Clinical Research

The Prevalence of Clinical and Biochemical Markers Related to Cardiovascular Disease: Design and Preliminary Results from the ATTICA Study

Demosthenes B. Panagiotakos, Christina Chrysohoou, Christos Pitsavos, John Skoumas, Akis Zeibekis, Ioanna Papaioannou, Lambros E. Papadimitriou, Marina Toutouza, Pavlos Toutouzas, Christodoulos Stefanadis

Department of Cardiology, Athens Medical School, Hippokration Hospital, Athens, Greece

Introduction: We present the design and preliminary results from the "ATTICA" epidemiological study, conducted by the Cardiological Clinic of University of Athens, in order to evaluate the association between several clinical, biochemical, nutritional and psychological markers and cardiovascular mortality and morbidity.

Methods: The study population includes 1658 randomly selected males and females, from several regions of Attica province. The parameters presented in this work are the serum values of total cholesterol, HDL-cholesterol, triglycerides, glucose, hs-CRP, homocysteine, fibrinogen, and blood pressure levels. Additionally, we evaluated the nutrition habits of the population of this study.

Results: The age-adjusted prevalence of coronary artery disease was 599/10000 persons (6%), and the prevalence of stroke was 112/10000 persons (1%). Thirty three persent 33% of the male population and 24% of the females had elevated blood pressure levels, 39% of men and 37% of women had hypercholesterolaemia, while 8% of the males and 7% of the females had diabetes mellitus. Additionally, 45% of the males and 35% of the females were defined as current smokers, and 57% of the males and 64% of the females reported physical inactivity (exercised less than once per week). Finally, 44% of the males and 25% of the females had at least three of the above mentioned risk factors.

Conclusions: The presented data are preliminary, but the increased prevalence of the common cardiovascular risk factors leads to the thought that actions for primary prevention have become more imperative than ever.

During the past years several clinical and epidemiological studies suggested factors that are more or less related to the development of cardiovascular (CVD) disease[1-4]. However, many epidemiologists revealed differences of the effect of CVD risk factors on the development of the disease, between populations as well as among individuals within populations[1]. A potential explanation, regarding the previous outcome, can be attributed to several lifestyle characteristics and environmental conditions. Moreover, during the past years, there has been a significant change in the prevalence of various lifestyle habits (i.e. unhealthy diet, smoking, sedentary life, psychological stress) that could influence the lipid levels

and other CVD risk factors, with a consequent rise of the percentage of people in the "high risk" group.

At the beginning of 2001 the Cardiology Clinic of the University of Athens (under the auspices of the Hellenic Cardiological Society) conducted an epidemiological study, the "ATTICA" study. The main goal of this study is to record, and evaluate the distribution of several lipids, inflammatory and thrombotic factors in addition to the conventional cardiovascular risk factors and several lifestyle habits, in a representative, stratified and randomly selected sample from the general population of the Attica province. The secondary goal will be the development of the *Greek Global CVD Risk Model* based on 5-year and 10-year incidence rates and the meta-analysis of the findings of this study with other similar international cohorts.

In this work, we present briefly the design and the methodology of the study, as well as the first descriptive results, from several demographic, biochemical and clinical markers, related to CVD.

Methods

Study design

The "ATTICA" study is a health and nutrition survey, which is being carried out in the province of Attica (including 78% urban and 22% rural areas), where Athens is a major metropolis. The multistage sampling was based on the city, sex and age distribution according to data provided by the National Statistical Service census of 2001. The final sample will enroll 3075 participants, 1476 of them will be men (48%) and 1599 women (52%). The sample size was decided through power analysis, in order to evaluate differences in the 10-year hazard ratio greater than 10%, with statistical significance <5% and statistical power >80%, for two-sided tests. The contribution of the regions of Attica in the final sample of the study will be the following: municipality of Athens (20%), municipality of Piraeus (8%), general district of the capital (41%), "rest" of Attica province (29%) and islands (2%). The protocol of the study suggests the random selection of one person per block, building and family. The association between the investigated parameters, and the hazard of developing fatal or not, CVD and cancer, will be evaluated through the 10-year follow-up. All data will be collected in a standard questionnaire from the study investigators and then they will be encoded in a special and confidential database. All persons have been informed about the purposes of the study and have agreed to participate.

