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Drug therapy may help obese patients, with diet, exercise, and behavior modification. The role of drugs was questioned because of concerns about efficacy, safety, and high recurrence rates.

The initiation and choice of drug therapy should be made only after evaluation of risks and benefits. Patient evaluation includes determination of the body mass index (BMI), waist circumference, and comorbid conditions such as diabetes, dyslipidemia, hypertension, sleep apnea, and heart disease.

GOALS OF THERAPY AND CRITERIA FOR SUCCESS:

The goal of treatment must be realistic. The physician and the patient need to come to a mutual understanding of the realities of weight loss. Success may be measured by the degree of weight loss and improvement in associated risk factors.

To be considered effective, weight loss should:

- Exceed 2 kg during the first month of therapy,
- Fall > 5% below baseline by 3-6 months, and
- Remain at this level.

A weight loss of 5-10% significantly reduces the risk for diabetes and cardiovascular disease. The maximal duration of published treatment results is 2 y for Sibutramin and 4 for Orlistat. If response is good and the patient wishes to continue, this may be considered after acknowledging the lack of longer term data. Obese subjects given drugs should be advised that when the maximal therapeutic effect is achieved, weight loss ceases. When drug therapy is discontinued, weight is regained.

Achieving and maintaining weight loss is hampered by reduction in energy expenditure associated with weight loss.

PRACTICE GUIDELINES

The American College of Physicians (ACP) has issued clinical practice guidelines for the pharmacologic and surgical management of obesity in primary care [1]. These guidelines are based upon the results of two meta-analyses [2,3] and the existing guidelines from the US Preventive Services

The guidelines include four recommendations:

1. Counsel all obese patients (BMI \geq 30 kg/m²) on diet, lifestyle, & goals.
2. Drug therapy may be offered to those who failed to achieve weight goals by diet and exercise.
3. Drug options include sibutramine, orlistat, phentermine, diethylpropion, and two drugs that are not approved for obesity treatment, fluoxetine and bupropion.
4. Bariatric surgery should be considered with BMI 40 kg/m² who failed diet and exercise (\pm drug

therapy) and obesity-related comorbidities (hypertension, diabetes mellitus, dyslipidemia, sleep apnea). It should be performed at high-volume centers with experienced surgeons.

SYMPATHOMIMETIC DRUGS:

1. Stimulate the release of norepinephrine or inhibit its reuptake into nerve terminals (phentermine, and diethylpropion, benzphetamine, phendimetrazine)
2. Block norepinephrine and serotonin reuptake (sibutramine)
3. Directly act upon adrenergic receptors (phenylpropanolamine - now withdrawn from the market)

Sympathomimetic drugs reduce food intake by causing early satiety. They may increase BP All are rapidly absorbed, peak plasma concentration in 1-2 hours. Plasma half-lives are short, except sibutramine (the only drug in this group that has active metabolites). All are metabolized in the liver.

Sibutramine (Meridia®, Reductil®) [4-17]: specific inhibitor of norepinephrine, serotonin and dopamine reuptake. It does not bind to any known CNS receptors.

In a meta-analysis of sibutramine therapy of obesity, at about 1 y, the mean difference in weight between treatment and placebo was 4.5 kg, pts on placebo lost about 5.5 kg. Patients would expect to lose 10 kg if treated for 1 year. Weight loss is associated with decrease in triglyceride and LDL cholesterol [12]. The initial weight loss predicts the long-term response.

Obese diabetics also benefit from therapy. Pts lose weight and waist circumference and reach lower HbA1c, triglycerides, and higher HDL cholesterol, without change in blood pressure.

Hypertensives treated with sibutramine had a significant increase in diastolic, not systolic, BP.

The addition of lifestyle changes to sibutramine is more effective than either therapy alone.

Safety: In the clinical trials of sibutramine, systolic and diastolic BP increased by 1-3 mmHg and pulse increased about 4-5 beats/minute. The drug should not be used with uncontrolled hypertension. I also avoid sibutramine with a history of coronary disease, heart failure, arrhythmia, stroke, and with selective serotonin reuptake inhibitor (risk of serotonin syndrome), and temporarily stop it in patients taking erythromycin and ketoconazole (metabolized by the cytochrome P450 enzyme system) There is no evidence that sibutramine causes cardiac valve disease or pulmonary hypertension.

The recommended starting dose is 10 mg QD, with titration up to 15 mg QD or down to 5 mg QD based upon the response.

Not recommended:

1. Phentermine and diethylpropion: potential for abuse
2. Phenylpropanolamine: removed, risk of hemorrhagic stroke.
3. Ephedrine and Ephedra alkaloids, removed, cardiovascular adverse effects

Orlistat (Xenical®)[18-28]: The only drug that alters fat digestion by inhibiting pancreatic

lipases. In normal subjects eating a diet that contains 30% fat, 50-200 mg with each meal causes a dose-dependent increase in fecal fat. Less than 1% is absorbed. Orlistat does not alter the pharmacokinetics of digoxin, phenytoin, warfarin, oral contraceptives, alcohol, furosemide, captopril, nifedipine, or atenolol. However, absorption of fat-soluble vitamins may be decreased.

In a 4 year double-blind randomized controlled trial on 3304 overweight patients (21% with impaired glucose tolerance -IGT), weight loss of > 11% in the orlistat group & 6% in the placebo-treated group was seen during the first year. Over the remaining years of the trial, there was a small regain in weight, by the end of 4 years, the orlistat-treated patients were 6.9% below baseline, compared with 4.1% for those receiving placebo. There was 37% reduction in the conversion of patients from IGT to diabetes. In diabetics, orlistat led to more weight loss, and a decrease in hemoglobin A1c at one year than placebo [26]. Meta-analysis of trials with diabetics and non-diabetics, the difference in weight loss due to orlistat was about 3 kg, the loss on placebo was about 5.5 kg, i.e. the expected loss of about 9 kg in compliant patients.

Orlistat also improves some serum lipids beyond weight reduction alone [27].

