344

The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

Short title: Obesity phenotypes and metabolic syndrome

Keyword: Obesity phenotypes, Metabolic syndrome, Adolescence, Adult, TLGS

Abstract

Obesity phenotypes can be regarded as an indicator of cardiovascular disease risk factors. The aim of this study was to determine the prevalence of adolescents with different obesity phenotypes and the role of obesity phenotypes in prediction of metabolic syndrome (MetS) in adults. For this population-based cohort study, 2159 adolescents aged 11-18 years were included. Subjects were divided into four obesity phenotype groups: Metabolically healthy normal weight (MHNW), metabolically healthy obese (MHO), metabolically unhealthy normal weight (MUNW), and metabolically unhealthy obese (MUO). Cox proportional hazard modeling was used to estimate incidence of MetS in adults after a median follow-up of 11.3 years. The incidence rate of MetS in early adulthood was 111.6 per 10000 person-year (95% CI: 98.7-126.3), with higher values in boys [210.1 (95% CI: 183.0-241.3)], compared to girls [39.7 (95% CI: 30.2-52.1)]. In the age- and adult BMI-adjusted model, the HR of MetS in adulthood for boys was 3.33 (95% CI: 2.08-5.32) among MUO phenotype followed less than 6 years, 1.71 (95% CI: 1.01-2.90) among MHO, and 2.52 (95% CI: 1.72-3.68) among MUNW. All associations were attenuated in girls except for MUO phenotype followed less than 6 years [5.72 (95% CI: 2.14-15.3)]. In conclusion, MUNW and MHO phenotypes in boys, but not in girls and MUO phenotype in both sexes with less than 6 years of follow up increased risk of adult MetS compared to MHNW. It seems that lack of obesity at least in boys does not protect them from development of MetS in adulthood.

Introduction

Over recent years, the growing prevalence of obesity and metabolic syndrome (MetS), as predisposing factors of non-communicable chronic diseases (NCDs), is of particular global concern and affects both children and adults [1]. A recent nationwide study provided evidence that based on ATP III criteria, 14.1% of adolescents in Iran have MetS [2]. Moreover, it can be argued that children with overweight are at higher risk to become adults with obesity than those with normal weight and consequently are more susceptible to cardio-metabolic abnormalities and shorter lifespan [3; 4; 5]. While some studies have investigated the status of childhood MetS as a risk factor for adult MetS, the clinical utility of identifying MetS in children and its association with future cardio-metabolic risk factors is a matter of debate and some studies showed that adolescent with overweight or obesity is a better predictor of cardiovascular risk factors and adult MetS in comparison to pediatric MetS [6; 7]. However, it is not yet known whether childhood obesity predicts future cardio-metabolic risk factors independent of adulthood body mass index (BMI) or not.

Obesity phenotypes can be regarded as an indicator of interactions between BMI and CVD risk factors. Thus, individuals can be divided into different subtypes based on their obesity phenotypes: some individuals with obesity are not affected by metabolic abnormalities associated with their body fat. These 'metabolically healthy obese' (MHO) subjects display a favorable metabolic status. On the other hand, some other individuals known as 'metabolically unhealthy normal weight' (MUNW) suffer from metabolic abnormalities despite their normal weight profile.

A series of previous studies in adults have indicated that MUNW subjects carry an elevated risk of developing type 2 diabetes and cardiovascular events compared with MHO subjects [8; 9; 10]. Furthermore, a systematic review and meta-analysis of observational studies showed that MHO group compared with the MUNW and 'metabolically unhealthy obese' (MUO) counterparts, had higher CV events [11].

In spite of the large body of evidence regarding the predictive role of different phenotypes of obesity on development of CVD outcomes in adults, there is paucity data in adolescents. However, it is important to note that there are several studies on metabolic risk factors or obesity

individually in prediction of adult MetS [12; 13; 14]. In fact, existing data are limited to only one population-based study that has demonstrated positive associations between cardiovascular risk and metabolic complications in adulthood and pediatric obesity phenotypes [11].

To facilitate more data emphasizing the importance of obesity phenotypes in adolescents, this population-based cohort study, aimed to determine the prevalence of obesity phenotypes including 'metabolically healthy normal weight' (MHNW), MHO, MUNW, MUO among 10-18-year-old adolescents, and to investigate the role of obesity phenotypes in prediction of adult MetS, in the context of the Tehran Lipid and Glucose Study (TLGS), during a median follow-up of 11.3 years.