Study variables

The studied variables express demographic characteristics, daily lifestyle habits, like smoking, physical exercise, dietary particularities, the presence of psychosocial stress and depression, detailed personal and family history of CVD, hypertension, dyslipidemia and diabetes mellitus, as well as more than 250 biochemical measurements. The presence and the therapy of hypertension, diabetes mellitus and of hyperlipidemia will be also recorded. The survey is carried out by a special team of investigators (the "ATTICA" study group) that includes cardiologists, general practitioners, psychiatrists, biopathologists, nurses and nutritionists.

Clinical measurements

Blood pressure is measured on the left arm (for the right-handed) of calm subjects in a sitting position. Three measurements were made and the mean value was estimated. If for any reason, the measurement was from the right arm, this was noted on the questionnaire. If any difference was perceived in the pulse between the two radial arteries, blood pressure was measured on both arms. The systolic pressure level corresponds to the indication of the manometer, when the first sound (of tapping quality) is heard. The diastolic blood pressure level is determined when the sounds cease to be tapping in quality and become fully muffled. Also, all participants had a 12-lead electrocardiogram.

Biochemical measurements

Blood samples are collected after a 12-hour fasting. We perform biochemical tests for the assessment of urea, uric acid, total bilirubin - direct bilirubin, creatinine, SGOT, SGPT, γ-GT, alkaline phosphatase, CPK, glucose, total serum cholesterol, high density lipoprotein cholesterol, and triglycerides (using the automatic biochemical analyser RA 1000 of Techikon, chromatometric enzymatic method). We also measure apolipoproteins A1 and B, lipoprotein-a, high sensitivity (hs) C- reactive protein (CRP), fibrinogen (by the automatic nephelometer BNII of Dade Behring), homocysteine, oxidized-

LDL, total thrombotic factor and the serum amyloid A (by the automatic immunological analyzer Axsym of Abott, polarized immunofluorescence technology, FPIA). Moreover, samples are kept at –80° C, in order to use them in the future for genetic evaluation (following DNA isolation).

Anthropometric measurements

Height is measured once, without shoes, with the back square against the wall tape and the eyes looking straight ahead and a right-angle triangle resting on the scalp and against the wall. In this manner, visual axis is horizontal, since the top of the external auditory meatus is at the same level with the inferior margin of the bony orbit. Height is measured to the nearest 0.5 cm. Weight is measured once, to the nearest 100g, without shoes in light undergarments. Body mass index (BMI) is calculated as weight (in kilograms) divided by height (in meters) squared (Kg/m^2).

Evaluation of lifestyle

Participants are grouped in accordance to their physical activity into three groups: people who do not exercise at all are defined as sedentary life (group I), participants who exercise a little (group II) and those who exercise systematically (group III). This classification is based on the answers of the participants to questions related to any type of daily leisure time- and occupation related physical activities. The information concerning the smoking habits are collected based on a standard questionnaire, which was designed for the study and evaluates the years of smoking, the daily number of cigarettes, the quitting reasons (if any) and the exposure to second hand cigarette smoke as well.

Nutritional evaluation

We apply a validated nutrient questionnaire (from the EPIC project) that was provided by the laboratory of Hygiene and Epidemiology of the Medical School of the University of Athens.

Statistical analysis

In this study continuous variables are presented as mean values±one standard deviation. The categorical variables are presented as absolute and relative (%) frequencies. Associations between the conti-nuous and the categorical variables were evaluated through the Student's t-test or the analysis of variance (ANOVA), after checking for equality of variances (homoscedacity). Associations between categorical variables were tested by the use of the chi-squared test, without the correction of continuity. Associations between continuous variables were tested by the use of Pearson's correlation co-efficient.

All statistical calculations were performed on the SPSS version 10.0 software (SPSS Inc, Illinois, U.S.A.).

