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Nutritional Status and Calcium Levels in Serum and Urine of Primary Hypertensives and Normotensives

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This research has been designed for the determination of the nutritional status, serum and urinary calcium (UCa) levels and correlation between UCa and blood pressure (BP). 31 Volunteers' patients with essential hypertension (HT) and 31 age- and gender-matched normotensive (NT) control subjects living in Ankara and Nigde from Turkey have participated in this study. 62 Subjects' 24 h urinary samples were collected. Besides, for determination of the correlation between 24 h UCa and overnight UCa (12 h UCa), 38 urine samples (9 patients and 29 control subjects) were collected. Nutritional status was evaluated according to their food consumption and physical examination. After analyzing the collected blood and urinary samples, the mineral status was also evaluated. Urinary and serum Ca levels were determined by flame atomic adsorption spectroscopy. The mean 24 h urinary Ca excretion rates (24 h UCaV) in hypertensive and normotensive subjects were 101 ± 69 and 128 ± 85 mg/day, respectively ($n = 62$, $t = 1.378$, $p > 0.05$). The mean serum Ca levels in HT was significantly decreased when compared with the controls ($n = 26$, $t = 2.54$, $p < 0.05$). In NT subjects systolic blood pressure (SBP) correlated positively with diastolic blood pressure (DBP) ($r = 0.588$) and UCaV ($r = 0.516$) and negatively with dietary Ca ($r = -0.617$, for all $p < 0.05$). In NT group DBP correlated positively with body mass index (BMI) ($p < 0.05$). In HT subjects, SBP correlated positively with DBP ($r = 0.741$) and negatively with serum Ca ($r = -0.633$), DBP also correlated negatively with serum Ca ($r = 0.727$). Among the 38 subjects, overnight calcium excretion rate (12 h UCaV) was correlated with the 24 h UCaV ($y = 0.8279x + 42.735$, $r = 0.81$, $R^2 = 0.653$, $p < 0.05$).

Key Words: Serum Ca, Urinary Ca excretion, Atomic absorption spectrometry, Food consumption.

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INTRODUCTION

Essential hypertension (EH) or primary hypertension (PH or HT) refers to lasting increase in blood pressure (BP) with heterogeneous genetic and environmental causes. Its prevalence rises with age, irrespective of the type of BP measurement and the operational thresholds used for diagnosis. The specific causes of EH remain incompletely understood. In cross-sectional and longitudinal population studies, systolic blood pressure (SBP) increases with age until the eight decade of life. By contrast, diastolic blood pressure (DBP) rises only until 50 years of age, after which it either becomes constant or even decreases slightly. Below age 50 years, DBP was the strongest predictor. Systolic and diastolic blood pressure thresholds used in clinical medicine are boundaries in normotension: optimum <120/<80, normal 120-129/80-84, high-normal 130-139/85-89, in hypertension: grade I (mild) 140-159/90-99, subgroup borderline 140-149/90-94, grade II (moderate) 160-179/100-109, grade III (severe) >180/>110, isolated systolic hypertension >140/<90, subgroup borderline 140-149/<90 mm Hg[1]. Hypertension is an asymptotic and important disease of modern civilized life. The overall prevalence of hypertension has been reported to range from 6-32 %[2]. Much epidemiologic data confirm the relevance of nutritional factors in determining BP. Factors epidemiologically related to BP such as weight, caloric intake and the minerals sodium, potassium, calcium and magnesium also have been the focus of therapeutic intervention trials. These trials have shown that lowering dietary calorie and/or sodium (salt) content and providing increased amounts of calcium, potassium or magnesium may lower BP in at least some sensitive subjects[3]. Calcium supplements have been found to lower BP in hypertensive and salt sensitive humans and attenuate the hypertensive effect of a high sodium diet. Recent research suggests abnormalities in Ca metabolism in both hypertensive humans and animals. The increased risk of elevated blood pressure with lower calcium intake appears to be related to an apparent deficit in calcium metabolism that occurs in a subset of hypertensive individuals. That deficit appears systemically as an increased urinary calcium excretion[4].

The body of an adult normally contains about 1200 g of Ca. At least 99 per cent of this amount is present in the skeleton, where Ca salts (chiefly phosphate) held in a cellular matrix provide the hard structure of the bones and teeth. Obviously all of this Ca comes from the diet. Among common foods, milk is much the richest source, which is one reason why milk and milk products are especially valuable for growing children and for females during the all live. Regularly Ca intake during life span prevents osteoporosis and teeth decay might develop in the future[5,6]. Blood pressure is affected by nutritional status[7,8]. Essential hypertension is associated with disturbed Ca metabolism. An increased calciuria could be a feature of the essential hypertension. Some authors have reported lower concentration of serum Ca in the HT than in NT subjects[2,9]. Young *et al.*[10] tested the hypothesis that the calciuria of hypertension is due to dietary factors and evaluated several alternate mechanisms. In the present investigation, the relationship between the blood pressure and nutritional status, serum and urinary Ca levels were studied in essential hypertensive patients and normotensive subjects.

EXPERIMENTAL

31 Volunteers' primary hpypertensive patients (22 females and 9 males, mean resting BP ≥ 140/90 mm Hg and with medically diagnosed hypertension and/or on antihypertensive medications and on antihypertensive diet) and 31 normotensive (15 females and 16 males, blood pressure < 140/90 mmHg) from Hacettepe University internal diseases polyclinic, asylum for the aged of Keçiören (Ankara) and Nigde University, aged 21-79 years of old participated in the first step of research. During the first step of research at same time daily energy and nutrient intakes were determined and 24 h urine were collected. On the following day, for the determination of the relationship between 24 h urine and overnight urine Ca concentration; 9 HT patients and 29 NT subjects from these groups participated for the second stage of research. At the second stage of research overnight urines samples were collected. Physicians from Hacettepe University and Nigde State Hospital examined all the subjects' health who joined at the first and second steps of research.