Side effects: major side effects are intestinal cramps, flatus, fecal incontinence, oily spotting, and flatus with discharge (15-30%) [29]. These complaints are usually mild and subside after several weeks of treatment. Absorption of vitamins A and E and beta-carotene may be slightly reduced in some studies of patients receiving orlistat. Orlistat only impairs absorption of cyclosporine.

The recommended dose is 120 mg TID.

Sibutramin-Orlistat combination [30,31]:

The addition of orlistat to sibutramine did not significantly enhance weight loss.

ANTIDEPRESSANTS

Fluoxetine and sertraline, selective serotonin reuptake inhibitors (SSRI), may facilitate weight loss in the short run. Fluoxetine as an anti-obesity drug (60 mg/d,

three times the usual dose for depression) produced variable weight loss.

Trials of fluoxetine for obesity showed weight loss at 6 & 12 m of 4.8 & 2.4 kg, respectively, vs. 2.2 & 1.8 kg on placebo. The regain of 50% of the lost weight during the second 6 m on fluoxetine suggest limited long term utility [32]. In obese patients with depression, SSRIs are preferred over other antidepressants, many of which cause weight gain.

Bupropion is used for depression and to prevent of weight gain when trying to stop smoking [33]. In a 6 m trial of bupropion (300 or 400 mg/d) versus placebo with a six-month blinded extension where all patients received active medication, both doses of bupropion produced significantly more weight loss than placebo [34]. During the six-month extension the weight loss was largely maintained. Because of the small number of subjects in the clinical database for weight loss, this drug is not recommended for use in obesity at this time.

ANTIPILEPTIC DRUGS

Topiramate & Zonisamide are currently investigational, not for clinical use.

DIABETES DRUGS

Metformin: In the Diabetes Prevention Program, metformin-treated patients with impaired glucose tolerance lost 2.5% of their body weight at about 3Y follow-up. It is a useful choice for obese patients at high risk for diabetes.

DIETARY SUPPLEMENTS — Over-the-counter dietary supplements are widely used by individuals attempting to lose weight, but evidence to support their efficacy and safety are limited. Examples of dietary supplements include Ephedra (no longer available), green tea, chromium, chitosan, ginseng, guar gum, and calcium supplement (all useless).

EMERGING DRUGS

Rimonabant (Acomplia)[35-39], a cannabinoid receptor antagonist, was investigated for both smoking cessation and obesity.

In a one-year trial of 1507 patients with BMI >30 kg/m² (or >27 kg/m² with treated or untreated dyslipidemia, hypertension, or both) who were randomly assigned to receive rimonabant (5 or 20 mg/day) or placebo in

addition to a hypocaloric diet (600 kcal/day deficit), the following results were seen [35]:

Variable	Rimonabant 20 mg	Placebo
Mean weight loss	6.6 kg ± 7.2	1.8 kg ± 6.4
% with > 5% Wt loss	51	19
% with > 10% Wt loss	27	7

The 20 mg dose also produced significantly greater improvements in waist circumference, HDL, triglycerides, insulin resistance, and prevalence of the metabolic syndrome. Adverse effects, included mood changes, nausea, vomiting, diarrhea, headache, dizziness, and anxiety. However, dropout rates were similar in all groups.

Thus, rimonabant 20 mg/day resulted in clinically meaningful weight loss, reduction in waist circumference, and improvements in several metabolic risk factors.

In a trial, on about 3000 obese / overweight patients, during the first year, those on rimonabant lost more weight than on placebo.

In the second y, rimonabant-treated patients were re-randomized to placebo or continue on the same dose, the placebo group continued with placebo [38]. Patients continued on rimonabant (20 mg/day) maintained weight loss, while those randomized to placebo regained their weight. Effects on lipids, insulin resistance, waist circumference, and BP were similar to previous trials. Commonest adverse effect on rimonabant 20 mg was nausea (11.2% vs. 5.8% on placebo). Psychiatric disorders were also more common (6.2 vs. 2.3%).

In summary, rimonabant 20 mg/day for 2 years, with diet and exercise, causes modest sustained weight loss, and favorably changes waist circumference and cardiometabolic risk factors. However, the trial was limited by a very high drop-out rate (nearly 50% by 1 year).

Rimonabant is also effective in obese patients with type 2 diabetes. It was associated with significant weight loss and improvements in cardiovascular risk factors and HbA1C [37].

Peptides: There are several peptides that result in weight loss, either by reducing food intake (Leptin, peptide YY, Oxyntomodulin, Melanocortin), or increasing energy expenditure. None are currently approved by the FDA.

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Bone Mineral Density And Carotid Atherosclerosis In Postmenopausal Women

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Abstract

Objective: The relationship between bone mineral density & atherosclerosis in older postmenopausal women is a subject of increasing concern. This study is aimed to assess the presence of such relation. **Design:** 150 postmenopausal women, aging from 55 years old and over, were recruited from the outpatient geriatric clinic and osteoporosis unit attendants. All participants were subjected to; detailed medical history taking, complete physical examination, and measurement of total cholesterol and triglycerides levels. Measurement of bone mineral density of lumbar spine (L2-L4) and left sided proximal femur (neck) was done using dual-energy X-ray absorptiometry. Intima-media thickness of both common carotid arteries and internal carotid arteries, presence of plaques and degree of stenosis were determined by using carotid ultrasonography. **Results:** there was no statistically significant relation between the presence of thickened mean intima-media of both common carotid arteries and internal carotid arteries and bone mineral density of lumbar spine and femur neck. On the other hand, statistically significant relation was observed between the presence of plaques in the carotid arteries and low bone mineral density of both lumbar spine $P=0.00$ and femur neck $P=0.02$. The Multinomial logistic regression analysis showed that, after controlling for age and duration of menopause, those with plaques have 3.28 and 2.54 folds risk to have osteoporosis of femur neck and lumbar spine respectively. Sub-analysis done for those not having known cardiovascular risk factors have even shown increased risk to 3.52 and 4.05 respectively. **Conclusion:** This result supports the aimed hypothesis that there is a relationship between bone mineral density and atherosclerosis in postmenopausal women.