Results

In this report we present the findings of the first pilot analysis concerning 1660 participants who entered the study during 2001-2002 (participation rate 87%). Of the 1660 people eight hundred and four were men (48.4%), with a mean age of 49±14 years (from 18 to 89 years) and 856 (51.6%) were women, with a mean age of 48±14 years (from 18 to 90 years). Seventy-three percent of the interviewed subjects were married, 17% single and the rest of them divorced (4%) or widowed (6%). The average number of children was 2 per family. According to the "ATTICA" study investigators there was no difference as far as the socio-economic status is concerned, between the participants and those who did not agree to participate (Table 1).

The age-adjusted prevalence of coronary heart disease (defined by the presence of ischemic type changes or scar of previous myocardial infarction in the ECG, or/and the report of a clinical syndrome that resemble angina or is its analogue, during the detailed medical history), was 620/10000 (6.2%) men and 400/10000 (4.0%) women. The age-adjusted prevalence of stroke was 100/10000 (1.0%) in men and 110/10000 (1.1%) in women (but this information may be limited due the inability of people with major stroke to participate). Thirty one present of the interviewed participants reported positive family history of coronary heart disease (CHD) in 1st or 2nd degree relatives (<55 years old for men, <65 years old for women).

Smoking habits (current or in the past) are presented in table 2 concerning smoking at least one cigarette per day and for a period of at least one year. We observe that 64.6% (520/804) of men and 40.5% (347/856) of women, reported being or having been smokers (P<0.01). 55.3% (445/804) of men and

Table 1. Stratification of the study sample (n=1660 participants), per sex, age and geographic area.

Municipality of Athens	Men	Women	Total	Rest of Attica	Men	Women	Total
20-29	60	65	125	20-29	42	44	86
30-39	57	62	119	30-39	40	42	82
40-49	48	52	100	40-49	34	36	70
50-59	52	57	109	50-59	37	39	76
60-69	44	47	91	60-69	31	32	63
70-79	25	28	53	70-79	18	19	37
>80	14	15	29	>80	9	10	19
Total	**300**	**326**	**626**	**Total**	**211**	**222**	**433**

Municipality of Piraeus	Men	Women	Total	Piraeus Region	Men	Women	Total
20-29	23	24	47	20 -29	36	37	73
30-39	22	23	45	30 -39	34	36	70
40-49	18	19	37	40 -49	29	30	59
50-59	20	21	41	50 -59	31	33	64
60-69	17	17	34	60 -69	26	27	53
70-79	9	10	19	70 -79	15	16	31
>80	5	6	11	>80	8	9	17
Total	**114**	**120**	**234**	**Total**	**179**	**188**	**367**

62.8% (538/856) of women reported that they do not exercise even once per week (P<0.01).

Figure 1 illustrates the distribution of body mass index (BMI) in men and women. In the younger and middle aged groups, the difference in the distribution of the BMI between men and women is confirmed, while it is refuted in older ages. Another measure for the evaluation of the anthropometric characteristics is the waist to hip ratio, which is described in figure 2.

The prevalence of hypertension (SBP/DBP> 140/90 mm Hg or use of anti-hypertensive treatment) was 38.5% in men and 24.1% in women (P<0.05). Thirty eight persent of the hypertensives followed a

Table 2. Smoking habits (active or former smokers) and sedentary life in the sample of study.

Smoking		20-29	30-39	40-49	50-59	60-69	70-79	>80	Total
	Men	59%	62.1%	75.2%	72.9%	64.4%	55.2%	50%	64.7%
		95/161	95/153	97/129	102/140	76/118	37/67	18/36	520/804
	Women	46.5%	53.4%	49.6%	42.7%	34.1%	9.5%	0%	40.5%
		79/170	87/163	68/137	64/150	42/123	7/73	0/40	347/856
Sedentary Life									
	Men	49.1%	60.1%	72.1%	56.4%	51.7%	40.3%	38.9%	55.3%
		79/161	92/153	93/129	79/140	61/118	27/67	14/36	445/804
	Women	62.4%	62.0%	70.8%	60.7%	62.6%	59.9%	57.5%	62.8%
		106/170	101/163	97/137	91/150	77/123	43/73	23/40	538/856