Anthropometrics measurements: Anthropometrics measurements were applied for body weight and height by using inflexible measure and sensitive balance, respectively. These values were used for calculation of the body mass index (BMI) as follows:

$$\text{BMI} = \text{Body weight (kg)/height}^2\text{ (m)}$$

Evaluation of body sculptures was used in WHO standard[11].

Blood pressure measurements: After urine samples had been returned to the data collection laboratory, BP was measured according to Charlton *et al.*[12] on each of the three occasions with an automated Braun electronic blood pressure manometer. Blood pressure measurements were taken three times from the left arm, with the palm upward, which rested on a table or a support at the level of the heart, after the participant had been seated for at least 5 min.

Dietary survey: Weighed intake method is used to determine individual food consumption for 31 HT and 26 NT subjects. Weighed intake method most often is limited to research studies[13]. Dietary intake was assessed by using an interviewer-administered student, 24 h food consumption method was applied for the determination of daily energy and nutrient intakes. All the ingredients were weighted and recorded before and after cooking. All the meals also were weighed and recorded before and after eating[14]. The food contents of meals were taken from Standard Meal Book[15,16]. The amount of nutrients, in consumed foods were calculated by using Bestuk computer programme[17]. The basal metabolic rates (BMR) and energy requirements of subjects were calculated from the method recommended by WHO[11]. Daily energy intakes were calculated from amounts of protein, carbohydrate and fat by multiplying at water factors[18].

Sample collection: The blood samples (10 mL) were collected after overnight starvation from the antecubital vein. After 2 h, serums were separated by centrifugation at 3000 rpm and then were kept in stopped tubes at -20 ºC until assayed. All urine specimens were obtained in polyethylene terephthalate (PET) vessels during food consumption research (5 L for 24 h or 2.5 L for 12 h). Also samples of drinking water (n = 59), tea (n = 60) and bread (n = 25) purchased from home and/or market. All vessels utilised in this research were demineralized with 6 N HNO_3 during 6 h. Serum samples were digested with 24 % trichloro acetic acid (TCA) at 90 °C and then centrifuged at 3500 rpm for 10 min. Urine sample (100 µL) was diluted fifty or one hundred fold with 0.2 % La (prepared with La_2O_3 + HCl), mixed well and then centrifuged at 3500 rpm for 10 min. Bread, tea and water samples were prepared (digested with 1 V $HClO_4$ + 3 V H_2O_2) for analysis using the method of wet digestion. Bread, tea, water, serum and urinary Ca levels and also Na, K and Fe concentrations of bread, tea, water were determined by flame atomic absorption spectrophotometer (Shimadzu AA-6501) according to the method of Perkin-Elmer using the method of standard additions, as described elsewhere[19,20]. For K analysis emission mode was utilized. The added standards of different Ca concentrations (*i.e.*, 0.50, 1.00, 2.00, 3.00 and 4.00 µgCa/mL) were prepared from stock standard. The standards and samples were read against the blank solution (0.2 % La). The optical density of samples, standards and blank was noted. The concentration of Ca in the samples were calculated by reading from the standard curve[20].

Data analysis: Daily dietary intake of energy and nutrients were compared with recommended daily allowances (RDA) for Turkish people[21] using following equation:

$$[(X^1–A)/(SD^1/n^{½})]$$

where A is RDA.

Data differences were compared by unpaired Student's t-tests for hypertensive *versus* normotensive subjects[22]. The relations of blood pressure to other variables were analyzed by linear regression and Person correlation coefficients. Data are expressed as the mean ± SD[22].

RESULTS AND DISCUSSION

Patients with hypertension (HT) have been reported to have higher levels of UCaV than normotensive persons. In this work, we have been tested the hypothesis that the calciuria of hypertensives is due to dietary factors. Urinary calcium excretion was studied in 31 patients with HT compared with 31 NT control subjects, aged 21-79 years. Demographic and basal biochemical data are displayed in Tables 1 and 2 for the 62 subjects. According to WHO the range of age is 18-30, 30-60 and 60 and plus[11]. However the great majority of present studied groups fall within 30-60 years. These values are 62 % for HT and 73 % for NT group. As seen in Table-1, mean BMI, DBP and SBP values in HT females were statistically found to be different from those of NT females ($p < 0.01$). Similar results about DBP and SBP were obtained for males (Table-2).

The mean 24 h UCaV levels in HT and NT subjects were 101 ± 69 and 128 ± 85 mg/d, respectively ($n = 62$, $t = 1.378$, $p > 0.05$). Urinary Ca_T is usually expressed as a 24 h excretion rate. The excretion depends on many variables including dietary intake of Ca. Ninety per cent of healthy men excrete < 7.5 mmol (300 mg) per day and 90 % of healthy women excrete < 6.25 mmol (250 mg) per day[23]. According to Davidson *et al.*[5] the urine normally contains 100 to 350 mgCa/day. The amount varies greatly from person to person and is higher in the summer. In women the fasting overnight level of Ca excretion increases after the menopause. This may provide an explanation for postmenopausal osteoporosis. Resnick *et al.*[3] studied 158 subjects [26 hypertensive (HT), 45 hypertensive hyperlipidemic (HTHL) and 87 normotensive hyperlipidemic (NTHL)] on no antihypertensive or antilipemic medications and reported that body mass index did not differ significantly among the groups. Blood pressure was higher among HT and HTHL compared with NTHL, but did not differ between HT and HTHL subjects. Both diets significantly lowered BP in all positions in all subjects. For all subjects, a greater fall in SBP (-6.2 ± 1.0 *vs.* -4.2 ± 0.8 mmHg, $p = 0.033$) and DBP (-4.2 ± 0.7 *vs.* -2.3 ± 0.4 mm Hg, $p = 0.02$) was observed on Campbel Center for Nutrition and Wellness (conform to the RDA standards) *versus* the self-sellected diet. Zozaya *et al.*[24] have found an increase in calcium excretion in the 24 h urine collections of the hypertensive group.

