

## Original Research

## Leontio Lyceum ALbuminuria (3L Study) Epidemiological Study: Aims, Design and Preliminary Findings

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**Introduction:** The significance of microalbuminuria (MA) in paediatric essential hypertension has yet to be established. The Leontio Lyceum ALbuminuria Study (3L Study) was designed to determine the prevalence of MA among Greek schoolchildren and to evaluate these rates in relation to the children's anthropometric and lifestyle characteristics, and dietary habits.

**Methods:** During April 2009, 498 students from the Leontio Lyceum, aged 12-17 years (7th-12th grade), were asked to participate in the 3L Study. For each child a questionnaire was completed that was developed for the purposes of the study to retrieve information on socio-demographic and lifestyle characteristics, as well as dietary habits (through a semi-quantitative Food Frequency Questionnaire), and physical activity status. Overweight and obesity were defined using the international body mass index cut-off points established for children and young people. Office blood pressure (BP) was measured on two different occasions and those students who had BP >95th percentile for gender, age and height on both occasions were considered as hypertensives. Microalbuminuria was determined as albumin to creatinine ratio  $\geq 22$  mg/g in boys and  $\geq 31$  mg/g in girls in a morning spot urine sample using a quantitative assay (DCA 2000).

**Results:** The prevalence of MA was found to be 12.9% and that of childhood hypertension 5.2%. The prevalence of overweight status was 25.8% and 5.8% of the students were classified as obese. Low physical activity was reported by 7% of boys and girls, while 46.5% of the students reported participation in vigorous physical activities during a normal week. Based on the KIDMED score of each student, only 6% of them were classified as high adherers to a Mediterranean diet and 41.9% were classified as having very low diet quality.

**Conclusions:** In this paper we present the aims, design and preliminary results of an epidemiological study on MA, hypertension, increased body size and lifestyle characteristics among Greek schoolchildren.

**P**rospective and epidemiological studies in adults have found that microalbuminuria (MA) constitutes an established marker of endothelial dysfunction and cardiovascular disease.<sup>1-5</sup> Even levels of albuminuria below the conventional MA cut-off points are independently predictive of cardiovascular events.<sup>6</sup> Data from large population-based studies

indicate a prevalence of MA around 20-30% in patients with diabetes mellitus, 11-17% in hypertensives and 5-15% in the general population.<sup>7,8</sup>

MA has been utilised as a screening test for the presence of diabetes-related kidney disease in children with type 1 diabetes mellitus.<sup>9</sup> Moreover, increased urinary albumin excretion in children has been associated

with obesity-related cardiovascular risk factors, such as impaired glucose tolerance and hypercholesterolaemia.<sup>10</sup> The prevalence of MA in children differs from that in adults. It seems to be greater in girls and in lower age-groups, while a great percentage of children with MA (30-50%) may have transient, non-repetitive MA due to strenuous exercise and emotional stress.<sup>8,11-14</sup> There are few data concerning the urinary albumin excretion rate in non-diabetic children and adolescents, and consequently there is no generally accepted normal range. Furthermore, there are absolutely no studies correlating MA with levels of blood pressure (BP) and body size, or with dietary characteristics, physical activity, and sedentary behaviour in school age adolescents.

Taking everything into account, the significance of MA in paediatric essential hypertension has yet to be established. Thus, the Leontio Lyceum ALbuminuria Study (3L Study) was designed to determine the prevalence of MA among Greek schoolchildren and to evaluate these rates in relation to anthropometric, lifestyle characteristics and dietary habits of the children. This report illustrates the 3L study's aims, design and methods used, as well as the status of various baseline characteristics of the participants.

## Methods

The Leontio Lyceum ALbuminuria Study (3L Study) study is an epidemiological cross-sectional survey that has as main research focus the prevalence of albuminuria in Greek adolescents in relation with their BP levels, level of physical activity, dietary habits, and socio-demographic characteristics.

