IntRoductIon

The continuous increase in mortality indices from cardiovascular diseases, in middle-aged men and women in Greece, is closely associated with risk factors that initiate in childhood. The gradual detachment from the traditional lifestyle and diet of the Greek population over the past decades has been stated to be responsible for the rapid increase in premature deaths from cardiovascular disease (CVD).[1,2]

Greece is one of the leading EU countries in the prevalence of childhood obesity.[3] The presence of overweight and obesity in children often co-exists with other CVD risk factors like hyperlipidemia, increased arterial blood pressure, and increased fasting glucose. Coexistence of three or four of these cardiovascular risk factors in children has been found to be related to an increase in fibrous plaque within the arterial endothelium, which may lead to the development of atheromatique plaques in adulthood.[4,5]

Overweight and obese children [body mass index (BMI) > eighty-fifth percentile] have a much higher possibility of increased total cholesterol (TCHOL) when compared to children under the eighty-fifth percentile, while they are also at a risk for the development of a non-ideal lipid and glycemic profile.[4] In addition, studies have pointed out that positive anthropometric indices for overweight and obesity are associated with at least two other atherogenic risk factors in half of the preschool children.[6] This is of
public health concern, as the importance of the monitoring of atherogenic risk factors from the age of preschool was elucidated within the Bogalusa Heart Study, where serum lipoproteins followed the same trend from the preschool period all the way into adulthood.\(^7\)

Taking this into account, the present study has aimed to identify the distribution, clustering, and characteristics of atherogenic risk factors among preschool children of six municipalities in the prefecture of Chania, Crete, Greece.

**Materials and Methods**

This cross-sectional study was conducted in 2005 in all public kindergartens of six municipalities out of total 23 in the prefecture of Chania that cover a population of 81,719 inhabitants in Chania, Crete, Greece.

**Sampling**

The duration of the study was 4 months (January-May 2005), with detailed evaluation of health, nutrition and development of a representative sample of 1189 children (591 boys and 598 girls), aged 4-7 years (mean age 5.3 ± 0.6 years).

Children’s examinations were conducted in the preschools with the approval of the Greek Ministry of Education and the Ethics Committee of the +University of Crete. Parents gave written consent for the children’s participation after having been fully informed. Further information on the sampling framework can be found elsewhere.\(^8\)

**Training**

Prior to data collection, the research team (comprised of pediatricians, nurses, health visitors, and social workers) underwent training by the study group of the Preventive Medicine and Nutrition Clinic, at the University of Crete, in January 2005, in order to examine all children.

**Blood collection and analysis**

Venous blood samples were taken from 603 preschool children, > five years old, for biochemical screening tests following a 12 h overnight fast. For ethical reasons we did not take blood samples from children < five years old. From each child 3 ml of blood, with Ethylenediaminetetraacetic acid (EDTA), was collected for hematological examinations, and 5 ml of blood, without anticoagulant, for serum separation. Glucose was measured by the enzymatical method GOD-PAP.\(^10\) The precise measurement method for TC\(HOL\), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) is described in previous published studies.\(^10,11\) Finally, the TCHOL / HDL-C ratio was estimated. The cut-off points used in hyperglycemia were blood glucose ≥ 100 mg / dl,\(^12\) and in hypertriglyceridemia as TG ≥ 100 mg / dl and low HDL-C till < tenth percentile, for age and gender.\(^13\)

**Anthropometric measurements**

Weight, height, waist circumference (WC) and hip circumference (HC) were measured in 1135 children, aged 4-7 years, while the waist to hip circumference and waist circumference to height ratios were calculated. The analytical description of the anthropometric methodology is described in depth elsewhere.\(^14\) BMI was calculated by dividing weight (kg) by height squared (m\(^2\)). The anthropometric categorization of children was made according to International Obesity Taskforce.\(^15\) Central obesity was defined as WC higher than the 90th percentile for age and sex.\(^14\)

**Arterial blood pressure measurement**

Blood pressure was measured in 1134 children, aged 4-7 years, in a sitting position and after 5-10 minutes rest using a mercury manometer. Hypertension was defined as systolic blood pressure (SBP) and / or diastolic blood pressure (DBP) ≥ 95\(^{\text{th}}\) percentile for age, gender, and height on at least three separate occasions.\(^17\)

