

# MELEN Study: Rationale, Methodology and Basic Results



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## ABSTRACT

**Aim:** The aim of the Melen Study was to investigate the cardiovascular risk profile of Turkish adults by utilizing newest techniques. Besides prevalence and types of endemic goiter will be established which was an important health problem in the Black sea region

**Method:** The study was conducted on 2230 participants (1427 women, 803 men with a mean age of 49). The participants underwent a Doppler Ultrasound examination of carotid intima media thickness, echocardiographic examination, ECG recording, bioimpedance meter analysis of body composition, pulmonary function test and various biochemical analysis.

**Result:** Twenty nine percent of the population had hypertension, 12% had diabetes mellitus and 17% were smokers. Thyroid ultrasonography revealed that 29% of the cohort had goiter. Echocardiographic measurements showed that 39% of the participants had diastolic dysfunction. Comparison of males with females showed that men had significantly higher visceral fat, triglyceride, hemoglobin and CIMT whereas women had higher mean diastolic blood pressure, HDL and total cholesterol.

**Conclusion:** According to the histories of patients, hypertension, diabetes mellitus and smoking were very frequent among Turkish adults. Among the objectively measured variables, diastolic dysfunction, visceral adiposity and goiter were strikingly high.

**Key words:** Cardiovascular, risk, Turkish

## MELEN Çalışması, Mantık, Metot Ve Temel Bulgular

**Amaç:** En son teknoloji ile Türk erişkinlerinin kardiyovasküler risk profilini ortaya çıkarmak, Ayrıca endemik guatrın görüldüğü Karadeniz'de bölgemiz için guatr prevalansını saptamak

**Metod:** Çalışmaya 2298 kişi alındı. (1471 kadın, 827 erkek, ortalama yaş 49). Katılımcıların Doppler ultrason ile Karotis arter intima-medya kalınlık ölçümü, ekokardiyografi, EKG; biyoimpedans metre ile vücut kompozisyonu ölçümü, solunum fonksiyon testi ve kan tahlilleri çalışıldı.

**Bulgular:** Katılımcıların anamnez bilgilerinden hareketle hipertansiyon sıklığı %29, diyabet %12, sigara içiciliği %17 olarak saptandı. Tiroit ultrasonografi sonuçlarına göre guatr sıklığı %29'du. Ekokardiyografi ile %39 oranında diyastolik disfonksiyon saptandı. Kadınlar erkeklere göre daha yüksek diyastolik kan basıncı, HDL ve toplam kolesterol sahipken, erkeklerde ortalama karotis arter intima-medya kalınlığı viseral yağ oranı, trigliserit ve hemoglobin daha yüksekti.

**Sonuç:** Temel bulgulara göre hipertansiyon, diyabet ve sigara içme sıklığı Türk erişkinlerinde yüksekti. Kantitatif ölçümlerden viseral obezite, diyastolik disfonksiyon ve guatr çarpıcı biçimde sıklı. Melen çalışmasında halk sağlığı açısından çok önemli hastalık ve bileşenleri taranmıştır ve takip süresi arttıkça önemli sonuçlara ulaşılacağı tahmin edilmektedir.

**Anahtar kelimeler:** Kardiyovasküler, riski Türk

## INTRODUCTION

The global burden of cardiovascular diseases has been recently increasing rapidly in the middle-income countries (1). Turkish adults have been previously assessed in the Turkish Adult Risk Factor Study as demonstrating a high prevalence (2) of coronary heart disease and a high rate of mortality from this disease; furthermore, a considerable excess absolute risk was detected based on prospective evaluation of the Framingham risk function in the original cohort (3). Furthermore a continuous rise of coronary artery disease, diabetes mellitus, hypertension and metabolic syndrome has been shown (4). There is still lack of epidemiologic data for distinct cardiovascular risk factors in Turkish adults. The ability to follow-up and perform measurements on established population cohorts has provided insights into the occurrence of cardiovascular risk factors and the development of coronary heart disease over time. Therefore the aim of the Melen Study was to investigate the cardiovascular risk profile of Turkish adults by utilizing newest techniques including echocardiography, ECG, carotid intima media thickness measurement, bio impedance meter analysis of body composition, pulmonary function test and various biochemical analysis. Besides prevalence and types of endemic goiter will be established which was an important health problem in the Black sea region (5).