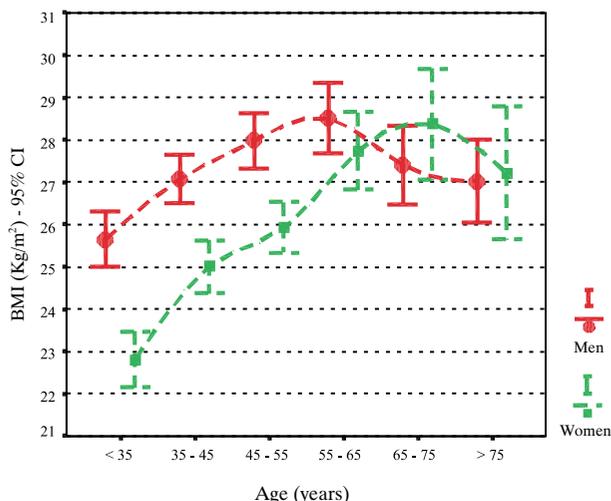The table header spans "Age group" above the age columns.

Figure 1. Body mass index (Kg/m^2) by sex and age.
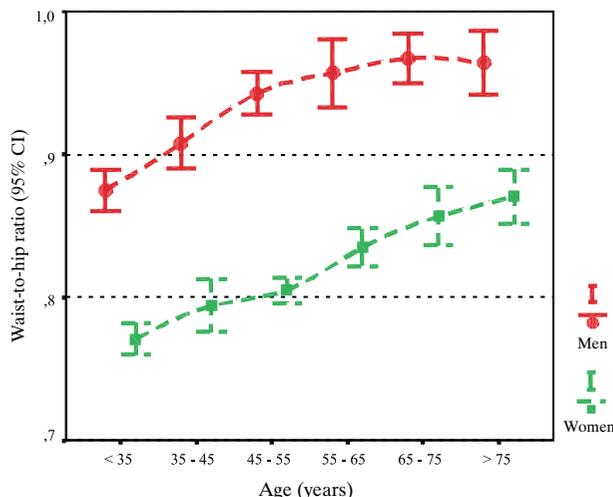

Figure 2. Waist - to - hip ratio by age and sex.

special diet, while 74% used anti-hypertensive medication. The levels of systolic and diastolic blood pressure are presented in figure 3.

Diabetes mellitus was present in 8% of men and 7% of women (P=0.43). Additionally, 6% of men and 7% of women had insulin resistance (serum blood glucose levels from 110 to 125 mg/dl). The diabetic participants controlled their glucose levels by (a) specific diet (70%), (b) oral medication (64%) or/and insulin injections (16%). The 27% of the diabetics had a family history of diabetes mellitus (P<0.001). Thirty nine persent of men and 37% of women had hypercholesterolemia (total serum cholesterol > 220 mg/dl or specific lipid lowering treatment, P=0.15). We also observed that 44% of men and 25% of women had at least three of the conventional risk factors (hyperten-

sion, hypercholesterolaemia, diabetes mellitus, smoking habits and sedentary life). The values of several investigated biochemical markers are presented in table 3.