The study, including data collection, was designed according to the principles of the declaration of Helsinki (1989). The study protocol was approved by the Education Institute of the Hellenic Ministry of Education (111799/Γ2/2-9-2008), as well as the ethics committee of our institution (ΕΣ 12/11-6-2008). The study was funded by research grants from the Hellenic Cardiological Society, Athens, Greece.

## Study sample

During April 2009, 650 students from the Leontio Lyceum, aged 12-17 years (7th-12th grade), were asked to participate in the 3L Study. The adolescents were enrolled on a voluntary basis. Prior to acceptance, the children's parents or guardians were fully informed about the objectives and methods of the study and gave

their informed written consent. Of the initial student population, 498 finally agreed to participate in the study (response rate almost 80%). All participants were interviewed and evaluated by trained personnel (cardiologists, internists, paediatricians, and general practitioners) who used a standard questionnaire with closed-ended questions.<sup>15</sup> The questionnaire was developed for the purposes of the study in order to retrieve information about demographic, socio-economic and lifestyle characteristics, as well as dietary habits and physical activity.

The number of enrolled children was adequate (statistical power: 80%) to evaluate two-sided hypotheses regarding standardised differences between various balanced groups (such as obese vs. non-obese children, physically active vs. inactive, etc.) greater than 0.5 at the probability level of  $p < 0.05$ .

## Anthropometric and socio-demographic measurements

Two trained physicians were in charge of measuring standing height using stadiometers (Raven Equipment Limited, Essex, UK) to the nearest 0.1 cm, with the students wearing light clothing and without shoes, and body weight to the nearest 0.1 kg on calibrated digital scales (Seca, Hanover, Germany). The stadiometers and balances were periodically calibrated. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest and hip circumference was measured at the trochanter level. Both circumferences were measured to the nearest 0.5 cm with a plastic tape and the ratio between them provided the waist/hip ratio. The measurements took place in the school setting before the interview. Overweight and obesity were defined using the international body mass index (BMI) cut-off points for children and young people proposed by the Childhood Obesity Working Group of the International Obesity Task Force (IOTF).<sup>16</sup> These cut-off points are based on health-related adult definitions of overweight ( $\geq 25 \text{ kg/m}^2$ ) and obesity ( $\geq 30 \text{ kg/m}^2$ ) but are adjusted to specific age and sex categories for children. Complete growth measurements were recorded in all participants (N=498).

## Measurement of BP

Office BP was measured by trained physicians on two different occasions during a routine school day. The first measurement was obtained at the end of the interview, after completion of the study questionnaire, and the second one at the end of the study protocol.

Measurements were taken using validated automated oscillometric devices (Omron 705 IT; Omron Healthcare Europe BV, Hoofddorp, The Netherlands) with the student in the sitting position after 5 minutes' rest. Three consecutive measurements of BP and heart rate were taken at heart level on each arm (a total of 12 measurements for every student). For an accurate measurement an appropriate cuff size was used. The bladder of the cuff covered at least 80% and up to 100% of the arm circumference and the width about half of its length. In the paediatric population the equipment necessary included three paediatric cuffs of different sizes (inflatable bladder size 9 × 16 cm, 13 × 23 cm, 15 × 30 cm), one standard adult cuff and one oversized cuff. For each student, after calculating the mean BP value and the respective standard deviation for each adolescent separately, we excluded those values exceeding mean BP value ± two standard deviations. Consequently, the average of the remaining measurements was computed for each occasion separately (BP1 and BP2, respectively), as well as the average of BP1 and BP2. According to the 2004 guidelines of the US National Heart, Lung, and Blood Institute for hypertension in children and adolescents, those students who had BP >95th percentile for gender, age and height on both occasions (BP1 and BP2) were considered as hypertensives.<sup>17,18</sup>

### ***Demographic, socio-economic and lifestyle characteristics***

Using a questionnaire developed for the purposes of the study, we retrieved information about age, sex, number of siblings, several indicators of the family's socio-economic status (i.e. the existence of a separate bedroom for each child in the family) and several adolescents' lifestyle characteristics, such as their smoking habits (i.e. if they were current smokers and how many cigarettes smoked per day), time spent on sedentary activities (i.e. watching television, working on a computer, playing video games and studying), the frequency and duration of adolescents' physical activities (brisk walking, swimming, etc), as well as information on the children's hospitalisations. The socio-economic status indicators have been extensively used as indices of a family's economic status in developed societies.