Keywords:

Bone mineral density
Osteoporosis
Intima-media thickness
Atherosclerosis
Menopause

Introduction

Atherosclerotic disease and osteoporosis are two common degenerative processes that contribute in great measure to the decline in performance and quality of life of the elderly population (1). Both osteoporosis and atherosclerosis increase in frequency with advancing age, and both appear worse (or at least more frequent) in postmenopausal women (2). Osteoporotic women are at significantly greater risk for cardiovascular disease than age matched controls (3).

For years, osteoporosis and cardiovascular disease were thought to be two independent consequences of aging; however, mounting evidence supports an association between these diseases (4). Hirose et al., 2003 found an association between increased bone loss and early stages of atherosclerosis (5). In another study, the presence of decreased bone mass or osteoporotic vertebral fractures was associated with an increased cardiovascular mortality. Calcaneal bone loss of 1 SD (standard deviation) as measured by

osteodensitometry was associated with a 1.31 times increased risk for the occurrence of stroke (6). Biological interaction between the bone and the blood vessels are gradually being clarified and evidence is accumulating for the link between the vascular and bone disease (7). The association between arterial calcification or atherosclerosis and osteopenia is documented to be more prominent in females than in males and this relation is stronger after the menopause. This evidence suggests the presence of common or related mechanisms, which may be accelerated after menopause, controlling both atherosclerosis and osteoporosis from the early stages (8). So the aim of this work is to assess the hypothesis that there is an association between bone mineral density and atherosclerosis among postmenopausal women.

Methodology

Participant selection criteria

Using the data from previous studies, the sample size of this study was calculated to be 144 candidates with a study power of 80%, confidence level 90% and Odd's ratio 2.5. Indeed, 150 participants were involved in this study. They were recruited from the outpatient geriatric clinic and osteoporosis unit attendants. The subjects were all postmenopausal females [postmenopausal woman is the woman who did not have a menstrual period for one year or longer (9)], aging 55 years old and over.

Tools of Assessment:

All participants were assessed using detailed medical history taking, and complete physical examination.

Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA); measurements were done at 2 sites: lumbar spine (L2-L4) in the anteroposterior position and proximal femur (neck) on left side using LUNAR DPX-MD+ densitometer. Quality control procedures were followed in accordance with the manufacturer's recommendations. Daily routine calibration was done using the standard phantom supplied by manufacturer.

Carotid ultrasonography was done using B-mode ultrasound device LOGIC 500

of GE Medical Systems. Sonography and reading were done by trained and certified sonographers and ultrasound readers with regular quality control at the radiology department. They were not aware of the aim of the study. Scanning protocol involved studying the right and left common carotid arteries (CCAs), and studying the right and left internal carotid arteries (ICAs) in all subjects. Patients were examined in the supine position, and each carotid wall and segment was interrogated independently from continuous angles to identify the thickest intima-media site.

Intima-media thickness (IMT) was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of near and far walls. Observations led to estimate that, on average, a healthy person reaches an IMT of 0.78mm at the age of 76 years (10). But in many studies, the maximum thickness of the intima-media complex of healthy elderly was 1.0mm and IMT \geq 1mm represents a risk of myocardial infarction and/or cerebrovascular disease (11). Therefore, we defined thickened IMT as when the axial thickness of the intima-media complex was \geq 1.0 mm.

A plaque was defined as a localized protrusion of the vessel wall into the lumen with the focal thickening $>$ 50% of the surrounding wall of adjacent segments (12, 13). The maximum percent stenosis was assessed and the patient was considered to have hemodynamically significant stenosis if its degree was \geq 70% (14, 15).

Laboratory investigations were done including; total cholesterol and triglycerides levels after 14 hours fasting. Fasting blood glucose was also done to detect diabetic patients not already diagnosed.

Data processing & statistical analysis:

Data collected was revised, coded, tabulated & introduced to PC for statistical analysis. All data manipulation & analysis were performed using the 11th version of SPSS (Statistical Package for Social Sciences). Qualitative data was presented in form of frequency tables (number and percentage). Quantitative data was

presented in form of mean \pm standard deviation and range.

Transformation of some variables was carried out in some instances for example to classify patients according to their T-score of the DEXA measurements into "Normal BMD", "Osteopenia" and "Osteoporosis". Pearson correlation coefficient was performed to test correlation between 2 quantitative variables, while Pearson chi-squared was used with correction to test association between 2 qualitative variables. Independent sample-t test was also used to compare two groups with quantitative continuous variables.

Multinomial logistic regression analysis was done to determine the independent association of different factors. All the included variables were made qualitative variables. P value was always set as significant at 0.05.
Results:

The sample included 150 postmenopausal women with a mean age of 66.18 ± 7.18 , and range 55 - 89 years and with BMI (body mass index) of 30.66 ± 7.06 and range 16 - 54. 34% (n=51) were married, 0.7% (n=1) single, while 65.3% (n=98) were either widow or divorced.

The age at menopause of the studied sample was 49.24 ± 5.2 years with a range of 30-58 years. The mean duration of menopause was 16.65 ± 7.89 years with a range of 1-35 years. Early age of menopause among some of the participants (n=11, 7.3%) was due to bilateral oophorectomy or hysterectomy.

Concerning co-morbid diseases, 25.3% (n=38) were diabetic, 40.7% (n=61) were hypertensive, 16.7% (n=25) had coronary artery disease (CAD), and 14.7% (n=22) had history of either stroke or TIA.

Patients were considered to be diabetic if they were being treated with anti-diabetic medications or if they had serum fasting blood sugar levels > 126 mg% (16). Patients were considered to be hypertensive if they were being treated with antihypertensive medications or if they had a systolic blood pressure ≥ 140 &/or diastolic blood pressure ≥ 90 on twice measurement (17). Patients were considered to have CAD if they were being treated with anti-ischemic medications or if they had ischemic symptoms.

When assessing the frequency of hyperlipidemia among the investigated sample of postmenopausal women, 48% (n=72) had hypercholesterolemia, and 24.7% (n=37) had hypertriglyceridemia. Hypercholesterolemia was considered if total cholesterol > 200 mg/dl while hypertriglyceridemia was considered if total TG level > 150 mg/dl after 14 hours of fasting (18).

