Effect of Relative Weight Group Change on Nuclear Magnetic Resonance Spectroscopy Derived Lipoprotein Particle Size and Concentrations among Adolescents

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Objective To examine whether longitudinal changes in relative weight category (as indicated by change in body mass index [BMI] classification group) were associated with changes in nuclear magnetic resonance (NMR)–derived lipoprotein particles among US youth.

Study design Secondary analysis of data from a clustered randomized controlled trial. BMI and fasting blood samples were obtained from 2069 participants at the start of the 6th grade and end of the 8th grade. BMI was categorized as normal weight, overweight, or obese at both time points. Lipoprotein particle profiles were measured with NMR spectroscopy at both time points. Regression models were used to examine changes in relative weight group and change in lipoprotein variables.

Results A total of 38% of participants changed relative weight category (BMI group) during the 2.5-year study period. Low-density lipoprotein (LDL) cholesterol and non–high-density lipoprotein (HDL) cholesterol decreased almost universally, but more with improved BMI category. There were adverse effects on LDL size and total LDL particles, HDL size, and cholesterol for participants who remained obese or whose relative weight group worsened. Changes in relative category had no impact on HDL particles.

Conclusion Improvement in relative weight group from 6th to 8th grade was associated with favorable changes in non-HDL cholesterol, very low-density lipoprotein size, LDL size, HDL size, and LDL particles but had no effect on HDL particles. Findings indicate that an improvement in relative weight group between 6th and 8th grade had an effect on NMR-derived particles sizes and concentrations among a large group of adolescents, which overrepresented low-income minorities. *(J Pediatr 2014;164:1091-98)*.

There is substantial evidence that insulin resistance in adults and children[1] is associated with high triglyceride (TG) levels and low levels of high-density lipoproteins (HDLs). Recent adult studies in which investigators used nuclear magnetic resonance (NMR) spectroscopy have suggested that low-density lipoprotein particle (LDL-P) concentration and size may have stronger associations with insulin resistance and the metabolic syndrome than traditional lipoprotein lipid measures.[2] LDL-P and LDL-P size may provide early markers for cardiometabolic risk and both incident type 2 diabetes and cardiovascular disease (CVD).[3,4]

Emerging evidence indicates that NMR lipoprotein profiles are associated with obesity and insulin resistance in children. For example, by comparing obese children with prediabetes, with obese children with normo-glycemia, Magge et al[5] reported there was no difference in levels of low-density lipoprotein cholesterol (LDL-C), HDL cholesterol (HDL-C), and TGs. However, participants with prediabetes had greater concentrations of small LDL-P and a smaller average LDL-P size. Moreover, a number of studies have shown that obese children have greater levels of small and total LDL-P than nonobese children.[6,7]

BMI	Body mass index	LDL-C	Low-density lipoprotein cholesterol
CVD	Cardiovascular disease		
HDL	High-density lipoprotein	LDL-P	Low-density lipoprotein particle
HDL-C	High-density lipoprotein cholesterol	LP-IR	Lipoprotein insulin resistance score
HDL-P	High-density lipoprotein particle	NMR	Nuclear magnetic resonance
HOMA-IR	Homeostatic model assessment of insulin resistance	TG	Triglyceride
		VLDL	Very low-density lipoprotein
LDL	Low-density lipoprotein	VLDL-P	Very low-density lipoprotein particle

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In adults, a diet and exercise regime that reduces total and visceral fat and improves insulin sensitivity has been shown to yield favorable changes in NMR-derived lipoprotein particles.[8] Longitudinal data have shown that nonobese adults who were overweight in childhood have levels of increased lipids comparable with individuals who had a healthy weight at both time points.[9] Thus, we examined the associations between 2.5-year changes in relative weight category and changes in NMR-derived lipoprotein particles in 2069 6th graders, 75% of whom were black or Latino, and 50% of whom were overweight or obese.

Methods

This report is an analysis of stored blood from the HEALTHY Study, a US National Institute of Diabetes and Digestive and Kidney Diseases cluster randomized controlled trial that aimed to reduce the prevalence of risk factors for type 2 diabetes mellitus among middle school children (6th-8th grade). The main study design has been detailed elsewhere.[11] In brief, participants were recruited from 42 middle schools across the US. Schools had at least 50% of students eligible for free or reduced-price lunch or belonging to an ethnic minority group and an annual student attrition rate ≤25%. The intervention included changes to the physical education curriculum school food service, health education, and a school-wide social marketing campaign.[10-15] The sample for this study is limited to participants who provided parental consent and child assent for ancillary analyses of stored blood, and for whom complete data were available. The study was approved by the institutional review boards at each field center, and written informed parental consent and child assent were obtained.