**Statistics**

Means and standard deviations were used to describe anthropometric measurements, lipoproteins, glucose, and arterial blood pressure by sex. Spearman’s correlation coefficients by sex were also used for the above-mentioned parameters. The Chi-square (\(\chi^2\)) test was used for the children’s distribution by normal, increased, and pathological levels of lipoproteins, glucose, and anthropometric measures. The Student t-test was used to test lipoprotein and glucose levels within the sexes in two age groups: 5.1 – 6.0 and 6.1 – 7.0 years. Analysis of Covariance (ANCOVA) was used to estimate the linear trend in arterial blood pressure and anthropometric measures for normal, increased, and pathological levels of lipoproteins and glucose, with sex and age as covariates. Heterogeneity was tested by the Levene’s test. In children with pathological glucose and lipoprotein levels, logistic regression analysis was used for estimating the risk (Odds Ratios), as also 95% confidence intervals. The estimation was determined in relation to those children who had pathological levels of arterial blood pressure, central obesity, and BMI that assigned overweight and obesity. Age was the control variable. Finally, one sample Student test was used for the comparison of findings between the present study and other studies. Data were analyzed with the use of SPSS statistical package (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL, USA).
**Results**

In this study, hypertension was found in 7.4% of the boys and 7.9% of the girls. Overweight and obesity affected 16.6 and 10.8% of the boys and 19.5 and 9.0% of the girls respectively; these differences were not significant. Only 1.3% from the total children was found to have increased blood glucose levels.

Descriptive values of lipoproteins and glucose, per age and sex, of the preschool children (> 5 years old) of Chania, in 2005, are given in Table 1. Boys and girls had similar figures for TCHOL, LDL-C, TCHOL / HDL-C, but girls aged between five and six years, had significantly higher TG values ($P < 0.05$). Also, among girls, notable lower glucose values were found in all ages ($P < 0.05$).

The distribution of lipoprotein levels in children of the prefecture of Chania: Increased levels of TCHOL were observed in 12.7% of the boys and 16.3% of the girls [Table 2]; however, these differences were not statistically significant. Similarly, increased LDL-C levels were also noted in both boys (11.1%) and girls (16.7%). According to the above-mentioned results, we were able to classify the preschool children into groups according to the prevalence of risk factors for the development of atherogenesis, by which one factor was found in 16.8% ($n = 50$) of the boys and 19.9% ($n = 55$) of the girls, two of these factors were observed in 5.1% ($n = 15$) of boys and 6.9% ($n = 19$) of girls, while three or more factors were found in 2.7% ($n = 8$) of boys ($P = 0.026$).

Arterial blood pressure and anthropometric measurements, in relation to normal, increased, and pathological lipoprotein and glucose levels of the study participants are shown in Table 3. The glucose levels were significantly correlated with SBP ($P < 0.05$). Significant correlations were observed between TG and WC ($P < 0.05$), BMI ($P < 0.05$), and waist-to-length ratio ($P < 0.05$). Negative correlations were found between HDL-C and WC ($P < 0.05$), BMI ($P < 0.05$), waist-to-hip ratio ($P < 0.05$), and waist-to-height ratio ($P < 0.05$).

Logistic regression analysis showed that children with central obesity as a WC > ninetieth percentile were found to have 3.43 times increased risk (95% CI: 1.28 – 9.17, $P = 0.014$) for hypertriglyceridemia (data not presented). Children with BMI > 30 kg/m², had a 2.87-fold higher risk (95% CI: 1.05 – 7.85, $P = 0.041$) for hypertriglyceridemia.

**Discussion**

The present study indicated that 77% of the preschool children had an increment in at least one risk factor for atherogenesis. The factors with the highest prevalence were overweight (18.1%) and obesity (9.9%) followed by hyperlipidemia (about 15%) and hypertension (7.7%), while seven of the 603 children had increased blood glucose. The high frequency of risk factors for atherogenesis, from such a small age, consists of an alarming finding and confirms the rise of obesity and its accompanying atherogenic factors among young children in Greece.