As for DEXA data, participants were classified into; Normal: T-score + or -1 SD, Osteopenia: T-score - 1 to -2.5 SD, Osteoporosis: T-score -2.5 or less (19). 56% (n=84) were found to be osteoporotic at the lumbar spine, while this percentage was 32.7% (n=49) for the neck of femur (table 1).

Table (1): The frequency of osteopenia and osteoporosis in the investigated sample of postmenopausal women (Total = 150).

Site	Frequency n=	Percent
*Lumbar spine		
Normal BMD	21	14%
Osteopenia	45	30%
Osteoporosis	84	56%
*Femur neck		
Normal BMD	32	21.3%
Osteopenia	69	46.0%
Osteoporosis	49	32.7%

Age was found to be significantly and inversely correlated to BMD (Gm/cm² and T-score) of both lumbar spine and femur neck. Duration of menopause (how many years passed since menopause) & not the

age at menopause, was inversely related to BMD (Gm/cm² and T-score) of both lumbar spine and femur neck. This relation was statistically significant (table 2).

Table (2): Correlation between BMD of lumbar spine, left femur neck and age and menopause duration in the studied sample.

BMD	Age		Menopause duration	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
*Lumbar spine				
Gm/cm ²	-0.269	0.00*	-0.320	0.00*
T- score	-0.280	0.00*	-0.324	0.00*
*Femur neck				
Gm/cm ²	-0.469	0.00*	-0.348	0.00*
T- score	-0.500	0.00*	-0.405	0.00*

* P value was set as significant at 0.05 level.

As for the findings of carotid duplex, the mean CCA IMT was 0.98mm (ranging from 0.35 to 1.80) and the percentage of women with thickened CCA IM \geq 1mm was 48.7%. The mean ICA IMT was 0.97mm (ranging from 0.35 to 1.90) and the percentage of women with thickened ICA IM \geq 1mm was 50.7%.

Hemodynamically significant stenosis \geq 70% on either side was found in only 0.7%. As for plaques, carotid plaques on either side was found in 48% of the participants (4% were soft plaques and 44% were calcific) (table 3).

Table (3): The frequency of Carotid artery duplex measurements findings:

	Frequency n=	Percent
Thick MCCA.IM \geq 1mm	73	48.7%
Thick MICA.IM \geq 1mm	76	50.7%
Sig. Stenosis R. side \geq 70%	1	0.7%
Sig. Stenosis L. side \geq 70%	1	0.7%
Plaques of right carotid arteries		
• No plaques	107	71.3%
• Soft plaques	6	4.0%
• Calcific plaques	37	24.7%
* Plaques of left carotid arteries		
• No plaques	94	62.7%
• Soft plaques	7	4.6%
• Calcific plaques	49	32.7%
*Carotid Plaques on either side		
• No plaques	78	52.0%
• Soft plaques	6	4.0%
• Calcific plaques	66	44.0%

Using the independent sample T test, statistically significant relation was found between BMD with the presence of plaques but not with the thickened intima-media (table 4).

Table (4): The relation between thickened mean intima-media ($\geq 1\text{mm}$) of both CCA & ICA and the presence of plaques in the carotid system and BMD of lumbar spine and left femur neck in the studied sample.

		Thickened mean CCA intima-media		Thickened mean ICA intima-media		Presence of plaques in carotid arteries	
		<1mm	$\geq 1\text{mm}$	<1mm	$\geq 1\text{mm}$	-	+
Spine	Gm/cm ²	0.92±0.18	0.89±0.18	0.92±0.16	0.88±0.16	0.94±0.18	0.87±0.17
		t=0.855	p=0.39	t=1.273	p=0.21	t=0.39	p=0.02*
	T- score	-2.29±1.52	-2.54±1.41	-2.26±1.50	-2.57±1.40	-2.12±1.44	-2.73±1.45
		t=1.056	p=0.29	t=1.296	p=0.20	t=0.62	p=0.01*
Femur neck	Gm/cm ²	0.78±0.14	0.74±0.14	0.78±0.14	0.74±0.14	0.81±0.14	0.70±0.12
		t=1.724	p=0.09	t=1.416	p=0.16	t=0.32	p=0.00*
	T- score	-1.69±1.19	-1.92±1.14	-1.62±1.17	-1.97±1.14	-1.38±1.12	-2.26±1.04
		t=1.182	p=0.24	t=1.848	p=0.07	t=0.51	p=0.00*

* P value was set as significant at 0.05 level.

Although, there was no statistically significant relation between the presence of thickened mean CCA IM ($\geq 1\text{mm}$) and BMD of lumbar spine and femur neck, 33.3% of women with normal BMD of lumbar spine had thickened mean CCA Intima-media compared to 48.9% and 52.4% in women with

osteopenia and osteoporosis respectively ($\chi^2=2.44$, $p=0.29$) (figure1).

Also, 43.8% of women with normal BMD of femur neck have thickened CCA Intima-media compared to 46.4% and 55.1% in women with osteopenia and osteoporosis respectively ($\chi^2=1.27$, $p=0.53$) (figure1).

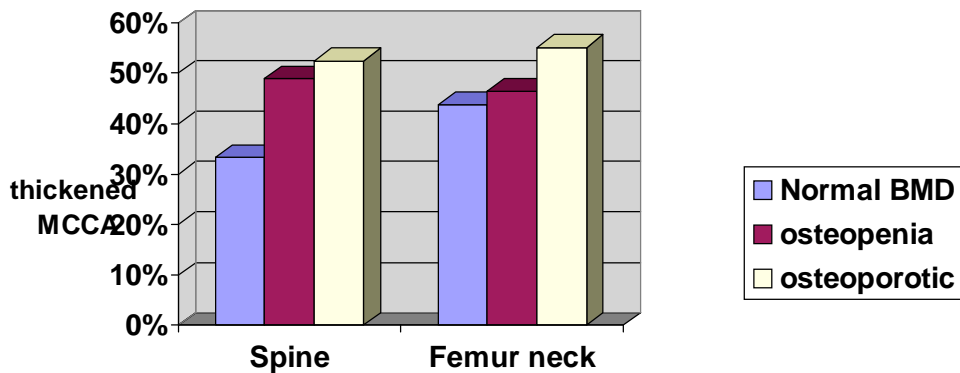


Figure 1: thickened CCA IM and the presence of osteoporosis in both lumbar spine and femur neck.