Materials and Methods

Study population

In the progression of our information in the TLGS data, in the current prospective study, we investigated the role of adolescence obesity phenotypes in the prediction of adult MetS. The TLGS is an ongoing a large scale community-based program for monitoring the trend of metabolic risk factors and developing healthy lifestyles to reduce these risk factors. [15]. Baseline data were collected from 15005 participants, aged ≥3 years, under coverage of three medical health centers residing in District No. 13 of Tehran. The participants were followed up every 3 years to update their data on demographics, lifestyle, biochemical and clinical information and anthropometric examination; the baseline survey was a cross-sectional study conducted from 1999 to 2001, and surveys 2 (2002-2005), 3 (2006-2008), 4 (2009-2011), 5 (2012-2015), and 6 (2016-2019) were prospective follow-up surveys. The cohort is still ongoing.

Of 15005 subjects recruited at the baseline examination of the TLGS, 3149 children and adolescents, aged 11-18 years from surveys 1 and 2 were included. After exclusion of those with missing anthropometric values and biochemical data (n=149), 3000 subjects remained. After a median of 11.3 years follow-up, 2159 subjects returned for reassessment. The design of this study was approved by the institutional ethics committee of the Research Institute for Endocrine Sciences, affiliated to the Shahid Beheshti University of Medical Sciences. All participants signed an informed written consent form to participate.

Measurements

Anthropometric measurements were obtained by qualified health care professionals according to standard protocols [15]; participants measured while they were minimally clothed and without shoes. Weight was measured to the nearest 0.1 kg with digital scale (Seca 707; range 0.1-150 kg, Hanover, MD). A tape meter stadiometer was used to measure height while the subjects were against the wall with their shoulders in normal alignment. Waist circumference (WC) was measured using an unstretched tape at the end of expiration at the narrowest level between iliac crest and lowest rib, while the participants were in standing position and there was no pressure to their body surface. Height and WC were recorded with an accuracy of up to 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2).

Systolic and diastolic blood pressures were determined by a physician using standard mercury sphygmomanometer (calibrated by Iranian Institute of Standards and Industrial Researches). After the subject remained seated for 15 minutes, blood pressure measurement was taken two times with at least a 30-second interval from the right brachial artery at the heart level. The mean values of measurements were considered as the individual's blood pressure.

After 12 to 14 hours fasting, a blood sample was taken between 7 to 9 am, based on the standard protocol. After centrifuging the collected blood sample, the serum was used for measurement and analyses of fasting plasma glucose (FPG) and lipid concentrations on the day of blood collection at the TLGS Research Laboratory, using commercially available laboratory kits (Pars Azmoon Inc, Tehran, Iran) adapted to a Selectra 2 auto analyzer. Plasma glucose concentration was measured by the enzymatic colorimetric method with glucose oxidase. Inter- and intra-assay CVs, were both 2.2% for FPG. For measurement of triglycerides (TG), we used an enzymatic colorimetric method with glycerol phosphate oxidase; inter- and intra-assay coefficients of variation (CV) for TG were 0.6 and 1.6%, respectively. Total cholesterol (TC) was assessed with cholesterol esterase and cholesterol oxidase using the enzymatic colorimetric method. High-density lipoprotein cholesterol (HDL-C) was assayed after precipitation of apolipoprotein B containing lipoproteins with phosphotungistic acid. Inter- and intra-assay CVs for both TC and HDL-C were 0.5 and 2%, respectively. When TG concentrations were< 400 mg/dl, the

Friedwald formula was used to calculate low density lipoprotein Cholesterol (LDL-C) from the serum TC, TGs, and HDL-C concentrations [16].

Definition

Obesity and overweight in adolescents were defined as age- and sex-specific BMI>95th and BMI between ≥85th and <95th percentiles in Iranian populations respectively [2].

Cardio-metabolic risk factors were defined as follows: Abdominal obesity as WC ≥ 90th percentile for age and sex, according to national reference curves [17]; hypertriglyceridemia as TGs ≥ 110 mg/dl [18]; low HDL-C as HDL-C <40 mg/dl [18]; hypertension as systolic and/or diastolic blood pressure ≥90th percentile for sex, age and height according to the Heart, Lung, and Blood Institute standards [19]; high FPG ≥ 100 mg/dl according to American Diabetes Association [20].