## MATERIALS AND METHODS

### Study population

The MELEN Study is a prospectively designed survey on the prevalence of cardio metabolic risk factors in

Turkish adults. The baseline visits were carried out in May and June, 2010 and biennial follow-up visits were planned. The name of the study comes from the geographic valley in north-east of Duzce, Turkey which is inhabitant of 21000 people. There is a town centre (Yigilca) and 37 villages. Health service of the region was supplied by six family physicians, each following up almost 2500 adults. The study was conducted in May and June, 2010 in the Social health center located in the town center. 400 adult subjects (>17 years old) from each family physician representatively stratified for sex, age and for rural-urban distribution were randomly assigned and invited to participate the study. A total of 2298 subjects with a mean age of 50 (age range 18 to 92) were interviewed. The study protocol was approved by the Ethics Committee of Duzce University and every subject signed a consent form. Data were obtained by a questionnaire, physical examination and measurements of carotid-intima media thickness, body fat composition, ECG, echocardiography and sampling of blood.

### Measurements

**Blood pressure recordings:** Measurement of the blood pressure was performed with a sphygmomanometer (Erka, Erlangen, Germany) after 10 min rest in seated position, and the left arm was used. The mean of the three measurements of each patient was recorded.

**Bioimpedance meter analysis:** Weight and visceral body composition was measured without shoes in light indoor clothes using a bio-impedance meter (Omron BF 510; Omron Corp. Kyoto, Japan). Waist circumference was measured with a tape, the subject standing and wearing only underwear, at the level midway between

**Table 1. Basic characteristics of the study population (diseases according to patient history)**

Variables	Results
Age, year	50±15
Gender (Female/male), n	1471/827
Family income, Turkish lira	796±640
Urban living, n (%)	418 (18%)
Number of children	4.4±2.8
Married, n (%)	1886 (82%)
Insurance cover, n (%)	2017 (88%)
Active smoker, n (%)	383 (17%)
Hypertension, n (%)	660 (29%)
Diabetes mellitus, n (%)	282 (12%)
Coronary artery disease, n (%)	139 (6%)
Chronic obstructive pulmonary disease, n (%)	166 (7%)
Alcohol consumption on a regular basis, n (%)	112 (5%)
Goiter, n (%)	665 (29%)
Systolic dysfunction (EF< 50), n (%)	144 (6%)
Diastolic dysfunction, n (%)	890 (39%)

the lower rib margin and the iliac crest. Body mass index was calculated as weight divided by height squared (kg/m<sup>2</sup>).

**Carotid intima media thickness measurement:** The participants underwent a Doppler Ultrasound examination (M Turbo, SonoSite Inc., Bothell, WA, USA) with a 5-12 MHz linear-array transducer. Ultrasonography was performed with the subject in the supine position. A careful search was performed to obtain optimal visualization of the vessel wall demonstrating the typical double lines representing the intima media layer. At least three consecutive longitudinal images of the common carotid artery were obtained. Measurements involved common carotid artery, bifurcation and origin (first 2 cm) of the internal carotid arteries. Carotid intima media thickness was measured from the thickest point on the far wall between the lumen-intima interface and the media-adventitia interface, using visual assessment (6). Measurements were done 3 times at a site free of plaque and the mean of the three measurements was recorded. No software analysis was used during and after the measurement process. All measurements were made by two experienced radiologist (F.H.B. and O.Y.). The interobserver coefficient of variation was 4.1%.

**Thyroid ultrasonography:** It was performed and interpreted by the same experienced physician, using the same equipment with a 5-12-MHz linear-array transducer (M Turbo, SonoSite Inc., Bothell, WA, USA). The

subjects were examined in the supine position with hyperextended neck. Images were obtained in the transverse and longitudinal planes. The length, width, and depth of each lobe were measured, and the volume was calculated by the mean of the elliptical shape volume formula ( $\pi/6 \times \text{length} \times \text{width} \times \text{depth}$ ). Size of the thyroid lobes and characteristics of thyroid parenchyma and nodules (e.g., with or without calcification, volume, echogenicity, structure, shape, dimensions) were determined. In order to establish the total volume of the thyroid, estimated volumes of the right and left lobes were added (7). Volume of the isthmus was not included in this calculation. Goiter prevalence was defined according to Gutekunst's criteria. Gutekunst reference values for adults (>18 cm<sup>3</sup> in women and >25 cm<sup>3</sup> in men) were used (8).

**ECG:** Standard 12 lead ECG recordings were made with a commercially available electrocardiography machine (Nihon Kohden, Japan) in 50 mm/sec velocity and 10 mV amplitude.