However in females, the difference between 24 h UCaV in the NT (106 ± 88 mg/d) and HT subjects (101 ± 78 mg/d) was not significant ($p > 0.01$, Table-1). While, in male subjects, the NT group showed higher 24 h UCaV (153 ± 83 mg/d) than HT group (105 ± 25 mg/d, $p < 0.05$, Table-2). Tillman and Semple[25] have reported that the hypertensive group showed higher urinary excretion of calcium (5.9 ± 3.0 mmol/24 h or 236 ± 120 mg/d) than controls (4.6 ± 1.7 mmol/24 h or 184 ± 68 mg/d).

TABLE-1
ANTHROPOMETRICS, BLOOD PRESSURE AND UCaV VALUES OF THE FEMALE SUBJECTS (n = 37)

Anthropometry	Normotensive (n = 15)			Hypertensive (n = 22)			Statistical analysis	
	X^1	SD^1	Average	X^1	SD^1	Average	t	p
Age (year)	43.8	11.7	21-52	50.0	7.1	45-55	1.820	ns
Height (cm)	162.0	7.4	152-175	159.1	7.3	148-170	-1.160	ns
Body weight (kg)	61.7	9.1	47-77	70.9	10.0	55-90	2.900	< 0.01
BMI (kg/m^2)	23.6	2.5	19-28	28.2	3.5	23-38	4.610	< 0.01
Diasystolic blood pressure (mmHg)	79.6	6.7	70-90	94.5	12.5	85-140	4.670	< 0.01
Systolic blood pressure (mmHg)	124.7	12.9	100-140	162.4	16.1	140-210	7.890	< 0.01
Urinary Ca (mg/d) (24 h)	106.0	88.0	65-277	101.0	78.0	78-278	-0.179	ns

TABLE-2
ANTHROPOMETRICS, BLOOD PRESSURE AND UCaV VALUES OF THE MALE SUBJECTS (n = 25)

Anthropometry	Normotensive (n = 16)			Hypertensive (n = 9)			Statistical analysis	
	X^1	SD^1	Average	X^1	SD^1	Average	t	p
Age (year)	43.0	11.9	23-54	59.5	9.5	47-79	3.45	< 0.01
Height (cm)	169.0	6.8	160-170	166.9	5.9	162-178	-0.74	ns
Body weight (kg)	67.3	9.5	51-80	66.0	9.6	53-84	-0.30	ns
BMI (kg/m^2)	23.3	3.6	20-29	24.3	4.6	20-35	0.56	ns
Diasystolic blood pressure (mmHg)	78.4	5.7	70-90	89.4	7.3	80-100	3.93	< 0.01
Systolic blood pressure (mmHg)	125.9	12.0	110-140	156.1	18.7	120-180	4.37	< 0.01
Urinary Ca (mg/d) (24 h)	153.0	83.0	102-302	105.0	25.0	69-133	-2.14	< 0.05

For daily urinary Ca excretion similar findings were described by Restnic *et al.*[3] These authors have reported that for subjects under self selected diet: 92-140 mg Ca/d and for subjects under a diet conforming to the RDA standards 115-136 mg/d. It has been reported that UCaV remained low despite a high intake of calcium. Leiba *et al.*[26] have reported that when the estimated calcium intake significantly increased from 933 mg/d to 1029 mg/d, UCaV remained the same during the study. Zozaya *et al.*[24] have also reported an increase in 24 h urinary Ca excretion in the hypertensive group. Simeckova *et al.*[27] have found, the age to have a marked influence on the value of urinary Ca and UCa was low both in the early and advanced age groups, while it reached peak values in subjects 18-35 years old. Because of 73 % of our NT group fall within 30-60 years, perhaps UCa in NT male group was higher than HT male group. Resnick *et al.*[3] reported a range of UCaV 115 -140 mg/d in NT hyperlipidemic group and 92-136 mg/d in HT and HT hyperlipidemic group, which agrees with our UCaV levels.

The mean serum Ca levels in hypertensive and normotensive subjects were 9.88 ± 1.59 mg/dL (2.47 ± 0.39 mmol/L range 6.9-11.8 mg/dL) and 11.05 ± 0.47 mg/dL (2.88 ± 0.12 mmol/L range 9.9-11.2 mg/dL), respectively. The mean serum Ca levels in hypertensive was significantly decreased when compared with the controls ($n = 26$, $t = 2.54$, $p < 0.05$). Mean plasma or serum Ca_T concentration for adults is 2.35 mmol/L (9.4 mg/dL) with 95 % confidence ranges from 2.20 to 2.55 mmol/L (8.8-10.2 mg/dL). 5 % Lower means have been reported[23] for persons over 50 year. According to Perkin Elmer[20], normal levels in serum are 9.0-11.0 mg% calcium (4.5-5.5 meqCa/L). Among 13 hypertensive subjects three women had serum Ca values lower than 8.8 mgCa/dL (6.9-7.9%). After 6 months treatment with Ca (Ca supplementation), serum Ca concentrations have increased to normal levels. The present study shows significantly reduced serum Ca in hypertensives and the results are in close agreement with that of others who have also found a significant decrease in serum Ca in patients with essential hypertension. Some authors have reported[2,24] lower concentrations of serum Ca in the hypertensives than in normotensive subjects. Sudhakar *et al.*[2] showed significantly decreased serum Ca levels in essential hypertensives (mean serum Ca; for hypertensive group 2.23 ± 0.36 mmol/L and for control group 2.52 ± 0.24 mmol/L) ($p < 0.01$). Reichel *et al.*[9] also reported reduced serum calcium in males with elevated diastolic blood pressure (2.41 ± 10 *vs.* 2.47 ± 0.10 mmol/L). Contrarily Kosch *et al.*[28] did not find any change in plasma Ca levels in hypertensives (2.59 ± 0.18 n = 34 *vs.* control 2.50 ± 0.16 mmol/L n = 34). Tillman and Semple[25] have reported similar result, they did not find a significant difference between serum total Ca concentration in the hypertensive (2.29 ± 0.09 mmol/L) and control (2.26 ± 0.07 mmol/L) subjects. However in our earlier publication[29], the lower serum Ca in the hypertensive pregnants than normotensive pregnants has also been reported.