A previous study, from 1982, carried out in Heraklion, Greece / Crete, on a cohort of children and adolescents aged between nine and sixteen years found that the incidence of overweight and obesity was 20%, while a comparable study published in 2002 showed that 40% of the children were overweight and obese. However, the body weight of younger children aged 5.5 – 7 years in the present study did not differ compared to the children of similar age in the prefecture of Chania, who were examined when registered in the first grade of all primary schools in the prefecture of Chania in 1992 – 1993.
The increase in atherogenic factors such as overweight or hypertension at young ages is also observed in other countries. In Germany, a cross-sectional study of 127,735 children aged five to six years conducted in Bavaria in 1997,\textsuperscript{[20]} showed that 12.3% of children were overweight in comparison to 8.5% in 1982. In the present study, in children of the same age group, 17.4% were overweight and 11% obese. Similar percentages were also noticed for overweight and obese children in Canada, among preschool children aged three to five years,\textsuperscript{[21]} (17.7 versus 18.1% and 8 versus 8.1%). In the Bogalusa study,\textsuperscript{[6]} only 6.5% of the children aged five to seven years were observed to be overweight. However, assessing the clustering of atherogenic risk factors in the Bogalusa study indicated that the odds ratio in overweight children (BMI > 95th percentile) was at a higher risk to develop hypercholesterolemia (TCHOL > 200 mg / dl), while such an association did not exist in the present study. Similar trends in BMI were observed among Indian children (18.2% were overweight and obese) putting them at increased risk for cardiovascular disease in their adulthood.\textsuperscript{[22]}

Regarding the levels of SBP and DBP in our study the children had significantly lower levels compared to those (5 – 6 years old) from Italy.\textsuperscript{[23]}

One study in Athens among children, in 1995 – 1996,\textsuperscript{[24]} indicated that those aged six to seven years had significantly lower TCHOL and LDL-C levels, compared to children of the same age in the present study, while they had significantly higher HDL-C levels and TG. Compared to children aged three to six years from another study conducted in Tehran involving a group of 3,148 children (3-19 years old), in 1999 – 2000,\textsuperscript{[25]} the children of our study had significantly lower TG and LDL-C levels and considerably higher HDL-C levels.

| Table 2: Distribution of lipoprotein levels in children in the prefecture of Chania, Crete, Greece in 2005 |
|---------------------------------------------------------------|
| Serum Lipoproteins | Boys | Girls | Total |
| TCHOL (mg / dl)  | N (%) | N (%) | N (%) |
| ≤ 170 | 169 (54.9) | 142 (48.1) | 311 (51.6) |
| 171 – 200 | 100 (32.5) | 105 (35.6) | 205 (34.0) |
| > 200 | 39 (12.7) | 48 (16.3) | 87 (14.4) |
| LDL-C (mg / dl) | N (%) | N (%) | N (%) |
| ≤ 110 | 205 (66.8) | 180 (61.2) | 385 (64.1) |
| 111 – 130 | 68 (22.1) | 65 (22.1) | 133 (22.1) |
| > 130 | 34 (11.1) | 49 (16.7) | 83 (13.8) |
| HDL-C (mg / dl) | N (%) | N (%) | N (%) |
| ≤ 40 | 13 (4.2) | 15 (5.1) | 28 (4.6) |
| 40 – 45 | 30 (9.7) | 27 (9.2) | 57 (9.5) |
| 45 – 60 | 161 (52.3) | 141 (47.8) | 302 (50.1) |
| > 60 | 104 (33.8) | 112 (38.0) | 216 (35.8) |
| TG (mg / dl) | N (%) | N (%) | N (%) |
| ≤ 80 | 284 (92.5) | 265 (90.1) | 549 (91.3) |
| 81 – 100 | 13 (4.2) | 14 (4.8) | 27 (4.5) |
| > 100 | 10 (3.3) | 15 (5.1) | 25 (4.2) |
| TCHOL / HDL-C | N (%) | N (%) | N (%) |
| ≤ 3.00 | 164 (53.2) | 152 (51.6) | 316 (52.4) |
| 3.01 – 4.00 | 124 (40.3) | 114 (38.6) | 238 (39.5) |
| > 4.00 | 20 (6.5) | 29 (9.8) | 49 (8.1) |