At baseline adolescents were categorized into four different obesity phenotypes groups based on their BMI and metabolic status as follows: MHNW: normal BMI (<85th percentile) and ≤ 1 the previously mentioned parameters of cardio-metabolic risk factors; MHO: overweight or obese (BMI ≥ 85th percentile) and ≤ 1 parameters of cardio-metabolic risk factors; MUNW: normal BMI (<85th percentile) and ≥ 2 parameters of cardio-metabolic risk factors; MUO: overweight or obese (BMI ≥ 85th percentile) and ≥ 2 parameters of cardio-metabolic risk factors.

Metabolic syndrome in adults was defined according to JIS criteria [21], as having ≥3 of following conditions: 1) TGs ≥ 150 mg/dl or drug treatment, 2) HDL-C <40 mg/dl in men and <50 mg/dl in women or drug treatment, 3) WC ≥89cm in men and WC ≥91cm in women [22], 4) systolic and/or or diastolic blood pressure ≥ 130/85 mmHg or drug treatment, and 5) FPB ≥ 100 mg/dl or drug treatment.

Statistical analysis

Continuous variables with normal and skewed distribution were expressed as mean (SD) and median (25-75 interquartile range), and differences were assessed using t-test and Mann–Whitney test, respectively. Categorical variables were reported as percentages, were compared using Pearson's χ^2 test. In this study, as the exact time of MetS incident was not known, this was

considered as interval-censored data. Interval censoring takes into account the event happening between two time periods. Considering alternate interval censoring approaches, results were investigated using mid-point censoring, which converts interval-censored data to the right-censored data problems. Mid-point censoring was set to the mid-point between the last negative and the most recent positive event time minus the first positive observation for the incidence of obesity and to the time span between the first and the last observation for censored subjects. Endpoints were considered as the time of incident adult MetS and censoring was defined as lost to follow up or end of follow up. Cumulative incidences of MetS with 95% confidence intervals (CI) were calculated as the number of new cases of MetS over the total number of subjects in that group minus half of the censored population. The person-year method was used to obtain MetS incidence rates (IRs); IR is reported as number of cases per 10000 person years. Cox proportional hazard modeling was used to estimate unadjusted, age- and adult BMI-adjusted hazard ratios (HRs) along with 95% CIs for baseline groups of obesity phenotypes. The proportionality assumption was verified by assessing the correlation between the Schoenfield residuals and person-days along with observing log minus log plots (considering different groups as strata variables). All proportionality assumptions were generally met with exception of the visual assessment for the MUO phenotype; it was noticed that the two curves are much closer until almost 6 years of follow-up, but they diverge greatly after that. Therefore, we performed an extended Cox model containing two heavy-side functions with the mentioned time cut-off. The corresponding model provides two hazard ratios for any time-dependent covariate, one that is constant above the cut-off time and the other that is constant below it. All analyses were performed using IBM SPSS for Windows version 20 (SPSS, Chicago, IL, USA) and STATA version 12 SE (STATA Inc., TX, USA), with two-tailed P-values of <0.05 being considered significant.

Results

In the current study, 2159 children and adolescents (975 boys) with a mean (±SD) age of 14.6 years were included. Subjects were divided into four groups based on their obesity phenotypes at baseline: MHNW (n=1211, 56.1%), MHO (n=177, 8.2%), MUNW (n=461, 21.4%) and MUO (n=309, 14.3%). Baseline characteristics, except for age, sex, BMI, and WC, between participants followed up and those missed to follow-up were not significantly different

(Supplementary Table 1). The statistically significant differences of age, sex, BMI, and WC were not clinically important.

Metabolically healthy obese and MUNW boys and girls were older and had higher prevalence of hypertension compared to MHNW ones (P<0.001). Furthermore, the prevalence of abdominal obesity was significantly higher in MHO subjects compared to those who were normal weight (P<0.001, Tables 1 and 2).

The incidence rate of MetS in early adulthood was 111.6 per 10000 person-year (95% CI: 98.7-126.3) with higher values in boys [210.1 (95% CI: 183.0-241.3)] compared to girls [39.7 (95% CI: 30.2-52.1)]. In boys, MHO and MUNW phenotypes predicted MetS in adulthood compared to MHNW in the fully adjusted model, with higher HR for MUNW (2.52 vs 1.71). In both sexes, adolescents in the MUO group had a statistically significant higher HR to predict MetS in adulthood than other groups. Regarding violation of proportionality assumption, based on the extended cox model, MUO phenotype in both sexes with less than 6 years of follow up, had significant HRs (3.33 in boys and 5.72 in girls) compared with the reference group after adjustment for adulthood BMI (Table 3).