With each subject shoeless, height and body mass were measured with the PE-AIM-101 stadiometer (Perspective Enterprises, Portage, Michigan) and the Alpha 882 electronic scale (SECA, Chino, California). Body mass index (BMI; kg/m^2) was calculated and converted to an age- and sex-specific BMI percentile using Centers for Disease Control and Prevention 2000 criteria.[16] Students with a BMI ≥5th percentile and <85th percentile were classified as healthy weight. The healthy weight range was then subdivided into 2 groups: BMI ≥5th percentile and <50th percentile and BMI ≥50th percentile and <85th percentile. Youth with BMI ≥85th percentile but <95th percentile were classified as overweight, those with BMI between 100% and 119% of the >95th percentile classified as obese and those at or greater than 120% of the 95th percentile classified as severely obese[17] and those with BMI <5th percentile were classified as underweight. Waist circumference was taken using a Gulick tape measure (G-tape) with a tension device on bare skin measured just above the iliac crest.

At baseline (beginning of 6th grade) and follow-up (end of 8th grade) fasting blood samples were collected from all participants. Standard lipid profiles including total cholesterol, TG, and HDL-C were measured by Centers for Disease Control and Prevention standardized direct assay at the University of Washington.[18] LDL-C was calculated via the Friedewald equation.[19] Analyses of glucose were performed with reagents from Roche Diagnostics Corporation (Indianapolis, Indiana). Insulin was measured with a 2-site immunoenzymometric assay. Fasting insulin (performed with an 1800 auto-analyzer; TOSOH Bioscience Inc, South San Francisco, California) and glucose (performed on a P module auto-analyzer by the hexokinase method; Roche) were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR) according to the formula: Glucose * insulin/[μU/L] 22.5.[20]

Lipoprotein particle profiles were measured by NMR spectroscopy with the LipoProfile-3 algorithm from LipoScience, Inc (Raleigh, North Carolina) on frozen EDTA plasma specimens of participants who provided informed consent for ancillary studies. Very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and HDL subclasses were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals, and weighted-average particle sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal.[19] Diameter range estimates for the subclasses were as follows: large VLDL particle (VLDL-P), >60 nm; medium VLDL-P, 35-60 nm; small VLDL-P, 29-35 nm; intermediate-density lipoprotein particles, 23-29 nm; large LDL-P, 20.5-23 nm; small LDL-P, 18-20.5 nm and very small LDL-P <20.5 nm; large HDL particles (HDL-Ps), 9.4-14 nm; medium HDL-P, 8.2-9.4 nm; small HDL-P, 7.3-8.2 nm. VLDL-P, LDL-P, and HDL-P are the totals of the particle numbers of the respective VLDL, LDL, and HDL subclasses. Also reported is a multivariate lipoprotein insulin resistance score (LP-IR), which is derived by combining the 6 lipoprotein subclass and size measures most strongly associated with insulin resistance (large VLDL-P, small LDL-P, large HDL-P, and VLDL, LDL, and HDL size).[21] LP-IR values range from 0 (most insulin sensitive) to 100 (most insulin resistant). Interassay reproducibility (coefficient of variation), determined from 80 replicate analyses of 8 plasma pools over 20 days, was 6% for LP-IR, 8%, 3%, and 2% for total VLDL-P, LDL-P, and HDL-P; 0.7% for LDL and HDL size; 4% for VLDL size; 7%, 13%, and 22% for large, medium, and small VLDL-P; 43%, 12%, and 10% for intermediate-density lipoprotein particles, large, and small LDL-P; and 9%, 14%, and 6% for large, medium, and small HDL-P, respectively.

Pubertal status was self-reported using the Pubertal Development Scale[22] and converted to pubertal stage groups consistent with the 5 pubertal stages outlined by Tanner.[23] Ethnicity and household education were obtained via parental report and sex was self-reported.

Statistical Analyses

We created 7 categories to examine shifts in relative weight categories from grades 6 through 8 (categories with small numbers were collapsed): (1) overweight to obese or severely obese; (2) healthy weight to overweight,