| Table 3: Arterial blood pressure and anthropometric measurements in relation to glucose and serum lipoproteins in children in the prefecture of Chania, Crete, Greece in 2005 |
|-------------------------------|
| N | SBP (mm Hg) | DBP (mm Hg) | Weight (kg) | Height (cm) | WC (cm) | BMI (kg / m²) | W / H | W / H |
|-------------------------------|
| Mean ± SD | 94.4 ± 0.5* | 60.9 ± 0.5 | 22.4 ± 0.2* | 117.1 ± 0.3* | 54.8 ± 0.4 | 16.2 ± 0.1 | 0.89 ± 0.01 | 0.47 ± 0.01 |
| GLU (mg / dl) | 295 | 233 | 50 | 98.7 ± 1.3 | 63.2 ± 1.1 | 24.1 ± 0.6 | 119.5 ± 0.7 | 56.4 ± 0.9 | 16.8 ± 0.3 | 0.88 ± 0.01 | 0.47 ± 0.01 |
| ≤ 50 percentile | 306 | 192 | 80 | 94.5 ± 1.0 | 60.3 ± 0.9 | 23.0 ± 0.5 | 117.2 ± 0.6 | 55.4 ± 0.7 | 16.7 ± 0.3 | 0.87 ± 0.01 | 0.47 ± 0.01 |
| 50 – 90 percentile | 171 – 200 | 80 | 95.4 ± 0.4 | 61.4 ± 0.3 | 23.2 ± 0.2 | 117.9 ± 0.2 | 55.9 ± 0.3* | 16.6 ± 0.1* | 0.89 ± 0.01 | 0.47 ± 0.01* |
| TCHOL (mg / dl) | 24 | 22 | 22 | 96.7 ± 1.9 | 62.3 ± 1.7 | 25.5 ± 0.9 | 118.7 ± 1.1 | 59.7 ± 1.4 | 17.9 ± 0.5 | 0.91 ± 0.02 | 0.50 ± 0.01 |
| ≤ 45 | 70 | 60 | 60 | 95.6 ± 0.5 | 61.1 ± 0.5 | 23.4 ± 0.2 | 117.8 ± 0.3 | 56.3 ± 0.4 | 16.7 ± 0.1 | 0.89 ± 0.01 | 0.48 ± 0.01 |
| TG (mg / dl) | 201 | 207 | 207 | 95.3 ± 6.1 | 66.6 ± 0.9 | 22.9 ± 0.3 | 117.7 ± 0.4 | 55.3 ± 0.4 | 16.5 ± 0.2 | 0.88 ± 0.01 | 0.47 ± 0.01 |
| 45 – 60 | 301 | 207 | 207 | 95.8 ± 0.5 | 61.5 ± 0.4* | 23.6 ± 0.2* | 118.2 ± 0.3* | 56.6 ± 0.3* | 16.8 ± 0.1 | 0.90 ± 0.01 | 0.48 ± 0.01* |
| ≥ 60 | 374 | 111 – 120 | 130 | 94.6 ± 1.0 | 65.9 ± 0.9 | 22.5 ± 0.5 | 116.3 ± 0.6 | 54.9 ± 0.7 | 16.4 ± 0.3 | 0.88 ± 0.01 | 0.47 ± 0.01 |

*P ≤ 0.05 for linear trend (Analysis of Covariance ANCOVA: sex and age were used as covariates. Heterogeneity was tested by Levene’s test, 1. Percentile (fiftieth percentile: Boys 82 / Girls 79, ninetieth percentile: Boys 91 / Girls 86)
In general, the findings of this study indicate that the rise in the levels of different atherogenic risk factors in preschool age are not independent of each other, but coexist, especially in case of overweight or obesity. Excess body weight in children can be affected by several factors, such as parental and grandparental characteristics, birth and growth-related variables, and lifestyle factors, such as dietary intake practices, television viewing, and physical inactivity.\(^{[26]}\) The abnormal lipid profile and hypertension observed here could be attributed to a Westernized diet probably consumed by the studied children.\(^{[26]}\) Adherence to the traditional Greek diet and adoption of moderate-to-vigorous physical activity could be two important steps toward the reduction of atherogenic risk factors in children, in Greece. The contribution of long-term education intervention programs in children, with an emphasis on nutrition and exercise issues,\(^{[27,28]}\) is important, and the benefits obtained from these programs are shown through their evaluation.

There were limitations in this study. We did not examine the other possible risk factors for atherogenesis in children, such as, parental BMI, physical activity and eating habits, lifestyle factors, and other early life factors such as birth weight, early weight gain, and breast feeding.

In conclusion, the findings of this study indicate that the rise in levels of different atherogenic risk factors at the preschool age are not independent of each other, but coexist, especially in children who are overweight or with obesity. These factors indicate that there is a need for an overall, joint action to combat the increasing risk factors for chronic diseases from early childhood, as there is a transition in the lifestyle of the Greek population.

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