The data analysis revealed that the values of triglycerides, serum glucose, and apolipoproteins are significantly different between the two sexes (P<0.01). Furthermore, 45% of the participants did not know which are the normal limits of total cholesterol, while 21% answered incorrectly to the respective question (i.e. >220 mg/dl). Fifty eight persent 58% of the participants declared checking the levels of their lipids, with regular visits to biochemical laboratories (annually or biennially). Figure 4 reveals the relation of total cholesterol, triglycerides, glucose and of Lp(a) with the age of the participants (P<0.01).
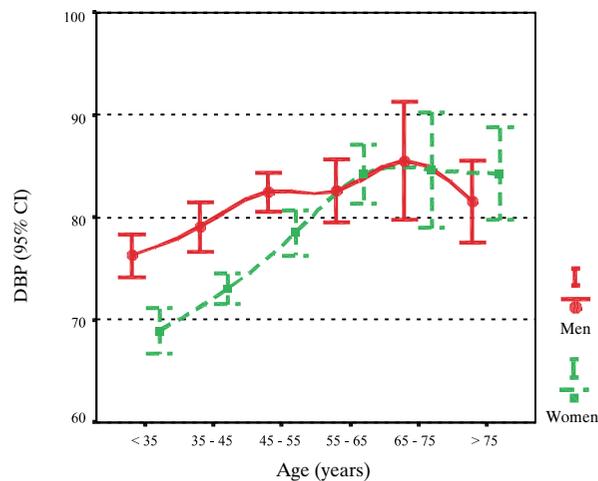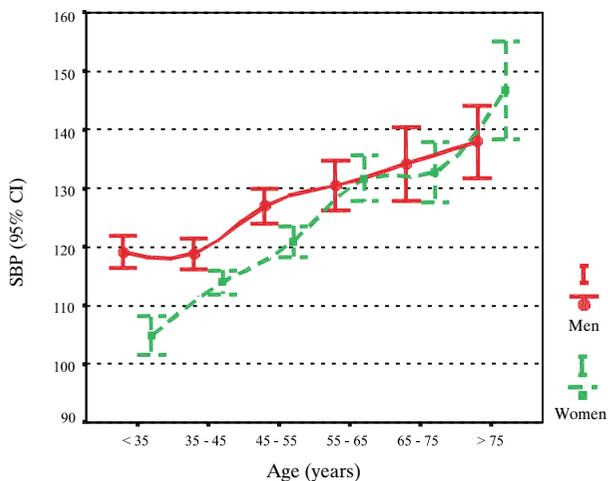

Figure 3. Systolic and diastolic blood pressures (mm Hg) by sex and age.

Table 3. Descriptive statistics of lipids and blood glucose, as well as indicators of inflammation and thrombosis in the sample of study.

	Mean value	Standard deviation	Quartiles		
			25%	Median	75%
Men					
Total serum cholesterol (mg/dl)	215.5	46.9	181.0	213.8	243.8
Triglycerides (mg/dl)	157.1[**]	116.8	92.0	127.0	188.0
Serum Glucose (mg/dl)	96.7[**]	38.4	82.0	91.0	101.0
HDL - cholesterol (mg/dl)	44.3[**]	18.0	35.0	41.0	50.0
Lp (a)	18.3[**]	22.3	4.5	10.6	22.5
APO-A1	141.3[**]	26.4	128.0	140.0	154.0
APO-B	118.6[**]	30.8	95.2	119.0	141.0
Fibrinogen (mg/dl)	306.4[**]	74.3	258.0	297.0	348.0
Homocysteine (μMol/l)	14.8[**]	6.8	11.1	13.1	16.5
hs -CRP (mg/dl)	2.3	3.5	0.6	1.2	2.5
Women					
Total serum cholesterol (mg/dl)	213.9	44.0	182.9	211.1	241.1
Triglycerides (mg/dl)	111.4	62.0	71.5	100.0	137.0
Serum Glucose (mg/dl)	90.6	21.1	79.0	86.0	96.2
HDL - cholesterol (mg/dl)	52.3	14.7	42.0	52.0	61.0
Lp (a)	20.2	26.5	4.5	11.4	23.9
APO-A1	160.1	59.2	142.0	158.0	174.0
APO-B	109.0	53.8	85.9	106.0	125.0
Fibrinogen (mg/dl)	341.2	214.3	279.0	318.0	367.0
Homocysteine (μMol/l)	10.5	3.4	8.3	10.1	12.5
hs - CRP (mg/dl)	2.3	3.2	0.4	1.2	2.8

*$p < 0.05$, **$p < 0.01$

Despite the strong statistical significance of the linear association between the aforementioned lipids and age, the correlation coefficient implies that age may explain only a small proportion of the variability of these lipids.

Finally, table 4 presents in brief the nutritional habits of the participants. Furthermore, the majority of men and women (95%) reported using olive oil in their daily cuisine and the consumption of small quantities of wine, usually with their meals (41% of men and 12% of women). Moreover, 84% of men and 55% of women consume junk food (fast food, grill restaurants, etc) at least once per week.

Discussion

In this work, the design and the results of a pilot analysis from the "ATTICA" study regarding various established and emerging cardiovascular risk factors, were presented. One of the most interesting, findings from a public health perspective, is that 44% of men and 25% of women participants had at least three of the conventional CVD risk factors (hypertension,

hypercholesterolaemia, diabetes mellitus, smoking habits and sedentary life).