Since 24 h urine collections are difficult to obtain and evaluate in free-living population groups[30], overnight and 24 h urine specimens were collected by 38 subjects (9 HT, 29 NT). Mean urine collection time and collected urinary volume were during overnight 805 min and 1232 mL (1.53 mL/min), during 24 h 1412 min and 2253 mL (1.60 mL/min). The mean calcium excretion rates were 137 ± 105 mg/d (0.285 ± 0.219 meq/h) and 157 ± 103 mg/d (0.327 ± 0.214 meq/h), respectively, during the overnight and 24 h period, overnight calcium excretion rate was statistically correlated with the 24 h excretion rate (n = 38, $p < 0.01$). This relationship is shown in Fig. 1. as a scatter diagram.

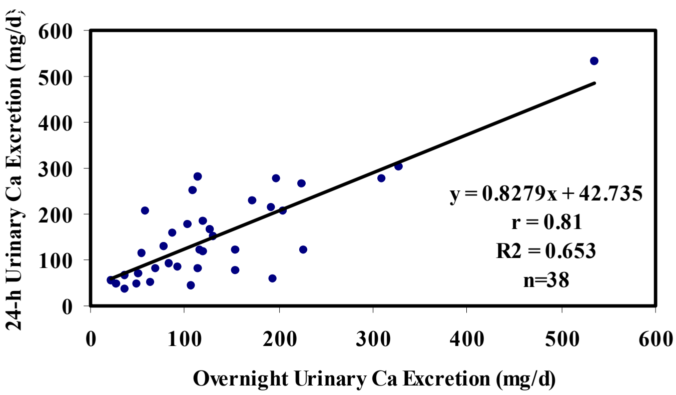

Fig. 1. Correlation between overnight urinary Ca excretion rate (mg/d) and 24 h urinary Ca excretion rate (mg/d)

Similar findings were described by Watson and Langford[30]. The authors reported mean calcium excretion rates 0.239 ± 0.45 meq/h and 0.257 ± 0.10 meq/h, respectively, during the overnight and 24 h period (r = 0.804, n = 29, a = 0.1230, b = 0.5583, $p < 0.01$). The present results are in the close agreement with that of Watson and Langford[30].

Daily dietary intake of energy and nutrients of the subjects and statistical results obtained dietary intakings compared with recommended daily allowances (RDA)[21] for Turkish people were showed in Tables 3 and 4. Total energy was obtained as 14.4 % from proteins, 29.6 % from fats and 56.0 % from carbohydrates for female NT subjects, 16.1 % from proteins, 27.5 % from fats and 56.4 % from carbohydrates for male NT subjects (Table-3). For HT subjects these values were 14.0, 32.8, 53.2 14.7, 27.2 and 58.1 %, respectively (Table-4). On the other hand, for both groups the dietary energy (except HT female and male groups), vitamin A, C, dietary fiber and protein (except HT female) were higher than RDA values. This might be due to calculation of vitamin A together with β-carotene, which are plant sources pro-vitamin, moreover, the other explanation of this situation was that the fruit, vegetables and cereals consumption were quite high in Turkey[31]. For NT female B_2 consumption was higher than RDA. For males B_1 consumption was lower than RDA (Tables 3 and 4). For both groups niacin and for HT groups energy and P consumption were in normal range[21] (Tables 3 and 4). Dietary fiber clearly has several important physiological role in the gastrointestinal tract with respect to lowering of plasma cholesterol, blunting the glycemic response, decreasing nutrient bioavailability (this deleterious effect diminishable by use leavened dough *vs.* unleavened dough) and having an impact on large-bowel function[32]. In Turkey, consumption of fruits (not fruit juice), vegetables, cereals, whole-grains (not milled grains) and legumes are abundant for this reason dietary fiber are also abundant[31,33].

Phosphorus intake was higher than RDA in NT group and about RDA in HT group. A phosphate intake of 0.2 mg phosphorus (P) per kilocalorie, assuming normal calorie requirements, is sufficient to meet the needs of the growing child, the adolescent and the adult[34]. In both groups of both sexes; P/energy ratio were higher than 0.2 (0.46, 0.53, 0.41 and 0.29). It is recommended[6,34] that Ca:P ratio in the diet be between 1.5 and 1.0, but not less than 0.5. In both groups in both sexes Ca/P ratio were higher than 0.5.

While intaking of iron was statistically lower among female, but male iron consumption was in normal range. The high RDA level of iron for women (18 mg/d) in Turkey is due to high birth rate.

In all groups, mean found intakes of Ca (569-720 mg/d) decreased far below the recommended intake of Turkish standards (about 60-70 % of the RDA of 1000 mg/d)[21] (Tables 3 and 4). Mean dietary Ca was higher in NT subjects than in HT subjects. Charlton *et al.*[12] also reported low mean dietary Ca (436-577 mg/d, about half of the daily recommended intake of 1000 mg/d).

After analyzed drinking water, tea and bread we obtained mean Ca concentration 6.5 mg (n = 59), 1.6 mg (n = 60) and 26 mg (n = 25) per 100 g, respectively. Mean drinking water consumption was 800 - 1500 g and than drinking water provided 50-100 mg Ca/d. These values are *ca.* 5-10 % of RDA [(50 mg Ca/1000 mg Ca RDA) × 100 = 5 %] and then not negligible compared to RDA. Therefore, Ca intakes showed in Tables 3 and 4 include Ca content of water. Daily tea consumption was *ca.* 200-400 g and then mean Ca from tea was 2-4 mg/d, these values are negligible. Daily mean bread consumption in four group (NT + HT) was 250 g. Mean Ca consumption from bread was 65 mg/d and then not negligible compared to RDA.