Also, 38.1% of women with normal BMD of lumbar spine had thickened ICA Intima-media compared to 44.4% and 57.1% in women with osteopenia and osteoporosis respectively ($\chi^2=3.43$, $p=0.18$). And, 43.8% of women with normal BMD of femur neck

have thickened ICA Intima-media compared to 49.3% and 57.1% in women with osteopenia and osteoporosis respectively ($\chi^2=1.49$, $p=0.47$) (figure 2). Yet, these results were not statistically significant.

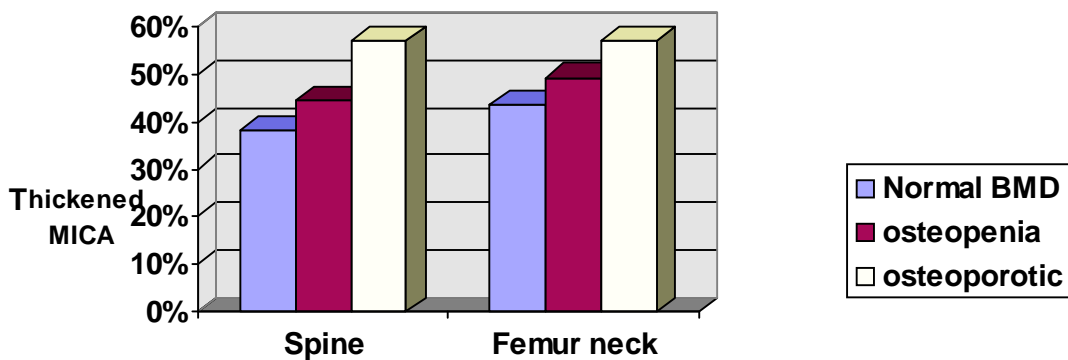


Figure 2: thickened ICA-IM and the presence of osteoporosis in both lumbar spine and femur neck.

Statistically significant positive relation was found between the presence of plaques in the carotid system and osteoporosis of both lumbar spine and femur neck. 33.3% of women with normal BMD of lumbar spine had plaques compared to 37.8% among osteopenics and 57.1% in

women with osteoporosis ($\chi^2=6.50$, $p=0.03$). And, 28.1% of women with normal BMD of femur neck had plaques compared to 42% and 69.4% in women with osteopenia and osteoporosis respectively ($\chi^2=15.03$, $p=0.00$) (figure 3).

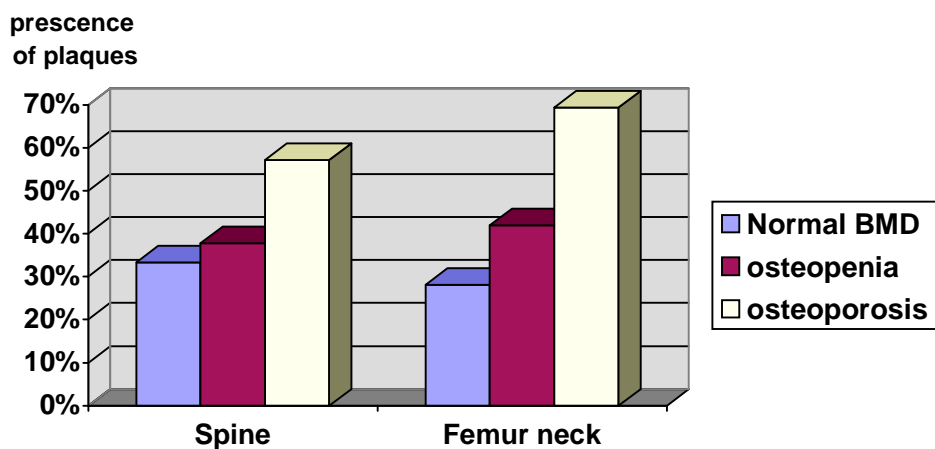


Figure 3: Carotid plaques and the presence of osteoporosis in both lumbar spine and femur neck.

In addition to plaques, age and menopausal duration, osteoporosis was found to have statistically significant relation with hypertension (only lumbar spine) (table 5).

Table (5): relation between osteoporosis and each of hypertension, diabetes mellitus and hypercholesterolemia.

	Site	Osteoporotic		Not osteoporotic		Statistical difference	
		N	%	N	%	X2	P
HTN	Femur neck	32	65.3	57	56.4	1.08	0.30
	Lumbar spine	56	66.7	33	50	4.25	0.04
DM	Femur neck	13	26.5	25	24.8	0.05	0.81
	Lumbar spine	22	26.2	16	24.2	0.07	0.78
Hypercho- Isterolemia	Femur neck	20	40.8	52	51.5	1.50	0.22
	Lumbar spine	36	42.9	36	54.5	2.03	0.15

Using Multinomial logistic regression, statistically significant association was found between the plaques and osteoporosis of both lumbar spine (p= 0.01, OR=2.54 (1.23-5.23)) and femur neck (p= 0.02, OR=3.28 (1.54-7.01)), independent of age, menopausal duration. Hypertension was also found to be an independent risk factor for osteoporosis of the spine (p= 0.01, OR=2.38 (1.17-4.83)).

An additional sub-analysis was done for those not having known cardiovascular risk factors (hypertension, DM, hypercholesterolemia, or pre-existing CVD). They were 79 postmenopausal women. 26.6% (n=21) of them had lumbar spine osteopenia while 62% (n=49) of them had spine osteoporosis. These percentages were

41.8% (n=33) and 35.4% (n=28) respectively for the femur neck. Significant negative correlation was found between the bone mass of the spine with both the age and menopausal duration (r=-0.313, p=0.005 – r=-0.341, p=0.002 respectively). This was the same for the bone mass of the femur neck (r = -0.582, p=0.000 – r=-0.447, p=0.000 respectively). As for the findings of carotid duplex, the percentage of women with thickened CCA IM \geq 1mm was 48.1% (n=38) and with thickened ICA IM \geq 1mm was 46.8% (n=37), whereas 50.6% (n=40) of them had plaques. Using the independent sample T test, statistically significant relation was found between BMD with the presence of plaques but not with the thickened intima-media (table 6).