### ***Evaluation of dietary habits***

The 3L Study also included a semi-quantitative Food Frequency Questionnaire (FFQ) that was distributed

to all adolescents and gathered information regarding the participants' dietary habits on a daily or weekly basis. The FFQ results have been found to be repeatable in previous applications.<sup>15,19</sup> The subjects were asked to recall the dietary habits that they had followed for the past year. Before completing the FFQ, an explanatory session on how the FFQ has to be filled out was given by trained physicians. Then, the interviewer went through the food list again to clarify entries. Various foods and beverages usually consumed in Greece and habits pertaining to mealtime behaviour were recorded in 63 detailed descriptive questions.

In particular, the measurements included the weekly or daily intake of dairy products with breakfast, the frequency of breakfast consumption, the frequency of consumption of cereals with breakfast, the daily consumption of meals including snacks (eating episodes), the frequency of consumption of foods outside of the home (including school canteens and meals not made at home), the family cooking method most often used, the type of oils/fat consumed, the frequency of sweet and "salty" snacks consumed, the frequency of consumption of certain specific items – fish, poultry, red meat, eggs, white bread, whole grain bread, potatoes, rice, fruits, vegetables, fruit juices, soft drinks, beverages – and of traditional Greek cooked meals (e.g. we asked questions such as, "How many glasses of milk do you usually drink?" with possible answers: none or 1 glass per month, 1 per week, 2-6 per week, 1 per day, 2-3 per day or ≥4 per day). Emphasis was placed on the seasonality of consumption with specific questions to the participants. The main categories (including their subcategories) of the abovementioned foods and beverages consumed were coded as follows: 1) dairy products: all kinds of milk, all types of yoghurt, and all types of cheese; 2) snack food was divided into two main categories: "salty" snacks (e.g. fast-food items such as a hamburger, slice of pizza, a hot dog, a toasted sandwich, a piece of cheese pie, a piece of spinach pie, all kinds of potato chips, savoury snacks, popcorn) and "sweet" foods (e.g. ice-cream, milk shake, all kinds of chocolates, croissant, cakes, biscuits); 3) all types of soft drinks (still, fizzy, diet, etc.); 4) fruit juices (fresh or ready to drink, 100% juice without added sugars); 5) beverages (tea, chamomile tea, etc.); and 6) traditional Greek cooked foods (moussaka, beans, etc.). When the FFQs were completed, the subjects indicated how often, on average, they consumed the amount of each food item. The categorisation of the frequency of

consumption was not uniform and varied according to the type of food/drink consumed.

In addition, based on the above questionnaire, an index (KIDMED) was developed that estimates the level of adherence to the Mediterranean diet. The index ranged from 0 to 12, and was based on a 16-question test. Questions denoting a negative connotation with respect to the Mediterranean diet were assigned a value of -1, and those with a positive aspect +1. The sums of the values from the administered test were classified into three levels: (1) >8, optimal Mediterranean diet; (2) 4-7, improvement needed to adjust intake to Mediterranean patterns; (3) ≤3, very low diet quality.<sup>20</sup>

### Evaluation of physical activity

Information about the frequency and duration of physical activities (brisk walking, swimming, etc.) and on the amount of time spent on sedentary activities (watching television, working on a computer, playing video games) was retrieved from the study's questionnaire.