**Echocardiography:** An echocardiography machine utilizing 2-5 MHz probe specific for field studies (M Turbo, SonoSite Inc., Bothell, WA, USA) was used. Echocardiographic measurements were performed according to the recommendations of the American Society of Echocardiography (9). All Doppler echocardiographic and tissue Doppler imaging (TDI) recordings were obtained during normal respiration. From the apical window, the pulsed Doppler sample volume was placed at the mitral valve tips and 5 to 10 cardiac cycles were recorded. Using continuous wave Doppler echocardiography, the cursor was positioned midway between LV outflow and mitral inflow to record the IVRT and IVCT. Left ventricular myocardial velocities were evaluated by pulsed TDI and sampled on the longitudinal axis from the apical four-chamber view. The TDI program was set to the pulsed wave Doppler mode. Filters were set to exclude high frequency signals, and the Nyquist limit was adjusted to a velocity range of -15 to 20 cm/s. Gains were minimized to allow for a clear tissue signal with minimal background noise. From the apical four-chamber view, a 5-mm sample volume was placed at the lateral corner of the mitral annulus and subsequently at the medial (or septal) corner. The resulting velocities were recorded for 5 to 10 cardiac cycles at a sweep speed of 100 mm/s.

The LV ejection fraction was calculated using the bi-

**Table 2.** Comparison of males with females according to basic measurements

Variables	Total	Women (n:1471)	Men (n:827)	P value (men vs women)
SBP, mmHg	126±24	127±25	126±23	NS
DBP, mmHg	79±13	80±14	78±13	0.023
Body mass index; kg/m	29±6	31±6	27±5	<0.001
Waist girth, cm	95±13	95±15	94±12	NS
Visceral fat mass, %	10±4	9±3	11±5	<0.001
Total body fat mass, %	34±11	40±8	24±8	<0.001
Total skeletal mass, %	29±6	26±4	35±5	<0.001
Fasting plasma glucose; mg/dL	113±49	116±51	114±48	NS
HDL; mg/dL	45±12	47±12	42±11	<0.001
LDL; mg/dL	103±33	103±34	102±32	NS
Total cholesterol; mg/dL	181±39	182±40	178±38	NS
Triglyceride; mg/dL	175±118	169±112	187±128	<0.001
Hemoglobin; g/dL	13±2	13±1	14±1	<0.001
CIMT, mm	0.61±0.19	0.59±0.18	0.64±0.2	<0.001

(SBP: Systolic blood pressure, DBP: Diastolic blood pressure, CIMT: carotid artery intima media thickness)

plane Simpson's method. The mitral inflow velocity was traced and the following variables derived: peak velocity of early (E) and late (A) filling, deceleration time of the E wave velocity. The peak systolic (S), peak early diastolic (lateral and mitral E') and peak late diastolic velocities of the lateral (lateral A') and septal mitral annulus (mitral A') by pulsed TDI were measured and the average value was calculated and used in all subsequent analyses (12). The ratio between the E and the E' wave (E/E') was calculated as a preload independent index of LV filling pressures.

**Biochemical analysis:** Ten milliliters of blood were drawn from the antecubital vein of each subject by applying minimal tourniquet force. The first 2 ml of blood, which was used for the full blood count, was drawn into a vacutainer tube containing 0.04 ml of the 7.5% K3 salt of ethylenediaminetetraacetic acid (EDTA). The 8 ml of blood was drawn into a vacutainer tube without anticoagulant. These blood samples were allowed to clot for 20 minutes prior to centrifugation. The blood tubes were centrifuged for 10 min at 1500 g and were processed within 30 minutes in place. Sera were aliquated. Sera were shipped within a few hours on cooled gel packs at 2-50 C and reached to the Duzce University central laboratory. Aliquots of the serum were kept in Eppendorf tubes frozen at -80 °C until the final analyses. Plasma concentrations of cholesterol, fasting triglycerides, HDL-cholesterol, glucose, electrolytes, liver function tests and other biochemical variables were measured by a Cobas 6000 auto analyzer using commercially available

kits (Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol values were computed according to the Friedewald formula.

**Complete blood count analysis:** Complete blood counts were done by CELL-DYN 3700 SL analyzer (Abbott Diagnostics, Chicago, USA). Blood sampling was done in the early morning (all of the patients were requested to be fasting) between 9:30- 10:30 am and the samples were analyzed at 16:00 pm.

#### Definitions

**Diabetes Mellitus:** Individuals with diabetes were diagnosed with criteria of the American Diabetes Association namely when fasting plasma glucose level was >126 mg/dl, or a Hemoglobin A1C was >6.5%, or a casual plasma glucose was >200 mg/dl with classic symptoms of hyperglycemia and/or the current use of diabetes medication (10).

**Metabolic Syndrome:** Individuals with metabolic syndrome were identified when 3 out of the 5 criteria of the National Cholesterol Education Program (ATP III) were met (11), modified for prediabetes (fasting glucose 100-125 mg/dl and further for abdominal obesity using as cutpoint 95 cm in men and 90 cm in women, as assessed in the Turkish Adult Risk Factor study (12).