Table (6): The relation between thickened mean intima-media (\geq 1mm) of both CCA & ICA and the presence of plaques in the carotid system and BMD of lumbar spine and left femur neck among those not having cardiovascular risks.

		Thickened mean CCA intima-media		Thickened mean ICA intima-media		Presence of plaques in carotid arteries	
		<1mm	\geq 1mm	<1mm	\geq 1mm	-	+
Spine	Gm/cm ²	0.90 \pm 0.19	0.85 \pm 0.18	0.91 \pm 0.19	0.84 \pm 0.17	0.94 \pm 0.18	0.82 \pm 0.18
		t=1.233	p=0.221	t=1.647	p=0.104	t=3.024	p=0.003*
	T- score	-2.49 \pm 1.58	-2.93 \pm 1.48	-2.45 \pm 1.61	-3.00 \pm 1.42	-2.19 \pm 1.45	-3.20 \pm 1.47
		t=1.294	p=0.199	t=1.661	p=0.101	t=3.081	p=0.003*
Femur neck	Gm/cm ²	0.78 \pm 0.16	0.74 \pm 0.14	0.79 \pm 0.15	0.73 \pm 0.14	0.83 \pm 0.14	0.69 \pm 0.12
		t=1.197	p=0.235	t=1.788	p=0.078	t=4.945	p=0.000*
	T- score	-1.66 \pm 1.33	-2.026 \pm 1.18	-1.59 \pm 1.29	-2.12 \pm 1.19	-1.20 \pm 1.15	-2.45 \pm 1.06
		t=1.304	p=0.196	t=1.888	p=0.063	t=5.035	p=0.000*

* P value was set as significant at 0.05 level.

Using the Multinomial logistic regression analysis for the chosen subgroup, statistically significant association was found between the presence of plaques and osteoporosis of lumbar spine (p= 0.01, OR=4.05 (1.32-12.41)) and osteoporosis of femur neck (p= 0.03, OR=3.52 (1.11-11.19)), independent of age, and menopausal duration.

Discussion:

Atherosclerosis and osteoporosis are major public health problems that frequently coexist and account for significant morbidity and mortality in the aging population (20). Vascular calcification is a common feature of atherosclerotic plaques and is regulated in a way similar to bone mineralization.

The current study was conducted to test the hypothesis that there is a relationship between these potentially related two diseases.

There were several limitations of this study. The cross-sectional design of our study makes it difficult to detect what come first atherosclerosis or osteoporosis. In addition, atherosclerotic risk factors were not fully assessed.

Although, the results of the current study revealed no statistically significant relation between the presence of thickened mean intima-media of both CCA and ICA and BMD of lumbar spine and femur neck, there was a statistically significant positive relation between the presence of plaques in the

carotid system and low BMD of both lumbar spine and femur neck.

Postmenopausal women with osteoporosis were shown to have higher risk of developing atherosclerotic plaques in the carotid arteries than the non-osteoporotic postmenopausal women. Also, we can say that those having carotid plaques are more liable to have osteoporosis than those not having plaques. The risk was also evident in the subgroup analysis, indicating that there is a relationship between BMD and carotid atherosclerosis in postmenopausal women regardless of the cardiovascular risk factors.

A cross-sectional, population-based study of 2,543 men and 2,726 postmenopausal women aged 55–74 years, revealed that increasing BMD is related to a decreasing prevalence of echogenic plaque (21). Another study revealed that atherosclerotic calcification is associated with bone loss in women (22). This is in accordance with earlier studies which suggested the presence of a relationship between osteoporosis and atherosclerosis (23, 24). The study of Uyama et al done on 30 postmenopausal women aged 67 to 85 revealed a significant correlation between carotid-plaque score and BMD (25).

Both age and menopause might explain the association between both atherosclerosis and BMD.

One possible explanation of the association between atherosclerosis and osteoporosis in postmenopausal women is estrogen deficiency status. Estrogen deficiency contributes to decreased BMD in post menopause and is reported to be associated with increased atherogenic oxidized LDL-cholesterol (26).

A recent data reported that age is associated with increase in vascular disease and low bone mineral density. And it is a major risk factor for both conditions (20). However, most studies (27, 28), but not all (29) have indicated that, after adjustment for age, arterial calcification is associated with low bone mass.

In the current study, the association between osteoporosis and presence of plaques was independent of age and

menopausal duration, as shown by multinomial logistic regression. This finding suggests that for atherosclerosis and osteoporosis, there are other common etiologic factors (21). So, there is an age-independent association between low BMD or bone loss and the prevalence of echogenic plaques and progressive atherosclerotic calcification, even after adjustment for age and several other confounders (21, 27).

Recently, converging lines of evidence suggest that hyperlipidemia contributes not only to atherosclerotic plaque formation, but also to osteoporosis, following a similar biologic mechanism involving lipid oxidation. In vitro studies indicate that lipid products of oxidation promote osteoblastic differentiation of vascular cells and inhibit such differentiation in bone cells (3). However, in this current study, hypercholesterolemia was not shown to be significantly related to osteoporosis.

Although, no statistically significant negative relation was found in the current study between hypercholesterolemia and BMD of lumbar spine, results of recent studies demonstrate that the lipid profile is strictly related to bone mass in both men and women (30). Many retrospective, observational data and longitudinal studies indicate that anti-lipid therapy (statins) exerts a beneficial effect on BMD (31). There are some early clinical data to complement these findings, suggesting that statins increase bone density and bisphosphonates may have a beneficial effect in vivo on plasma lipid levels and on the atherosclerotic process (32).