To ascertain the physical activity status, we used the short version of the International Physical Activity Questionnaire (IPAQ) as an index of weekly energy expenditure, using frequency (times per week), duration (in minutes per time) and intensity of sports or other habits related to physical activity.<sup>21-23</sup> Specifically, intensity was gradated in qualitative terms, such as light (expended calories <4 Kcal/min, i.e. walking slowly, stationary cycling, light stretching, etc.), moderate (expended calories 4-7 Kcal/min, i.e. walking briskly, cycling outdoors, swimming with moderate effort, etc.) and high (expended calories >7 Kcal/min, i.e. walking briskly uphill, long-distance running, fast cycling or racing, swimming fast crawl, etc.). For each participant, data collected with the IPAQ form were reported as median MET-minutes for walking, moderate-intensity activities and vigorous-intensity activities, using standard formulas. An overall total physical activity MET-minutes/week score was also computed as the sum of Walking + Moderate + Vigorous intensity activities. Based on the IPAQ criteria, there are three levels of physical activity proposed to classify populations: low, moderate, and high.

### Albuminuria assessment

In all students, urinary albumin excretion was determined in a morning spot urine sample using a quantitative assay (DCA 2000, Bayer Diagnostics Europe, Dublin, Ireland) and expressed as the albumin-to-crea-

tinine ratio (ACR). Microalbuminuria was defined as ACR ≥22 mg/g in boys and ≥31 mg/g in girls.<sup>24</sup>

### Statistical analysis

Continuous variables are presented as mean values ± one standard deviation, skewed variables are presented as median and quartiles, while qualitative variables are presented as absolute and relative frequencies. Contingency tables with the calculation of chi-square test were used to evaluate the associations between categorical variables. All reported p-values are based on two-sided tests and were compared to a significance level of 5%. SPSS version 11.0 software (Statistical Package for Social Sciences, SPSS Inc., Illinois, USA) was used for all the statistical calculations.

## Results

### Anthropometric and BP characteristics of the participants

A total of 498 students were recruited: 304 boys (61.2%) and 194 girls (38.8%). Mean age was 14.1 ± 1.6 years (range 12-17.9 years), height 165.9 ± 9.6 cm (142-94 cm), weight 60.7 ± 13.6 kg (27-107 kg), waist circumference 76.8 ± 9.6 cm (53-116 cm), and mean BMI was 21.85 ± 3.5 kg/m<sup>2</sup> (13.3-36.9 kg/m<sup>2</sup>) (Table 1). According to the international BMI cut-off points for children and youth, 25.8% of the studied adolescents were overweight and 5.8% of them were classified as obese (Figure 1).

The average systolic BP of BP1 and BP2 was 116.5 ± 11.3 mmHg; average diastolic BP was 67.2 ± 6.9 mmHg, average pulse pressure 49.3 ± 8.6 mmHg and average heart rate 84.2 ± 11.9 /min (Table 1). Based on levels of BP1, 12.9% of the studied population ex-

**Table 1.** Anthropometric, demographic and blood pressure characteristics of the studied adolescents.

Variable	Mean ± SD
Age (years)	14.1 ± 1.6
Males (%)	304 (61.2%)
Height (cm)	165.9 ± 9.6
Weight (kg)	60.7 ± 13.6
Waist (cm)	76.8 ± 9.6
Body mass index (kg/m <sup>2</sup> )	21.85 ± 3.5
Average systolic blood pressure (mmHg)	116.5 ± 11.3
Average diastolic blood pressure (mmHg)	67.2 ± 6.9
Average pulse pressure (mmHg)	49.3 ± 8.6
Average heart rate (/min)	84.2 ± 11.9