Discussion

The current study provides evidence regarding the role of adolescent obesity phenotypes in prediction of adulthood MetS. Metabolically unhealthy normal weight and MHO phenotypes in boys, but not in girls and MUO phenotype in both sexes with less than 6 years of follow up increased risk of adult MetS compared to MHNW, independent of adult BMI.

While some studies showed a positive correlation between higher childhood BMI and developing MetS as adults later in life [12; 13; 14; 23], the predictive value of childhood BMI for developing adult MetS is a controversial issue. Lyold et al, in their systematic review reported that childhood obesity was associated with increased risk of adult MetS without adjustment of adult BMI; however, after adjustment for adult BMI, the association was ameliorated or inversed [24]. In an analysis of four prospective cohort studies, adolescents who had an increased BMI and remained obese or overweight as adults had an elevated risk of developing type 2 diabetes, hypertension, dyslipidemia, and carotid-artery atherosclerosis [25]. The role of childhood MetS in prediction of

adult MetS is also unclear. While some studies provided evidence for tracking of MetS from childhood into adulthood [26], the debate continues about the clinical utility of identifying MetS in children and its association with future cardio-metabolic risk factors [27].

Obesity phenotypes, in other words, an interaction between BMI and CVD risk factors, have been used in a series of previous studies conducted in adults. Roberson et al showed in their systematic review that the MHO obesity phenotype is an emerging phenotype with higher CVD risk than healthy normal weight phenotypes [28]; however, to the best of our knowledge, only one study has examined the predictive role of adolescent obesity phenotypes in development of adult MetS prospectively [11] and there is still conflicting as to whether children with MHO and MUNW phenotypes are more susceptible to future cardio-metabolic risk factors or not [29].

In our study, the MHO phenotype in boys was associated with increased risk of adult MetS; however, in girls, after adjustment for adult BMI, this association was disappeared. In the young Finns study, before adjustment of BMI, adolescents with MUNW, MHO, and MUO phenotypes had increased risk of adulthood MetS; however, after adjustment for adult BMI, only the MUNW and MUO groups showed significant associations with development of adult MetS [11]. The discrepancy between findings of the Koskinen et al study, compared to ours may be explained by: 1) the baseline older age of participants who had passed unstable stage of puberty, 2) longer follow-up led to older age for outcome assessment, 3) different definition of "healthy metabolic status" in adolescents which was "not having any components of MetS", and last but not least 4) not using WC in the definition of adolescent MetS.

It is important to note that in our study despite the positive association between the MHO phenotype and adult MetS in boys, the strength of association was weaker than that of the MUNW one (1.71 vs 2.52), highlighting the importance of metabolic abnormalities compared to BMI in predicting adult MetS. In the current study the incidence rate (per 10000 person-year) of MetS was found to be significantly lower in girls compared to boys (39.7 vs 210.1 per 10000 person-year), similar to previously reported findings [30; 31]. This lower prevalence might be due to easier access of boys to junk food, and eating more out of home. Moreover, it has been found that among boys TV watching >2 h/day, leisure time computer working >2 h/day, and screen time >4 h/day were dramatically higher than girls [31]. Similarly, a study among US adolescents

aged 12-19 years from the National Health and Nutrition Examination Survey (1999-2002), using the Healthy Eating Index to assess the overall picture of diet quality showed that girls had better scores for fruits, vegetables, saturated fat, cholesterol, and sodium than boys [30]. Higher body fat and lower muscle mass, as a result of diverse sexual maturation, and body image and physical fitness in girls might be another reason [32; 33; 34].