However, a longitudinal study did not support the hypothesis that statins protect against early postmenopausal bone loss (33). In another study, the association between echogenic plaques and BMD was essentially unchanged when adjusted for use of statins (21). This hypothesis needs further assessment.

Another possible link between atherosclerosis and low BMD is diabetes mellitus (DM). DM adversely affects bone metabolism and vascular function and may

play a causal role in both these disease entities (20). However, such association was not evident in our study especially with all women in the studied group being having type (II) DM.

Elevated blood pressure was shown to be an independent long-term predictor of IMT in both men and women (34). Another study showed that hypertension was found to be associated with progression of extracranial carotid IMT in healthy populations (35). Also studies have suggested an association between increased bone loss and elevated blood pressure (36). This may provide another explanation for the association between Low BMD and carotid atherosclerosis.

Hypertension was found to be significantly related to lumbar spine osteoporosis but such relation was independent from the association with the plaques.

In addition, the association between osteoporosis and plaques was evident after exclusion of these cardiovascular risk factors; whether hypertension, diabetes mellitus or hypercholesterolemia. So such relation is independent of these factors.

Several factors can explain the association between atherosclerosis and low BMD. Physical activity plays a role that influences both cardiovascular and bone health, especially in postmenopausal women (4). Cigarette smoking was also found to be

associated with progression of extracranial carotid IMT in healthy populations (35) and showed an adverse effect on BMD (37). Also, alcohol was found to be associated with progression of extracranial carotid IMT in healthy populations (35) and with a significant increase in osteoporotic and hip fracture risk (38).

However, in this study, non of these factors could be assessed because none of the studied women followed a regular physical activity, though all ambulant, only four of them were light smokers and none were taking alcohol.

There are other confounding risk factors for atherosclerosis such as, oxidative stress, inflammation, hyperhomocystinemia which have also been associated with increased risk of low BMD (20). But assessment of these factors was beyond the scope of the current study.

Conclusion:

It is important to emphasize that the current evidence linking both of these diseases is far from conclusive. Therefore, additional research is necessary to further characterize the relationship between atherosclerosis and low BMD.

Finally, enhanced understanding of the mechanisms of vascular and bone mineralization might lead to the development of therapeutic agents that effectively target these two processes.

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Changes in baroreflex sensitivity
In L-NAME Hypertension

By

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Abstract

Administration of L-NAME (20mg/kg/d) for 1, 2 & 4 weeks caused a time-dependent systolic and diastolic hypertension. The maximal SBP responses to phenylephrine (PE) and angiotensin II (ang II) were exaggerated, suggesting an important role of sympathetic nervous system (SNS) and renin angiotensin system (RAS) in the pathogenesis of this model of hypertension. The responses to bradykinin (BK) were peculiar being pressor, instead of depressor as in the control group, indicating that the depressor effect of BK is dependent upon the instantaneous release of endogenous nitric oxide (NO) which mediates its depressor action.

When L-arginine was combined with L-NAME for 2w, it prevented the development of hypertension and kept the responses to PE and ang II similar to the control group and the response to BK was depressor again; but dipyridamole combined with L-NAME for 2 w failed to prevent hypertension, and was similar to the group receiving L-NAME alone for 2w as regards its responses to PE, ang II and BK.

Baroreflex sensitivity (BRS), measured by correlating net pressure increments (d SBP) and net HR decrements (d HR), was deteriorated in L-NAME 1 & 2 w groups, but was markedly improved in L-NAME 4 w group even better than control group. Combination with L-arginine for 2 weeks improved BRS but dipyridamole had minimal or no effect.

NO metabolic products, estimated as nitrate, in rats treated with L-NAME 1, 2 & 4 weeks were markedly and significantly reduced in the aorta, and in plasma and was significantly reduced in L-NAME 2w group. Such reduction was abolished both in aorta and in plasma in rats treated with L-NAME combined with L-arginine, but not with dipyridamole.

Introduction

The endothelial production of NO is essential for the maintenance of normal blood pressure, and several disease states including essential hypertension have been associated with defects in the production or action of NO^(1,2). A characteristic feature of L-NAME hypertension is that the severity increases progressively and the eventual development of irreversible target organ damage, particularly renal injury, occurs earlier than in other models of hypertension⁽³⁾. Changes in baroreflex sensitivity which is one of the important mechanisms involved in L-NAME hypertension are controversial. Some studies have found that L-NAME treatment is accompanied by a progressive attenuation in sinoaortic baroreflexes, which might contribute to vasoconstriction and hypertension⁽⁴⁾, others have found that L-NAME treatment is accompanied by normal⁽⁵⁾ or even augmented⁽⁶⁾ baroreflexes.

This work was planned to study baroreflex sensitivity and other mechanisms involved in the cardiovascular changes induced by L-NAME treatment for 1, 2 & 4 weeks duration, and whether they can be prevented or modified by combination of L-NAME with either L-arginine or dipyridamole.

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Materials and Methods

This work was performed on Wistar rats of both sexes weighing 150-220 gm, purchased from Research Institute of Ophthalmology, Giza and maintained in the Animal House of the Physiology Department under standard conditions of boarding and feeding. Rats were allocated into 6 groups: A control group and 3 groups of rats which received N^G-nitro-L-arginine methyl ester (L-NAME) at a dose of 20 mg/kg dissolved in distilled water by gavage daily for 1, 2 and 4 weeks. In the 5th and 6th groups L-NAME was given in the same dose for 2 weeks combined with L-arginine (40 mg/kg/d) or dipyridamole (3 mg/kg/d).

