Physical Activity, Fitness, and Serum Leptin Concentrations in Adolescents

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Objective To examine the association of physical activity and fitness with leptin concentrations in European adolescents, after taking into account several potential confounders including total body fat (TBF).

Study design We conducted a cross-sectional study in a school setting for the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. This study included 902 (509 girls) adolescents aged 12.5-17.5 years. Weight, height, and TBF (sum of 6 skinfold thickness) were measured, and fat free mass and body mass index were calculated. Physical activity was assessed by accelerometry. Physical fitness was assessed by the handgrip, standing long jump, 4 × 10-m shuttle run, and 20-m shuttle run tests. Serum fasting leptin, insulin, and glucose concentrations were measured, and homeostasis model assessment was computed. Multiple linear regression models were used.

Results Vigorous physical activity and fitness tests (all $P < .05$) were negatively associated with leptin, independently of several confounders including TBF and homeostasis model assessment. These associations remained significant after further controlling for each other (physical activity and fitness).

Conclusion These results suggest that vigorous physical activity and fitness moderate the levels of leptin concentrations, regardless of relevant confounders including TBF. Intervention programs addressed to increase high intensity physical activity and fitness as well as to assess its impact on leptin concentration are required. *(J Pediatr 2012;160:598-603)*.

Leptin is a cytokine primarily expressed by adipose tissue, which was described as an important regulator of energy homeostasis by means of informing the brain about the body's energy store.[1] Increased serum leptin concentrations are prominent in obese youth.[2] Leptin concentrations are associated with cardiovascular disease risk factors, insulin resistance, and type 2 diabetes in children[3,4] and is an independent risk factor for coronary heart disease in adults.[5]

Physical activity seems to attenuate the negative influence of fatness on several cardiovascular disease risk factors in adolescents.[6,7] The association between physical activity (PA) and leptin in adolescents is contradictory. Several studies showed that PA assessed by questionnaire[8-10] or accelerometers[11] was inversely associated with leptin in adolescents, whereas another found no association between objectively assessed PA and leptin concentrations.[12]

Physical fitness is considered an important marker of health in children and adolescents.[13] We observed negative associations between cardiorespiratory fitness and insulin resistance, blood pressure, and low-grade inflammatory proteins in youth with relatively high levels of total and central body fat.[14-16] However, few studies have analyzed the relationship between fitness and leptin concentrations among adolescents.[11,17,18] In fact, most of these studies are focused on obese adolescents and they used only one fitness component (mainly cardiorespiratory fitness[17] or muscular strength[18]). Studies analyzing the role of objectively measured PA and the main health-related physical fitness components (muscular

FFM	Fat free mass
HELENA-CSS	Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study
HOMA	Homeostasis model assessment
MPA	Moderate physical activity
MET	Metabolic equivalent
MVPA	Moderate to vigorous physical activity
PA	Physical activity
SLJ	Standing long jump
SRT	Shuttle run test
TBF	Total body fat
VPA	Vigorous physical activity

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strength, speed-agility, and cardiorespiratory fitness) on leptin concentrations in a large sample of European adolescents are scarce.

The aim of the present study was to examine the association of PA and fitness with leptin concentrations in European adolescents, after taking into account several potential confounders including total body fat (TBF).

Methods

The Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) is a multicenter study of nutritional habits and patterns, body composition, and levels of PA and fitness in European adolescents aged 12.5-17.5 years.[19] The total sample of the HELENA-CSS included 3528 adolescents; a subset of 1089 of had blood samples. The present study is confined to a sample of 902 adolescents, with complete data on leptin and at least 1 physical fitness test. The adolescents from this subsample of the HELENA-CSS included in the analyses did not differ from those excluded in the study variables (ie, age, sex, weight, height, PA levels, fitness tests) (all $P > .1$).

Ten cities in 9 different European countries included in the HELENA-CSS were chosen to get a rough geographical balance across Europe—Stockholm (Sweden), Athens (Greece), Heraklion (Greece), Rome (Italy), Zaragoza (Spain), Pecs (Hungary), Ghent (Belgium) Lille (France), Dortmund (Germany), and Vienna (Austria).[19] Data collection took place between October 2006 and December 2007. Parents and adolescents gave their written informed consent to participate in the research. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), the Good Clinical Practice, and the legislation regarding clinical research in humans in each of the participating countries. The protocol was approved by the Human Research Review Committees of the involved centers.