An unexpected finding, in both sexes, after adjusting for adult BMI, the risk for developing adult MetS in MUO phenotype was observed only in adolescents followed less than 6 years. It seems that in those individuals who had more follow-up time, contribution of adult BMI would be more prominent than in those with less follow-up time, i.e. those with < 6 years follow-up time had more adolescent metabolic characteristics than those with longer follow-up time. In fact, majority of adolescents with obesity in the category with < 6years follow-up remained obese (60.9%) than those with > 6years (35.7%) at the end of study (data not shown). The reason for this finding might be the instability of MetS components in children and adolescents and occurrence of physiological insulin resistance in adolescents [35]. An increased risk of adulthood MetS in adolescents with unhealthy metabolic characteristics, regardless of obesity status was reported in a prospective longitudinal study with 25-30 years of follow-up [36], a finding illustrating the importance of adolescents' un-healthy metabolic status, despite of having normal weight. We can hypothesize that a considerable proportion of MUO subjects with more follow-up time achieved favorable BMI at the end of study. Accordingly, we have previously reported that adolescent overweight or obesity did not predict early adult MetS independent of adult BMI [27].

Different cardio-metabolic risk factors have different impacts on the incidence of adult MetS, e.g. high WC/low HDL-C was the strongest predictor of adults MetS [6]. However, as the current study was conducted on the subgroups of obesity phenotypes, we lacked sufficient power to determine which cardio-metabolic risk factors has higher impact on development of adult MetS.

Of the several limitations in this study, we did not have data regarding puberty, nutrition intake, and physical activity of participants. Furthermore, serum insulin was not measured to calculate insulin resistance as metabolic risk factor; however, its predictive value for developing metabolic syndrome is a controversial subject due to passing the pubertal status and instability of insulin

concentration. Strengths of this study deserve comments as well. Having the prospective nature of the study and its length of follow-up are main strengths. Nation-based definitions and cut-off points were used in this study. Data and measures were obtained by trained technicians in order to reduce subjective errors and no self-report measure was used. Another important advantage of current study is the use of WC which indicates the actual adiposity mass of body as a metabolic risk factor more accurately than BMI, used previously in several studies.

In conclusion our study evidently is the first in the Middle East North Africa (MENA) region, prospectively investigated the role of adolescent obesity phenotypes in prediction of MetS in adulthood, independent of adult BMI. The current study gives deeper insight on the interaction of childhood excess weight and unfavorable cardio-metabolic profiles, tracking BMI from childhood to adulthood. Obviously presence of obesity in adolescents is associated with major concern regarding development of MetS in early adulthood; however, from the practical point of view, the lack of obesity in adolescents does not protect them from development of MetS in future (especially boys). This finding highlights the importance of prevention, screening, and early control of metabolic abnormalities in adolescents even in the absence of obesity.

Acknowledgment

The authors express appreciation to the participants in the Tehran Lipid and Glucose Study for their enthusiastic support, and the staff of the Tehran Lipid and Glucose Study Unit of the Research Institute for Endocrine Sciences, for their valuable help. We would like to acknowledge Ms. Niloofar Shiva for critical edition of English grammar and syntax of the manuscript.

Financial Support

This work was funded by a grant from the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author contributions

GA and FH conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. SS and SH collected data, carried out the initial analyses, and reviewed and revised the manuscript. FA designed the data collection instruments, and

coordinated and supervised data collection, and critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