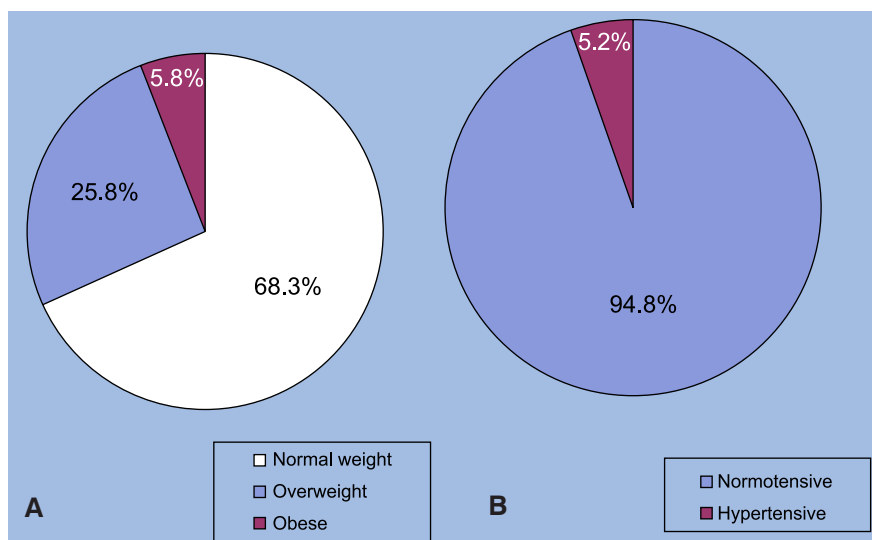


Figure 1. Pie charts depicting the prevalence of overweight/obesity status (A) and hypertension (B).

hibited values greater than 95th percentile for age, gender and height, 6.5% had values between 90th and 95th percentile, while 80.6% had BP <90th percentile. Based on levels of BP2, 8.5% had BP >95th percentile, 5.6% presented values between 90th and 95th percentile and 85.9% exhibited BP <90th percentile for age, gender and height. Accordingly, 26 students (5.2%) exhibited both BP1 and BP2 values >95th percentile for age, gender and height and therefore were considered as hypertensives (Figure 1).

**Socio-demographic and lifestyle characteristics of the participants**

Table 2 presents various socio-demographic and lifestyle characteristics of the children. A family history of hypertension (parents) was reported in 108 children (21.8%). Six out of 7 children (83.7%) reported that they had their own room, indicating moderate to good financial status. As far as smoking status is concerned, 84 students (16.8%) reported that they had tried smoking at least once, and 15 of them (3.0%) were current smokers. In addition, 64.2% of the children reported that at least one of their parents was a smoker.

Low physical activity was reported by 7% of boys and girls, while 46.5% of the students reported that they participated in moderate and 46.5% in vigorous physical activities during a regular week (Figure 2). The time spent on sports related activities during the week was 5 hours, and 3 hours were devoted to non-sports related activities. Children also reported that they spent 2 hours per day watching television or playing video games.

The percentage of students who reported having breakfast every day was 71.9%, while 59.5% had more than 3 meals per day, consumed on a weekly basis (Table 2). The average KIDMED score was  $4.05 \pm 2.2$ . Based on the KIDMED score of each student, only 6% of them were classified as high adherers to Mediterranean diet, 52.1% showed average values of the index and 41.9% were classified as having very low diet quality (Figure 2).

Finally, urine samples were provided by 465 students (93.4%) and the prevalence of MA was found to be 12.9%. With respect to gender differences, 11% of boys and 16.3% of girls exhibited increased urine ACR.