The role of smoking in the development of CVD has been reported in many population based observational studies, while there is increased scientific evidence for the effect of passive smoking in the risk of CVD and atherosclerosis[5-7]. The frequency of active smoking was found particularly high, confirming a recent epidemiological study[8] and data from other (unpublished) reports, like the Hellenic Society

Table 4. Weekly consumption of various food items consumed (servings/week).

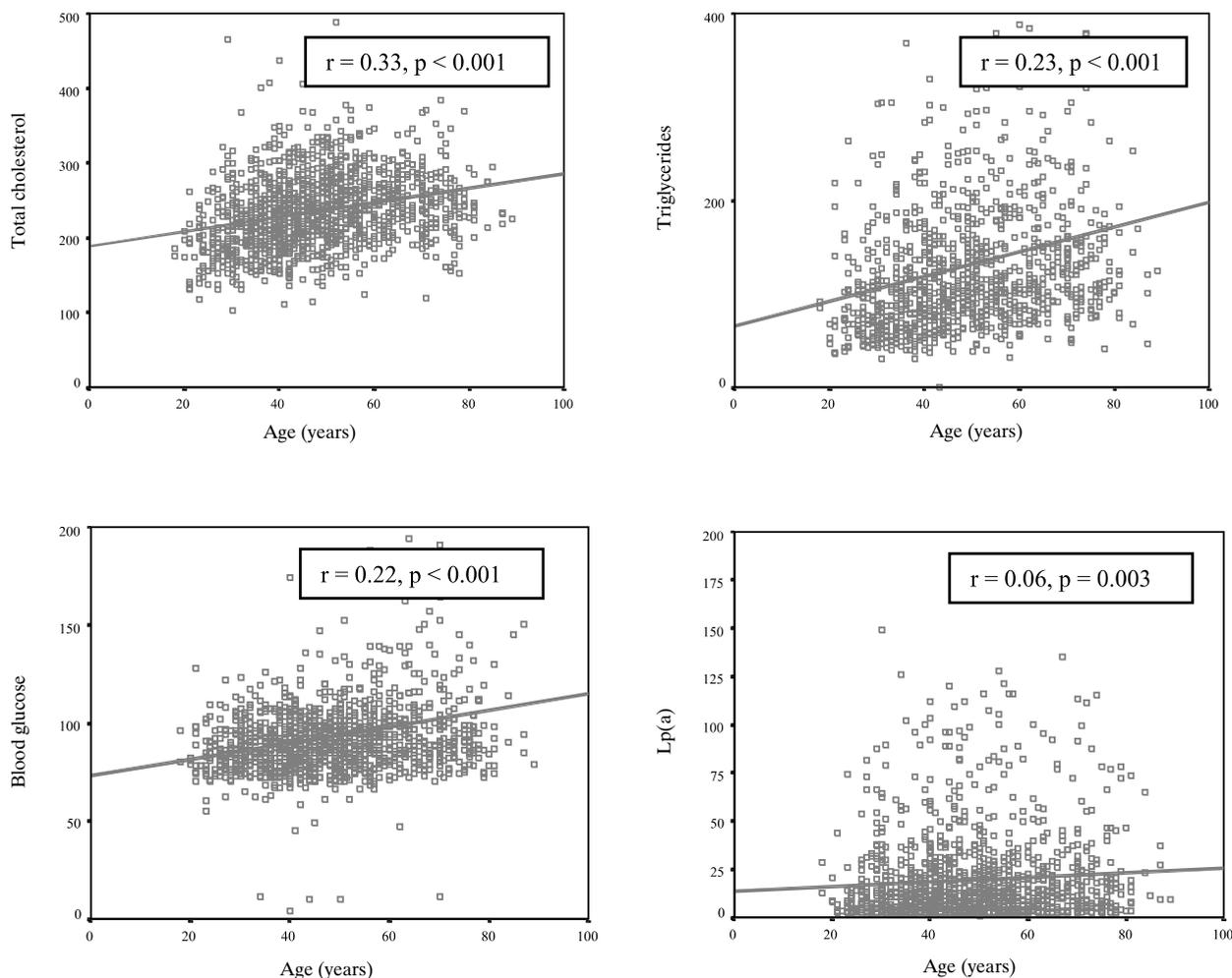|	Men	Women
Red meat	2.1±1.4	1.7±1.2
Chicken	1.3±0.8	1.3±0.8
Fish	1.3±0.9	1.4±1.0
Legumes	1.4±0.9	1.3±1.0
Vegetables	2.1±1.6	2.3±1.7
Pastas	1.8±1.7	1.7±1.3
Salads	5.5±2.0	5.6±2.1
Fruits (daily)	2.0±1.9	2.1±1.8