TABLE-3
DAILY ENERGY AND NUTRIENT INTAKES OF THE NORMOTENSIVE GROUPS (n = 26)

Energy and Nutrients	Female (n = 15)		Male (n = 11)		[21]Recommended daily allowances		Statistical analysis			
							Female		Male	
	X[1]	SD[1]	X[1]	SD[1]	Female	Male	t	p	t	p
Energy (Kcal)	2339	494	2159	343	2065	2623	2.15	<0.05	-4.49	<0.01
Total protein (g)	84	26	87	17	1g/kg body weight (mean 61.7)	1g/kg body weight (mean 67.3)	3.32	<0.01	3.84	<0.01
Total fat (g)	77	28	66	18	–	–	–	–	–	–
Dietary fiber (g)	39.5	17.5	52.0	16.5	25	29	3.20	<0.01	4.60	<0.01
Ash	15.4	1.4	20.2	7.7	–	–	–	–	–	–
*Ca (mg)	720	209	709	194	1000	1000	-5.19	<0.01	-4.98	<0.01
P (mg)	1088	294	1153	300	700	700	5.10	<0.01	5.01	<0.01
Ca/P	0.66	–	0.61	–	–	–	–	–	–	–
K (mg)	2995	710	3152	1184	–	–	–	–	–	–
**Total Na (mg)	3879	776	4064	1496	–	–	–	–	–	–
Na/K	1.14	0.38	1.54	1.2	–	–	–	–	–	–
Discretionary NaCl (g)	5.52	1.93	5.89	2.2	–	–	–	–	–	–
Na from Discretionary NaCl (%)	56		57		–	–	–	–	–	–
Fe (mg)	12.8	4.4	15.3	6.1	18	10	-4.58	<0.01	2.88	<0.05
***Vit A (μg)	1800	1703	2158	1236	700	700	2.50	<0.01	3.91	<0.01
Vit A (IU)	9004	8514	10792	6182	–	–	–	–	–	–
B_1 (mg)	1.05	0.31	1.01	0.28	1.1	1.2	-0.63	ns	-2.25	<0.05
B_2 (mg)	1.57	0.67	1.51	0.48	1.1	1.3	2.73	<0.05	1.46	ns
Niacin (mg)	12.7	5.3	15.8	7.1	14	16	-0.95	ns	-0.09	ns
Vit.C (mg)	163	84	187	77	90	90	3.36	<0.01	4.18	<0.01
Beverage (Drinking water, tea, coffee, *etc.*)	1244	224	1692	255						
Metabolic water (g)	298		275		–	–	–	–	–	–
****Total water	2681	689	2907	826	–	–	–	–	–	–
Water from beverage (%)	46	–	58	–	–	–	–	–	–	–
Total water/Energy	1.15	–	1.35	–	–	–	–	–	–	–

Energy %: Protein, Fat, Carbohydrate = Female/Male: 14.4/16.1, 29.6/27.5, 56.0/56.4

*Total Ca intake = Ca from food and drinking water; **Total Na intake = Discretionary (consumer controlled) + non-discretionary (commercially controlled and/or naturally occurring Na); ***VitA retinol equivalent is defined as 1 μg retinol, which is considered equal to 6 μg β-carotene or 12 μg of mixed pro vitamin A carotenoids. 3.33 IU vitamin activity from retinol, 10 IU vitamin A activity from β-carotene 5000 IU is equal to 1000 RE. If only supplements of preformed vitamin A are considered, however, the RDA is 3333 IU, not 5000 IU[21, 16]; ****Total water: Water from beverages + water from foods + metabolic water (ex: 1244 g + 298 g + 1139 g = 2681 g).

TABLE-4
DAILY ENERGY AND NUTRIENT INTAKES OF THE HYPERTENSIVE GROUPS (n = 31)

Energy and Nutrients	Female (n = 22)		Male (n = 9)		[21]Recommended daily allowances		Statistical analysis			
							Female		Male	
	X[1]	SD[1]	X[1]	SD[1]	Female	Male	t	p	t	p
Energy (Kcal)	1948	528	2318	644	2065	2250	-1.04	ns	0.32	ns
Total protein (g)	68	23	85	17	1g/kg body weight (mean 70.9)	1g/kg body weight (mean 66.0)	0.59	ns	3.36	<0.05
Total fat (g)	71	30	70	27	–	–	–	–	–	–
Dietary fiber (g)	35.9	20.5	48.0	27.5	25	29	2.49	<0.01	2.07	<0.05
Ash	12.7	5.0	13.0	3.7	–	–	–	–	–	–
Ca (mg)	569	287	602	293	1000	1200	-7.04	<0.01	-6.12	<0.01
P (mg)	794	407	669	357	700	700	1.08	ns	-0.26	ns
Ca/P	0.72	–	0.93	–	–	–	–	–	–	–
K (mg)	2393	1008	2862	938	–	–	–	–	–	–
Na (mg)	3173	1267	3506	1821	–	–	–	–	–	–
Na/K	1.39	0.69	1.19	0.25	–	–	–	–	–	–
Discretionary NaCl (g)	3.68	1.98	2.23	1.61	–	–	–	–	–	–
Na from discretionary NaCl (%)	46		25		–	–	–	–	–	–
Fe (mg)	12.5	6.1	14.8	6.0	18	10	-4.23	<0.01	2.04	ns
Vit A (μg)	1067	713	1755	1021	700	900	2.41	<0.01	2.51	<0.05
Vit A (IU)	5334	3566	8776	5104	3500	4500	–	–	–	–
B_1 (mg)	1.14	0.59	0.85	0.40	1.1	1.2	0.32	ns	-2.63	<0.05
B_2 (mg)	1.39	0.75	1.22	0.63	1.1	1.3	1.81	ns	-0.38	ns
Niacin (mg)	13.5	11.0	15.1	7.7	14	16	-0.21	ns	-0.35	ns
Vit.C (mg)	162	96	144	69	90	90	3.52	<0.01	2.35	<0.05
Beverage (Drinking water, tea, coffee, *etc.*)	1105	459	965	547	–	–	–	–	–	–
Metabolic water (g)	247		297		–	–	–	–	–	–
Total water	2131	646	2148	500	–	–	–	–	–	–
Water from beverage (%)	52		45		–	–	–	–	–	–
Total water/Energy	1.9		0.93		–	–	–	–	–	–