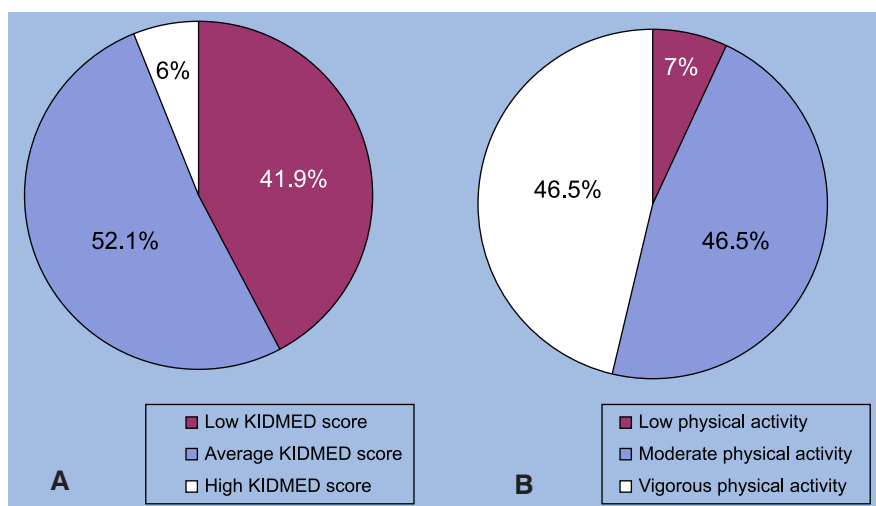
**Discussion**

In this paper we present the aims, design and preliminary results of an epidemiological study of MA, hy-

Table 2. Socio-demographic and lifestyle characteristics of the studied adolescents. Values other than percentages are given as median and quartiles.

Variable	
Own room (%)	83.7
Tried smoking (%)	16.8
Current smokers (%)	3
Parents smoke (%)	64.2
Hours of sports activities/week	5, 3-8
Hours of activities excluding sports/week	3, 0-6
Hours of watching TV/electronic games/day	2, 1-2
Eating breakfast every day (%)	71.9
Eating >3 meals per day (%)	59.5





**Figure 2.** Pie charts depicting the classification of schoolchildren according to their adherence to the Mediterranean diet (A) and according to their physical activity status (B).

pertension, increased body weight, and physical activity among schoolchildren, which was conducted in the Leontio Lyceum, Greece (3L Study). We also present the basic characteristics of children participating in the study regarding socio-demographic status, dietary and other lifestyle habits.

The prevalence of MA in the whole study population appears to be in the higher range, based on data derived from the general adult population, while the increased urine albumin excretion rate in girls compared to boys is in accordance with previous studies.<sup>8,11</sup>

The issue of hypertension in children has undergone substantial conceptual change during the past 2 decades. The prevalence of childhood hypertension depends on the methodology used and the number of measurements on different occasions, and is reported to vary from 5% in studies that included ambulatory and home BP monitoring up to 25% in studies based on the average of 3 measurements on a single occasion.<sup>25-27</sup> This was also the case in our study population, since the prevalence of hypertension was around 5%, taking into account two separate measurement occasions.

With respect to the prevalence of obesity and overweight status in the present study, our findings are in line with the existing research evidence in Greece, which suggests an alarmingly high prevalence of increased body size among Greek schoolchildren,<sup>15,28-35</sup> although not all studies were based on the IOTF criteria. In addition, only 6% of our study population were classified as high adherers to the Mediterranean diet, while 42% had a poor KIDMED score, values similar to those reported in a recent survey among Cypriot children.<sup>36</sup>

A number of different measurement approaches have been described for assessing children's physical status. We chose the short-form IPAQ, since it has been used more often in young populations. This instrument provided satisfactory results for physical activity levels in the Greek schoolchildren of our study.

The present study has certain limitations, which should be taken into account before conclusions are drawn from its results. The cross-sectional nature of the study does not permit causal inferences, but indications can be derived that may be valuable in future investigations. Furthermore, although we made every effort to get as accurate data as possible, there is a possibility that misreporting has occurred, which might have influenced our findings.

To the best of our knowledge this is the first epidemiological study to evaluate BP levels, body size, dietary habits and physical activity in relation to the prevalence of MA among school-aged children in Greece. We anticipate that further analysis of the collected data will provide current, novel and valuable information on the interrelations between diet, lifestyle, physical activity, obesity, hypertension and urine albumin excretion in Greek school children. Public health policy makers and other health care professionals should urgently focus their attention on schoolchildren in order to reduce the burden of cardiovascular risk factors in the future.

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