The anthropometric measurement protocols followed in the HELENA-CSS were described in detail by Nagy et al.[20] Weight was measured in underwear and without shoes with an electronic scale (Type SECA 861; Seca, Hamburg, Germany) to the nearest 0.05 kg, and height was measured barefoot with the head in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. Body mass index was calculated as body weight (kg) divided by the height (m) squared (kg/m^2). Skinfold thicknesses were measured to the nearest 0.2 mm in triplicate in the left side at biceps, triceps, subscapular, suprailiac, thigh, and medial calf with a Holtain Caliper (Crymmych, United Kingdom).[21] The sum of 6 skinfold thickness measurements was used as an indicator of TBF. We calculated body fat percentage using skinfold thickness from the Slaughter equation,[22] and fat free mass (kg) (FFM) was derived by subtracting fat mass from total body weight. In every city, the same trained investigator made all skinfold thickness measurements. For all the skinfold thickness measurements, intraobserver technical errors of measurement were <1 mm and reliability was >95%. Interobserver reliability for skinfold measurements was >90%.[20]

Pubertal status was assessed by a physician according to Tanner and Whitehouse.[23] Systolic blood pressure was measured with an automatic oscilometric device (M6, HEM-7001-E; Omron, Kyoto, Japan).

A detailed description of the blood samples analysis has been reported elsewhere.[24] Venous blood was obtained by venipuncture after an overnight fast. Serum was aliquoted and frozen at 18-25°C until assays were performed. The concentration of serum leptin (ng/mL) was measured using the RayBio Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay; RayBiotech, Norcross, Georgia) kit. The sensitivity of the leptin assay was <6 pg/mL, with intra-assay and interassay coefficients of variation of <10% and <12%. The homeostasis model assessment (HOMA) was calculated as [fasting insulin (μlU/mL) × fasting glucose (mmol/L)]/22.5.

The Actigraph accelerometer (Actigraph MTI, model GT1M, Manufacturing Technology Inc, Fort Walton Beach, Florida) was used to assess PA.[25] Prior to data collection, the adolescents were instructed on how to handle the accelerometer. Adolescents were asked to wear the accelerometer during the daytime for 7 consecutive days, except during water-based activities. The criterion for inclusion was to record at least 8 hours per day, for at least 3 days.[26]

In this study, the time sampling interval (epoch) was set at 15 seconds.[25] A measure of average volume of activity (hereafter called average PA) was expressed as the sum of recorded counts divided by total daily registered time expressed in minutes (counts/min). The time engaged in moderate physical activity and vigorous physical activity (MPA and VPA, respectively) was calculated and presented as the average time per day during the complete registration. The time engaged at MPA [3-6 metabolic equivalents] was calculated based upon a cut-off of 2000-3999 counts/min. The lower cut-off for MPA (2000 counts/min) is equivalent to walking at 3 km/h. The time engaged at VPA (>6 Metabolic equivalents) was calculated based upon a cut-off of ≥4000 counts/min. We calculated the time spent in at least moderate intensity level (>3 Metabolic equivalents) as the sum of time spent in moderate to vigorous physical activity (MVPA).[25]

The scientific rationale for the selection of the fitness tests, as well as their validity and reliability in young people, has been published elsewhere.[27,28] All the tests were performed twice and the best score was retained, except the 20-m shuttle run test (SRT), which was performed only once. Physical fitness was assessed by the tests described next.

Handgrip Test (Maximum Handgrip Strength)

A hand dynamometer with adjustable grip was used (TKK 5101 Grip D; Takey, Tokyo, Japan). The adolescent squeezes gradually and continuously for at least 2 seconds, performing the test with the right and left hand alternatively, using the optimal grip-span. The maximum score in kilograms for each hand was recorded. The sum of the maximum scores achieved by left and right hands was used in the analysis.