Energy %: Protein, Fat, Carbohydrate = Female/Male: 14.0/14.7, 32.8/27.2, 53.2/58.1

In this study, the range of the total daily dietary sodium intakes; discretionary (consumer controlled: salt added to food at the table and/or added during food processing by the consumer) and non-discretionary (commercially controlled and/or naturally occurring Na) was 3173 mg (138 mmol) to 4064 mg (177 mmol) per person, except HT male group, about 50 % occurring from discretionary salt (NaCl) (Tables 3 and 4). The Food and Nutrition Boards has determined[35] levels of Na intake which are safe and adequate. For adults this level is 1100-3300 mgNa/d. Some authorities[36] have recommended that dietary sodium and chloride intake should be curtailed to < 100 mmol/d in the hope that the development of hypertension, increase in BP with age and cardiovascular disease morbidity and mortality may be alleviated. In this study all subjects consumed higher than 1100 mg Na and 100 mmol Na per day.

Mean daily dietary Na intake equated to daily discretionary salt (NaCl) intakes of 8.07 [(58.5 mg NaCl/23 mg Na) × 3173 mg Na × (1 g NaCl/ 1000 mg NaCl) = 8.07 g NaCl], 8.92, 9.87 and 10.34 g NaCl in HT female, HT male, NT female and NT male subjects, respectively (Tables 3 and 4).

Discretionary salt consumption was 5.52, 5.89, 3.68 and 2.23 g in NT female, NT male, HT female and HT male subjects, respectively and NT groups consumed higher than HT groups (Tables 5 and 6). Discretionary Na intake made up 56 % [5.52 g NaCl × (23 mg Na/58.5 mg NaCl) × (1000 mg/1 g) × (1/3879 mg Na) × 100 = 56 %], 57, 46 and 25 % of total sodium intakes in NT female, NT male, HT female and HT male subjects, respectively (Tables 3 and 4). The discretionary (consumer controlled), non-discretionary (commercially controlled and/or naturally occurring) and total intakes of sodium and potassium by Americans have been estimated. Data have been obtained from production and sale of table salt, dietary recall and urinary excretion of sodium and potassium. The data suggest the total (discretionary plus non-discretionary) daily sodium chloride intake in the range of 10 to 14.5 g per person with about one-third occurring naturally in food, one-third added during food processing and one-third added by the consumer[35]. Total intake of sodium ion by the healthy men amounted to 5.55 g/d (14.0 g NaCl/d). Of this, the non-discretionary intake was 2.18 g Na (5.5 g NaCl). Authors[35] have estimated the discretionary NaCl intake to be 3 to 6.5 g/d.

With regard to non-discretionary sources of Na in the Turkish diet, bread and cereals by far constituted the largest contributor and meat products, processed food, breakfast cereals etc. constituted the lesser contributor. During our research published earlier[33], bread consumptions in pregnant and lactating women were 292 and 366 g/d, respectively. In this study daily mean bread consumption in four groups (NT + HT) was *ca.* 250 g. From bread analysis mean Na concentration of bread was 534 mg Na/100 g. Sodium intake from bread was 250 g bread × 534 mg Na/100 g bread ×

(58.5 g NaCl/23 mg Na) × 1 g/1000 mg = 3.4 g NaCl (discretionary NaCl). Daily mean discretionary NaCl consumption in four groups was 9.3 g. This is about 37 % discretionary NaCl. Charlton *et al.*[12] reported mean urinary Na excretion equated to daily salt (NaCl) intakes of 7.8-9.5 g and daily added salt (NaCl) amounts of 4.1-4.8 g. With regard to non-discretionary sources of Na in the diet of South Africans, bread and cereals by far constituted the largest contributor (45.9 to 48.6 %). Bread was identified as the single food item that provided the largest contribution to total dietary (non-discretionary) Na intake, particularly in the Black subsample. Other important food sources of Na in Black subjects included meat products, such as commercial meat pies, beef sausage and processed and soup powders and margarine.

According to Olson *et al.*[35] reported a positive correlation between dietary NaCl intake and the percentage of the population that is hypertensive in a series of different populations. The highest intakes were found in Northern Japan (28 g/d) where about 38 % of the population is hypertensive. In contrast, Alaskan natives rarely add salt to food (4 g/d) and rarely have hypertension. According to same literature, Olson *et al.*[35] reported hypertension in 22 of 100 healthy Bantu males whose mean daily NaCl intake was estimated to be 18.6 g/d from their mean 24 h urinary sodium output.

Potassium ingestion throughout the word ranges[36] from 50 to 200 mmol/d. During the present study, the daily mean K intake was 73-81 mmol/d, these values are in the range[36] of 50-200 mmol/d and similar[12] with 70-80 mmol/d. For the prevention of cardiovascular disease, the World Health Organization recommends a potassium intake that will keep the Na:K ratio close to 1, *i.e.*, 70 to 80 mM/d. We found Na:K ratio ranging from 1.14 to 1.54 in the present study (Tables 5 and 6). In the study of Charlton *et al.*[12] in all reported groups, mean reported intakes of K decreased far below the recommended intake of 90 mM/d and only 8 % of subjects met this recommendation. Daily means of dietary Na, K and Ca intakes in NT female subjects were found 3879, 2995 and 720 mg, respectively. From food composition analysis[19], daily means of dietary Na, K and Ca intakes for lactating women were found to be 5051 mg, 2358 mg and 942 mg, respectively. Consumption of Na and Ca in NT women were naturally lower than lactating women. About K intake result is discrepancy.

The consumption of legumes (dried bean, chic bean, broad bean, lentil), whole wheat flour, parboiled-pounded wheat that are rich sources of several minerals (Fe, Ca, Mg, K), B complex vitamins and fiber, are high all over Turkey. The consumption of fruit is also high all over Turkey that is reach source of K and fiber. Increased amounts of K may lower BP in at least some sensitive subjects[3].