Figure 4. Serum total cholesterol (mg/dl), triglycerides (mg/dl), blood glucose (mg/dl) and lipoprotein a (mg/dl) levels, by age.

against Cancer, which classify our country as one of the first with increased smoking prevalence between Europe. Thus, the suggestions of smoking cessation and the protection from secondhand smoke exposure should be one of the main goals of physicians for primary and secondary prevention. Moreover, the observed high frequency of smoking in younger women deserves particular attention (Table 2). The harmful effect of smoking correlates to the number of cigarettes and the duration of smoking, and therefore, a potential serious increase in the incidence of CVD is expected the next years, particularly in middle-aged women, when the natural protection of the female sex no longer exists because of the menopause[8].

The independent role of obesity in cardiovascular risk is well known[9-11]. In the young, an increase in weight implies an unhealthy way of life, like smoking and sedentary life[12-14]. The adoption of even light physical activity, by older people, seems to exert its bene-

ficial effects to the reduction of body weight (i.e. reduction of BMI and reduction of waist to hip ratio), reduction of total cholesterol and the control of hypertension in both sexes[12]. However, we observed that a significant percentage of men and women reported not having any physical exercise (Table 2). Consequently, the only solution is the utilization of leisure time physical activity, with programs of aerobic exercise, after taking into account the age and medical history of the subjects.

The importance of hypertension as a risk factor of CVD in men and women is already known by many epidemiological and clinical studies[15-17]. Although there has been progress in the pharmaceutical treatment of hypertension, there are significant difficulties in controlling hypertension at population level, while the adoption of healthy diet remains considerably low[17-19]. Furthermore, despite the efforts for informing the public, many people are

unaware of being hypertensives, and of those who are informed, very few are controlled[17]. The present study revealed surprisingly similar data.

Several population based meta-analyses underlined hyper-triglyceridaemia, as a risk factor of CVD[20,21], though there have been surveys that did not show a significant correlation[22,23]. The mean values of total cholesterol and triglycerides, in the Greek population, are undoubtedly higher than those of the American adults[24], a finding that can hardly be explained by the different nutritional habits of the two populations. Additionally, the women of the sample demonstrated higher values of apolipoprotein A1 and lower apolipoprotein B than men, which may explain the lower CVD risk among women[14]. Finally, as it is reported in the present study, as well as the international literature, an increase of hypertension and cholesterol is observed from the age of menopause, that is from the age of 45 years and thereafter. In USA, about the one half of women, >55 years old, have cholesterol levels higher than 240 mg/dl[30].

During last years, there is growing scientific evidence, that homocysteine constitutes an independent and strong risk factor for CVD[25-30]. There are experimental data reporting possible mechanisms that relate homocysteine and atherogenesis[25]. Considering the up to now results of our study, high levels (>10 μmol/L) of homocysteine are reported, which could be attributed to the lack of vitamin B-12 and of folic acid, and not to a genetic background. Two of the emerging risk factors that have been given much attention in the past are the levels of hs-CRP and fibrinogen. The present study reported higher levels of hs-CRP compared to other surveys. This is something that has to be further analyzed after the completion of the study sampling, for possible genetic, nutritional or / and other relations with the levels of hs-CRP and fibrinogen.

The presented data constitute the first results of the "ATTICA" study and may be partially modified by the completion of the sampling process. However, the fact that 44% of men and 25% of women had at least three of the conventional CVD risk factors, as well as the significant high percentage of under-treated subjects should alert the policy makers of prevention programs for the public health.

Acknowledgements

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