**Hypertension:** The individuals with arterial blood pressures higher than 140/90 mmHg, under antihypertensive drug therapy, or with hypertension history together with no use of antihypertensive drug were accepted hypertensive. Based on JNC VII criteria, measurements

of <140/90 mmHg in uncomplicated patients, <130/80 mmHg in diabetics, and <125/75 mmHg in chronic renal insufficient patients were accepted as under control.

**Obesity:** BMI>30 individuals were accepted as obese, according to WHO recommendations.

**Hyper and hypothyroidism:** A thyroid stimulating hormone (TSH) level of <0.35  $\mu$ IU/mL was accepted as hyperthyroidism and >4.5  $\mu$ IU/mL as hypothyroidism.

**Coronary Artery Disease:** Diagnosis of nonfatal coronary artery disease (CAD) was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG, or on a history of myocardial revascularization.

**Major depression:** The questionnaire included an extensive list of questions that operationalize DSM-IV criteria (13) for major depression. The questions concerning symptoms in the last month before the interview were the focus of this study. Consistent with DSM-IV, the diagnosis of major depression requires the presence of a chronic and persistent depressed mood for at least 2 weeks. Intermittent depressed mood was excluded. A minimum of 5 out of 9 possible depression symptoms were required to have occurred during the period of disturbance, and 1 of these must have been depressed mood or anhedonia. Social and/or occupational dysfunction must have also occurred.

**Thyroid abnormalities:** Ultrasonographic findings were grouped as follows: 1-Normal thyroid US (normal parenchyma, and volume), 2-Hyperplastic thyroid gland (increased thyroid volume but in normal thyroid parenchyma appearance), 3-Thyroiditis (non-homogeneous thyroid parenchyma (grade 2-3 heterogeneity) without nodules), 4-Nodular goiter (increased thyroid volume with only one nodule), 5- Multinodular goiter (increased thyroid volume with 2 or more that 2 nodules), 6-Recurrent nodular goiter 7-Totally thyroidectomised (no thyroid gland after surgery) 8-Others (Hypoplastic 4 participants), atrophic thyroid gland (2 subjects), hemigenetic thyroid gland (2 subjects ), subtotal thyroid operation (3 participants). Nodules diameter >5 mm accepted as real nodular appearance.

**Smokers and ex-smokers:** Smoking was defined as the constant use of at least one cigarette per day. Former smokers were defined as smoking previously at least one cigarette/day but who have no longer been smoking for at least three months. Attempt to quit was defined as

abstaining from cigarettes for at least three days with a definite intention to quit forever.

**Diastolic dysfunction:** It was defined, according to ASE guidelines (14) and taking into consideration the mean age of our population, as: a) E/A ratio <0.8 (impaired relaxation); b) E/A ratio  $\geq$ 0.8 and  $\leq$ 1.5 and E' velocity <8 cm/s (pseudo-normalized pattern); or c) E/A ratio >1.5 and E' velocity, <8 cm/s (restrictive pattern).

### Statistical analyses

Statistical Package for Social Sciences software (SPSS 12, Chicago, IL, USA) was used for analysis. Descriptive parameters were shown as mean $\pm$ standard deviation or in percentages. Two-sided t-tests and Pearson's chi-square tests were used to analyze the differences in means and proportions between groups. Abnormally distributed variables were compared using Mann-Whitney U test. A p value of < 0.05 was considered significant

## RESULTS

Characteristics and demographic findings of the study population were shown in Table 1. The final cohort was consisting of 2298 participants; 1471 women, 827 men with a mean age of 50 $\pm$ 15. According to the patient histories, 29 % of the population had hypertension, 12% had diabetes mellitus and 17% were smokers. Thyroid ultrasonography revealed that 29% of the cohort had goiter. Echocardiographic measurements showed that 39% of the participants had diastolic dysfunction. Mean body mass index was 29 $\pm$ 6 and CIMT was 0.61 $\pm$ 0.19 mm (Table 2). Comparison of males with females showed that men had significantly higher visceral fat, triglyceride, hemoglobin and CIMT whereas women had higher mean diastolic blood pressure, HDL and total cholesterol (Table 2).

## DISCUSSION

The present study showed that according to the histories of patients, hypertension, diabetes mellitus and smoking were very frequent among Turkish adults. Among the objectively measured variables, diastolic dysfunction, visceral adiposity and goiter were strikingly high.

The high prevalence of hypertension is not surprising since it is one of the most common cardiovascular risk factor throughout the world. The prevalence was 33.7%,

31.8%, 41.7% and 32.7% in four previously done nationwide epidemiologic surveys (TEKHARF, PATENT, METSAR, CREDIT, respectively) (15-18). Therefore, diastolic dysfunction was relatively high, possibly due to lower control rate of hypertension.

CONFLICT OF INTEREST: None declared

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