TABLE-5
STATISTICAL EVALUATION OF DAILY ENERGY AND NUTRIENT INTAKES IN FEMALE (n = 37)

Energy and nutrients	Hypertensive group (n = 22)		Normotensive group (n = 15)		Statistical analysis	
	X[1]	SD[1]	X[1]	SD[1]	t	p
Energy (Kcal)	1948	528	2339	494	-2.294	< 0.05
Total protein (g)	68	23	84	26	-1.857	ns
Total fat (g)	71	30	77	28	-0.707	ns
Dietary fiber	35.9	20.5	39.5	17.5	-0.573	ns
Ash	12.7	5.0	15.4	1.4	-2.404	< 0.05
Ca (mg)	569	287	720	209	-1.851	ns
P (mg)	794	407	1088	294	-2.551	< 0.05
Ca/P	0.72	–	0.66	–	–	–
K (mg)	2393	1008	2995	710	-2.131	< 0.05
Na (mg)	3173	1267	3879	776	-2.098	< 0.05
Na/K	1.39	0.69	1.14	0.38	1.436	ns
Discretionary NaCl (g)	3.68	1.98	5.52	1.93	-2.802	< 0.01
Na from discretionary NaCl (%)	46		56			
Fe (mg)	12.5	6.1	12.8	4.4	-0.176	ns
Vit A (IU)	5334	3566	9004	8514	-1.578	ns
B_1 (mg)	1.14	0.59	1.05	0.31	0.648	ns
B_2 (mg)	1.39	0.75	1.57	0.67	-0.765	ns
Niacin (mg)	13.5	11.0	12.7	5.3	0.323	ns
Vit.C (mg)	162	96	163	84	-0.013	ns
Beverage (Drinking water, tea, coffee, *etc*)	1105	459	1244	224	-1.208	ns
Metabolic water (g)	247		298			
*Total water	2131	646	2681	689	-2.45	< 0.02
Water from beverage (%)	52		46			
Total water/Total energy	1.09		1.15			
Energy %						
Protein	14.0		14.4			
Fat	32.8		29.6			
Carbohydrate	53.2		44.0			

*Total water: Water from beverages + water from foods + metabolic water

For healthy life, estimated requirement for water (W): 1 mL W/kcal of food is reasonable allowance for adults (higher intakes necessary when sweat looses are high). Metabolic W refers to W gained from the metabolism of food. Metabolism of 1 g protein yields 0.40 g W; of 1 g starch (carbohydrate) 0.56 g W and of 1 g fat, 1.07 g W[37-39]. In this study, water/energy ratio were higher than 1 mL water/kcal in NT groups (for female 1.15, for male 1.35) and HT female (1.09) and near in HT group for male (0.93) (Tables 3 and 4). This values are closed to as reported earlier[19]. The range of metabolic W was 247-298 g/d. This range close to that mean value reported in the literature[39].

For determine the influence of hypertension on daily energy and nutrients intakes Tables 5 and 6 were arranged. The dietary energy, ash, P, K, Na, table salt and total water in NT female were statistically higher than those HT female (Table-5). Some trials have shown[3] that lowering dietary calorie and/or sodium (salt) content and providing increased amounts of calcium and potassium may each lower BP in at least some sensitive subjects. Because of hypertensive subjects are under physicians control in general their intakes of energy and nutrient especially discretionary salt were lower than those normotensive subjects ($p < 0.05$, Table-5). The dietary ash, P, discretionary salt and total water in NT male were statistically higher than those HT male ($p < 0.02$, Table-6).

TABLE-6
STATISTICAL EVALUATION OF DAILY ENERGY AND NUTRIENT INTAKES IN MEN (n = 20)

Energy and nutrients	Hypertensive group (n = 9)		Normotensive group (n = 11)		Statistical analysis	
	X[1]	SD[1]	X[1]	SD[1]	t	p
Energy (Kcal)	2318	644	2159	343	0.671	ns
Total protein (g)	85	17	87	17	-0.292	ns
Total fat (g)	70	27	66	18	0.453	ns
Dietary fiber	52.0	16.5	48.0	27.5	0.404	ns
Ash	13.0	3.7	20.2	7.7	-2.735	< 0.02
Ca (mg)	602	293	709	194	-0.937	ns
P (mg)	669	357	1153	300	-3.241	< 0.01
Ca/P	0.90	–	0.61	–	–	–
K (mg)	2862	938	3152	1184	-0.610	ns
Na (mg)	3506	1821	4064	1496	-0.738	ns
Na/K	1.19	0.25	1.54	1.2	-0.918	ns
Discretionary NaCl (g)	2.23	1.61	5.89	2.2	-4.341	< 0.01
Na from discretionary NaCl (%)	25		57			
Fe (mg)	14.8	6.0	15.3	6.1	-0.217	ns
Vit A (IU)	8776	5104	10792	6182	-0.799	ns
B_1 (mg)	0.85	0.40	1.01	0.28	-1.013	ns
B_2 (mg)	1.22	0.63	1.51	0.48	-1.130	ns
Niacin (mg)	15.1	7.7	15.8	7.1	-0.214	ns
Vit.C (mg)	144	69	187	77	-0.714	ns
Beverage (Drinking water, tea, coffee, *etc*)	965	547	1692	255	-1.064	ns
Metabolic water (g)	297		275			
Total water	2148	500	2907	826	-2.53	< 0.02
Water from beverage (%)	45		58			
Total water/Total energy	0.93		1.35			
Energy %						
Protein	14.7		16.1			
Fat	27.2		27.5			
Carbohydrate	58.1		56.4			

The results obtained from regression analysis for NT subjects are given in Table-7. In NT subjects there were statistically significant positive correlations between energy, protein and fat intakes (Table-7). There was also a significant positive correlation between dietary Ca and K. Systolic blood pressure correlated positively with DBP ($r = 0.588$) and UCaV ($r = 0.516$) and negatively with dietary Ca ($r = -0.617$, for all $p < 0.05$). Epidemiological evidence has suggested[7] an inverse relationship between blood pressure and dietary calcium intake. Diastolic blood pressure correlated positively with BMI ($r = 0.494$, $p < 0.05$). Dietary intakes of Na and K, were associated with neither systolic nor diastolic blood pressure. Correlation between UCa and dietary Ca was not statistically significant ($p > 0.05$, Table-7). Serum Ca was not correlated statistically with BP ($p > 0.05$, data not shown).