References

1. Ng M, Fleming T, Robinson M *et al.* (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384**, 766-781.
2. Kelishadi R, Ardalan G, Gheiratmand R *et al.* (2006) Paediatric metabolic syndrome and associated anthropometric indices: the CASPIAN Study. *Acta Paediatr* **95**, 1625-1634.
3. Field AE, Cook NR, Gillman MW (2005) Weight status in childhood as a predictor of becoming overweight or hypertensive in early adulthood. *Obes Res* **13**, 163-169.
4. Singh AS, Mulder C, Twisk JW *et al.* (2008) Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev* **9**, 474-488.
5. Li L, Pinot de Moira A, Power C (2011) Predicting cardiovascular disease risk factors in midadulthood from childhood body mass index: utility of different cutoffs for childhood body mass index. *Am J Clin Nutr* **93**, 1204-1211.
6. Hosseinpanah F, Salehpour M, Asghari G *et al.* (2015) "Adolescent metabolic phenotypes and early adult metabolic syndrome: Tehran lipid and glucose study". *Diabetes Res Clin Pract* **109**, 287-292.
7. Magnussen CG, Koskinen J, Chen W *et al.* (2010) Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone: the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. *Circulation* **122**, 1604-1611.
8. Bell JA, Kivimaki M, Hamer M (2014) Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev* **15**, 504-515.
9. Voulgari C, Tentolouris N, Dilaveris P *et al.* (2011) Increased heart failure risk in normal-weight people with metabolic syndrome compared with metabolically healthy obese individuals. *J Am Coll Cardiol* **58**, 1343-1350.
10. Pajunen P, Kotronen A, Korpi-Hyovalti E *et al.* (2011) Metabolically healthy and unhealthy obesity phenotypes in the general population: the FIN-D2D Survey. *BMC Public Health* **11**, 754.
11. Koskinen J, Magnussen CG, Sabin MA *et al.* (2014) Youth overweight and metabolic disturbances in predicting carotid intima-media thickness, type 2 diabetes, and metabolic syndrome in adulthood: the Cardiovascular Risk in Young Finns study. *Diabetes care* **37**, 1870-1877.
12. Graversen L, Sørensen TI, Petersen L *et al.* (2014) Preschool weight and body mass index in relation to central obesity and metabolic syndrome in adulthood. *PloS one* **9**, e89986.
13. Sachdev H, Osmond C, Fall C *et al.* (2009) Predicting adult metabolic syndrome from childhood body mass index: follow-up of the New Delhi birth cohort. *Archives of disease in childhood* **94**, 768-774.
14. Liang Y, Hou D, Zhao X *et al.* (2015) Childhood obesity affects adult metabolic syndrome and diabetes. *Endocrine* **50**, 87-92.
15. Azizi F, Ghanbarian A, Momenan AA *et al.* (2009) Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. *Trials* **10**, 5.
16. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**, 499-502.
17. Kelishadi R, Gouya MM, Ardalan G *et al.* (2007) First reference curves of waist and hip circumferences in an Asian population of youths: CASPIAN study. *J Trop Pediatr* **53**, 158-164.
18. Cook S, Weitzman M, Auinger P *et al.* (2003) Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Archives of pediatrics & adolescent medicine* **157**, 821-827.
19. (2004) The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* **114**, 555-576.

20. Genuth S, Alberti KG, Bennett P *et al.* (2003) Follow-up report on the diagnosis of diabetes mellitus. *Diabetes care* **26**, 3160-3167.
21. Alberti KG, Eckel RH, Grundy SM *et al.* (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **120**, 1640-1645.
22. Delavari A, Forouzanfar MH, Alikhani S *et al.* (2009) First nationwide study of the prevalence of the metabolic syndrome and optimal cutoff points of waist circumference in the Middle East: the national survey of risk factors for noncommunicable diseases of Iran. *Diabetes care* **32**, 1092-1097.
23. Magnussen CG, Koskinen J, Chen W *et al.* (2010) Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus – But Is No Better Than Body Mass Index Alone: The Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. *Circulation* **122**, 1604-1611.
24. Lloyd LJ, Langley-Evans SC, McMullen S (2012) Childhood obesity and risk of the adult metabolic syndrome: a systematic review. *Int J Obes (Lond)* **36**, 1-11.
25. Juonala M, Magnussen CG, Berenson GS *et al.* (2011) Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med* **365**, 1876-1885.
26. Juhola J, Magnussen CG, Viikari JS *et al.* (2011) Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. *J Pediatr* **159**, 584-590.
27. Hosseinpanah F, Asghari G, Barzin M *et al.* (2013) Adolescence metabolic syndrome or adiposity and early adult metabolic syndrome. *J Pediatr* **163**, 1663-1669.e1661.
28. Roberson LL, Aneni EC, Maziak W *et al.* (2014) Beyond BMI: The "Metabolically healthy obese" phenotype & its association with clinical/subclinical cardiovascular disease and all-cause mortality -- a systematic review. *BMC Public Health* **14**, 14.
29. Li S, Chen W, Srinivasan SR *et al.* (2012) Relation of childhood obesity/cardiometabolic phenotypes to adult cardiometabolic profile: the Bogalusa Heart Study. *Am J Epidemiol* **176 Suppl 7**, S142-149.
30. Pan Y, Pratt CA (2008) Metabolic syndrome and its association with diet and physical activity in US adolescents. *Journal of the American Dietetic Association* **108**, 276-286; discussion 286.
31. Ahmadi A, Gharipour M, Nouri F *et al.* (2013) Metabolic syndrome in Iranian youths: a population-based study on junior and high schools students in rural and urban areas. *Journal of diabetes research* **2013**, 738485.
32. Lim JS, Hwang JS, Cheon GJ *et al.* (2009) Gender differences in total and regional body composition changes as measured by dual-energy x-ray absorptiometry in Korean children and adolescents. *J Clin Densitom* **12**, 229-237.
33. Kirchengast S, Marosi A (2008) Gender differences in body composition, physical activity, eating behavior and body image among normal weight adolescents--an evolutionary approach. *Coll Antropol* **32**, 1079-1086.
34. Voelker DK, Reel JJ, Greenleaf C (2015) Weight status and body image perceptions in adolescents: current perspectives. *Adolesc Health Med Ther* **6**, 149-158.
35. Steinberger J, Daniels SR, Eckel RH *et al.* (2009) Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. *Circulation* **119**, 628-647.