The results obtained from regression analysis for HT subjects are given in Table-8. There were statistically significant correlations between energy intake and some dietary nutrients: protein, fat, Ca, K and Na. There were also statistically significant correlations between dietary protein and some dietary nutrients: fat, Ca, K and Na ($p < 0.05$). Systolic blood pressure correlated positively with DBP ($r = 0.741$) and negatively with serum Ca ($r = -0.633$). Diastolic blood pressure also correlated negatively with serum Ca ($r = -0.727$). Correlation between UCa and dietary Ca was not statistically significant. Urinary Ca was not correlated statistically with BP ($p > 0.05$, Table-8). Leiba *et al.*[26] reported estimated Ca intake slightly correlated with 24 h urinary excretion ($r = 0.23$, $p < 0.01$). It seems that urinary calcium does not accurately reflect dietary Ca intake and should not be routinely evaluated in NT and HT subjects. Tillman and Semple[25] reported a significant correlation between total serum Ca and systolic pressure ($r = 0.23$, $p < 0.05$). Charlton *et al.*[12] reported that dietary Ca intake inversely associated with systolic blood pressure ($r = -0.254$, $p < 0.05$) and diastolic blood pressure ($r = -0.224$, $p < 0.05$) for white HT subjects, a similar trend for the other group, but statistical not significant ($r = -0.127$ for all groups, $p > 0.05$). Resnick *et al.*[3] reported a significant positive correlation between basal SBP and UCaV, particularly among HT hyperlipemic and NT hyperlipemic subjects.

In both groups were not observed significant correlation between UCa and diet Ca ($p > 0.05$). Similar findings were described by Leiba *et al.*[26]. They reported a weak correlation between estimated intakes of Ca and measured urinary excretion. Urinary calcium excretion remained low despite a high intake of Ca. Vaughan *et al.*[40] reported that a high Ca (1400 mg/d) and moderate Na (3300 mg/d) diet significantly decrease BP in mild hypertensive males *vs.* a low Ca (400 mg/d), moderate Na diet, the high Ca group show a nonsignificant increase in UCa. In this study the range of dietary Ca levels was 569-720 mg/d, this values are below 1400 mgCa/d.

TABLE-7
CORRELATION BETWEEN BLOOD PRESSURE AND ENERGY, NUTRIENTS, BODY MASS INDEX, SERUM AND URINARY CALCIUM LEVELS IN NORMOTENSIVE SUBJECTS (n = 31)

	Energy	Protein	Fat	Diet Ca	Diet K	Diet Na	DBP	SBP	BMI	UCaV
Energy	1									
Protein	0.483*	1								
Fat	0.550*	0.187	1							
Diet Ca	0.191	-0.227	-0.082	1						
Diet K	0.294	0.183	-0.197	0.559*	1					
Diet Na	0.108	0.326	-0.195	0.021	0.237	1				
DBP	0.195	0.358	0.048	-0.066	0.178	0.068	1			
SBP	0.086	0.343	-0.080	-0.617*	-0.075	0.062	0.588**	1		
BMI	0.107	0.041	0.060	0.003	0.121	0.011	0.494**	0.305	1	
UCaV	0.020	-0.179	0.340	-0.254	-0.300	-0.285	0.271	0.516**	0.074	1

*Correlation is significant at the 0.05 level (2-tailed) (n = 26); **Correlation is significant at the 0.05 level (2-tailed) (n = 31).

TABLE -8
CORRELATION BETWEEN BLOOD PRESSURE AND ENERGY, NUTRIENTS, BODY MASS INDEX, SERUM AND URINARY CALCIUM LEVELS IN HYPERTENSIVE SUBJECTS (n=31)

	Energy	Protein	Fat	Diet Ca	Diet K	Diet Na	DBP	SBP	BMI	UCaV	Serum Ca
Energy	1										
Protein	0.867*	1									
Fat	0.743*	0.666*	1								
Diet Ca	0.653*	0.596*	0.559*	1							
Diet K	0.689*	0.756*	0.537*	0.739*	1						
Diet Na	0.656*	0.564*	0.485*	0.501*	0.419*	1					
DBP	-0.283	-0.244	-0.198	-0.103	-0.044	-0.038	1				
SBP	-0.073	-0.051	0.098	0.107	0.085	0.067	0.741*	1			
BMI	-0.124	-0.019	0.226	0.136	0.060	-0.034	0.150	0.266	1		
UCaV	0.156	-0.011	0.033	-0.097	-0.268	0.213	-0.340	-0.315	0.335	1	
Serum Ca	0.056	0.208	-0.082	-0.264	0.218	-0.279	-0.727**	-0.633**	-0.250	0.020	1

*Correlation is significant at the 0.05 level (2-tailed) (n = 31); **Correlation is significant at the 0.05 level (2-tailed) (n = 13).

With regard to non-discretionary sources of Na in the diet of Turkey, bread and cereals by far constituted the largest contributor. For this reason we recommend that the food industry decrease the Na content and increase amounts of K, Ca and fiber in bread and that the effect of this intervention on blood pressure be tested in controlled studies.

Dietary magnesium may influence BP and urinary magnesium also influence the blood pressure. For this reason our researches about this case continue.

Conclusion

Correlation between UCa and dietary Ca was not statistically significant, so the urinary calcium excretion does not accurately reflect dietary Ca intake and there for should not be routinely evaluated in normotensive and hypertensive subjects. In NT subjects systolic blood pressure correlated negatively with dietary Ca and diastolic blood pressure correlated positively with BMI. Therefore, we recommend that normotensive subjects increase dietary Ca and decrease BMI.

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