36. Morrison JA, Friedman LA, Wang P *et al.* (2008) Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. *J Pediatr* **152**, 201-206.

Table 1- Baseline characteristics of boys based on different obesity phenotypes

	MHNW (n=568)	MHO (n=84)	MUNW (n=184)	MUO (n=138)	*P*-value*
Age (years)	14.5±2.3‡§	13.4±2.2§¶	14.9±2.2	14.8±2.1	<0.001
Body mass index (kg /m^2)	18.0±2.3‡§¶	22.9±2.2§¶	19.0±2.7¶	27.2±4.5	<0.001
Waist circumference (cm)	64.3±7.0‡§¶	74.8±7.8§¶	67.4±7.8¶	88.2±11.3	<0.001
Abdominal obesity (%)	0.0	3.5	0.0	42.0	<0.001
Systolic blood pressure (mmHg)	103.6±10.8§¶	107.3±11.2¶	108.3±12.8¶	114.2±12.7	<0.001
Diastolic blood pressure (mmHg)	68.5±8.9§¶	68.7±8.4§¶	72.8±9.5	74.1±8.6	<0.001
Hypertension (%)	0.9	1.2	3.3	4.3	0.019
HDL-C (mg/dl)	46.0±10.0§¶	47.0±9.3§¶	36.0±8.0	37.4±7.2	<0.001
Triglycerides (mg⁄dl) †	78 (59-96)	86 (65-104)	127 (111-156)	151 (121-200)	<0.001
Fasting plasma glucose (mg⁄dl)	88.3±9.1§¶	89.3±6.5§	92.2±9.0	91.0±7.4	<0.001

Data are given as the mean (SD) or median (IQ 25–75) unless otherwise indicated.
MHNW, metabolically healthy normal weight; MHO, metabolically healthy obese; MUNW, metabolically unhealthy normal weight; MUO, metabolically unhealthy obese; HDL-C, high-density lipoprotein cholesterol.
Abdominal obesity was defined as waist circumference ≥ 90th percentile for age and sex, according to national reference curves. Hypertension was defined as SBP and/or DBP ≥90th percentile for sex, age and height according to Heart, Lung, and Blood Institute standards.
*P values are for the comparisons across obesity phenotypes, with the use of analysis of variance.
† Log transformed values were used for comparison
‡Significantly different from MHO phenotype, using post hoc LSD analysis test
§Significantly different from MUNW phenotype, using post hoc LSD analysis test
¶Significantly different from MUO phenotype, using post hoc LSD analysis test

Table 2- Baseline characteristics of girls based on different obesity phenotypes.

	MHNW (n=643)	MHO (n=93)	MUNW (n=277)	MUO (n=171)	*P*-value*
Age (years)	14.9±2.3[§¶]	14.7±2.2	14.3±2.2	14.4±2.1	0.001
Body mass index (kg /m^2)	18.8±2.6[‡¶]	25.3±2.8[§]	19.1±2.7[¶]	25.8±3.0	<0.001
Waist circumference (cm)	66.1±6.8[‡§¶]	76.7±6.7[§¶]	68.1±7.6[¶]	81.4±7.4	<0.001
Abdominal obesity (%)	0.0	2.2	0.0	9.9	<0.001
Systolic blood pressure (mmHg)	101.2± 10.5[‡§¶]	106.2±10.4[§¶]	103.7±11.5[¶]	109.1±11.2	<0.001
Diastolic blood pressure (mmHg)	68.8±8.6[‡§¶]	72.3±7.6[¶]	72.3±10.2[¶]	75.1±8.5	<0.001
Hypertension (%)	0.8	0.0	1.8	2.9	0.078
HDL-C (mg/dl)	46.4±9.7[§¶]	44.8±8.1[§¶]	36.5±7.4	37.2±8.2	<0.001
Triglycerides (mg⁄dl)	84 (67-102)	89 (67-105)	133 (116-168)	136 (110-176)	<0.001
Fasting plasma glucose (mg⁄dl)	86.4±6.9[§¶]	88.0±7.0	89.3±8.4	88.8±9.3	<0.001

Data are given as the mean (SD) or median (IQ 25–75) unless otherwise indicated.
MHNW, metabolically healthy normal weight; MHO, metabolically healthy obese; MUNW, metabolically unhealthy normal weight; MUO, metabolically unhealthy obese; HDL-C, high-density lipoprotein cholesterol.
Abdominal obesity was defined as waist circumference ≥ 90th percentile for age and sex, according to national reference curves. Hypertension was defined as SBP and/or DBP ≥90th percentile for sex, age and height according to Heart, Lung, and Blood Institute standards.
[*]P values are for the comparisons across obesity phenotypes, with the use of analysis of variance.
[†] Log transformed values were used for comparison
[‡]Significantly different from MHO phenotype, using post hoc LSD analysis test
[§]Significantly different from MUNW phenotype, using post hoc LSD analysis test
[¶]Significantly different from MUO phenotype, using post hoc LSD analysis test

Table 3- Hazard ratio and 95% confidence interval (CI) for incidence of metabolic syndrome in early adulthood based on different adolescent obesity phenotypes in children and adolescents.

	MHNW	MHO	MUNW	MUO	
Total (n)	1211	178	461	309	
Number of person-years	13242.54	1867.73	4812.50	2740.68	
Number of events	72	27	60	94	
Incidence rate (per 10000 person-years)	54.4 (43.1-68.5)	144.6 (99.1-210.8)	124.8 (96.8-160.5)	343.0 (280.2 – 419.8)	
				≥ 6 years	< 6 years
Hazard ratios (95% CI)					
Unadjusted	1.00	2.69 (1.73-4.18)	2.30 (1.63-3.24)	2.98 (1.73-5.13)	8.68 (6.11-12.34)
Model 1	1.00	3.07(1.97-4.79)	2.43 (1.72-3.42)	3.22 (1.87-5.57)	9.21 (6.48-13.11)
Model 2	1.00	1.30 (0.81-2.1)	2.14 (1.51-3.02)	1.26(0.71-2.23)	3.65 (2.46-5.42)
Boys (n)	568	85	184	138	
Number of person-years	6007.88	857.78	1717.37	983.36	
Number of events	61	20	49	71	
Incidence rate (per 10000 person-years)	101.5 (79.0 – 130.5)	233.2 (150.4-361.4)	285.3 (215.6-377.5)	722.0 (572.2-911.1)	
Hazard ratios (95% CI)					
Unadjusted	1.00	2.30 (1.39 – 3.82)	2.81 (1.93-4.09)	2.95 (1.46-5.93)	9.18 (6.25-13.47)
Model 1	1.00	2.91 (1.74 – 4.85)	2.68 (1.84-3.90)	2.96 (1.47-5.96)	8.94 (6.09-13.14)
Model 2	1.00	1.71 (1.01 – 2.90)	2.52 (1.72-3.68)	1.12 (0.53-2.35)	3.33 (2.08-5.32)
Girls (n)	643	93	277	171	
Number of person-years	7234.66	1009.95	3095.13	1757.32	
Number of events	11	7	11	23	
Incidence rate (per 10000 person-years)	15.2 (0.8-27.5)	69.3 (33.0-145.4)	35.5 (19.7-64.2)	130.9 (86.9-196.9)	
Hazard ratios (95% CI)					
Unadjusted	1.00	4.67 (1.81-12.04)	2.34 (1.02-5.40)	5.04 (1.94-13.07)	14.59 (5.88-36.17)
Model 1	1.00	4.99 (1.93-12.90)	2.66 (1.15-6.17)	5.75 (2.21-14.99)	16.55 (6.64-41.25)
Model 2	1.00	1.30 (0.46-3.66)	1.87 (0.78-4.47)	1.74 (0.63-4.82)	5.72 (2.14-15.3)

Incidence rate, number of incident MetS cases divided by person-year follow up.
MHNW, metabolically healthy normal weight; MHO, metabolically healthy obese; MUNW, metabolically unhealthy normal weight; MUO, metabolically unhealthy obese.
Model 1 is adjusted for age; model 2 is adjusted for age and adult's body mass index.