On the day of sacrifice, overnight fasted rats were weighed, and injected intraperitoneally (i.p.) with 1000 I.U. heparin sodium (Nile Co). Half an hour later, the rats were anaesthetized using pentobarbital sodium (40mg/kg) i.p. An incision was made in the inguinal region and the femoral vein was exposed and cannulated with a polyethylene catheter (PE-50). A midline abdominal incision was made and the abdominal aorta was exposed and cannulated with polyethylene catheter (0.5 mm internal diameter), which was connected through a blood pressure transducer (Omeda DTX pressure transducer) to a pressure/heart rate computer BP-1 (Columbus instruments, Ohio, U.S.A). Digital readings were taken for basal SBP& DBP, together with simultaneous reading of HR. *Phenylephrine (PE)* was injected sequentially as bolus doses of 5, 10, 20 and 40 µg/kg⁽⁷⁾; following each dose readings were taken for simultaneous responses of SBP and HR. A ten minutes period was allowed between injection of different doses to enable blood pressure to reach a stable value before administering the next dose. *Baroreflex Sensitivity (BRS)* was determined by correlating delta changes in SBP (Δ SBP) vs. delta changes in HR (Δ HR) in response to the 4 doses of PE. BRS was represented in each group by the correlation coefficient (r) and was expressed numerically as the slope (b) of regression line^(8, 9).

Responses to Angiotensin II (ang II) and Bradykinin (BK): pressor responses to ang

II one bolus injection at a dose of 50ng/kg⁽¹⁰⁾ were recorded. After another ten minutes period of rest, depressor responses to BK one bolus injection at a dose of 0.5 µg/kg⁽¹¹⁾ were recorded.

Plasma samples and supernatants of aortic tissue after homogenation⁽¹²⁾ were subjected to nitrate assay⁽¹³⁾.

All drugs and chemicals used were supplied by, Sigma- Aldrich, Germany.

Statistical analysis : All statistical data, significance tests, correlations and regression lines were performed by using SPSS (version 8.0.1). Statistical significance was determined by:

1- Way ANOVA and by LSD to find inter-groupal significance. A probability of P< 0.05 was considered statistically significant.

Results:

As shown in table (1) basal SBP & DBP were significantly increased in L-NAME 1, 2 & 4 wks groups as well as L-NAME & dip 2w group, whereas basal SBP& DBP in L-NAME & L-arg 2w were significantly lower than L-NAME 2w and were not different from control group. Basal HR showed a general tendency for reduction but was not significantly different in all groups compared to control group, however HR was significantly decreased in L-NAME 4w and L-NAME & dip 2w groups compared to L-NAME 2w group.

Aortic tissue nitrate was significantly reduced in L-NAME 1, 2 & 4 weeks and L-NAME & dip 2w groups compared to control group. Also, plasma nitrate values were lower in all L-NAME-treated groups than control group but this was only significant in L-NAME 2w group. Such reduction was abolished both in aorta and plasma in rats treated with L-NAME combined with L-arginine but not with dipyridamole (table 1).

Figure (1) shows that in all groups, bolus injection of PE sequential doses significantly increased SBP and significantly decreased HR compared to pre-infusion values. Maximal SBP responses to PE were significantly higher in L-NAME 1, 2 & 4 wks & L-NAME & dip 2w group compared to control group; but in L-NAME & L-arg 2w group, SBP responses to PE were

significantly lower than in L-NAME 2w group, and not different from control group. In both L-NAME & L-arg 2w & L-NAME & dip. 2w, the maximal bradycardia in response to PE was significantly more than that in L-NAME 2w and/or control groups. Figure (2) illustrates the relationships and regression lines between Δ SBP and Δ HR in the different groups studied. The BRS in the control group is represented by a significant and negative correlation coefficient (r) and a high and negative slope. In the L-NAME-treated groups the BRS was deteriorated in 1w and 2w groups as shown by weak insignificant correlations. In L-NAME 4w group, BRS was improved even better than in the control group as shown by a more linear and significant correlation and a higher and more negative slope. L-arginine combination with L-NAME for 2w showed some improvement in BRS than in L-NAME 2w group with a higher slope and more linear correlation though still insignificant, whereas, combination with dipyridamole for 2w had minimal or no effect on BRS.

Figure (3) shows that SBP responses to ang II bolus injection were significantly increased

in all models studied than pre-injection values. SBP responses were significantly higher in L-NAME 1, 2 & 4 wks groups as compared to control group, and were greater in L-NAME 4w than 2w & than L-NAME 1w. In L-NAME & L-arg 2w group, SBP response became significantly lower than in L-NAME 2w and not different from control, but in L-NAME & dip 2w SBP response was not significantly different from L-NAME 2w but significantly higher than control. Also, figure (3) shows that in control group SBP was significantly decreased upon BK bolus injection i.e. the response was depressor. On the other hand, L-NAME 1, 2 & 4 weeks and L-NAME & dip 2w groups responded by increased SBP, even significantly, in L-NAME 2 & 4 w groups, from pre-injection levels, i.e. the response was pressor and not depressor. In the group of L-NAME & L-arg 2w, BK injection decreased SBP significantly from pre-injection values similar to control group, i.e. became depressor again and became significantly lower than that in L-NAME 2W group.

Table (1): Mean \pm SEM of basal systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), basal heart rate (HR, bpm), plasma nitrate (μ mol/L) and aortic tissue nitrate (μ mol/gm) in control rats and L-NAME – treated rats

	Control	L-NAME 1W	L-NAME 2W	L-NAME 4W	L-NAME & L-arg 2W	L-NAME & dip 2W
Basal SBP	108 \pm 3	119 \pm 4.1 ^a	128 \pm 3.4 ^a	138 \pm 2.5 ^{abc}	114 \pm 2.3 ^b	121 \pm 4.8 ^a
Basal DBP	86 \pm 2.7	95 \pm 3.8 ^{ab}	106 \pm 2.9 ^a	114 \pm 2.2 ^{abc}	90 \pm 2.7 ^b	101 \pm 5.3 ^a
Basal HR	352 \pm 15.9 (20)	336 \pm 16.8 (15)	360 \pm 11.4 (20)	318 \pm 13.9 ^b (21)	325 \pm 12.4 (15)	315 \pm 11.6 ^b (14)
Plasma nitrate	41.86 \pm 4.92 (9)	37.06 \pm 11.01 (6)	25.18 \pm 3.31 ^a (10)	26.88 \pm 3.94 (5)	38.8 \pm 10.0 (8)	25.77 \pm 4.57 ^a (9)
Aortic tissue nitrate	0.52 \pm 0.06 (10)	0.34 \pm 0.06 ^a (7)	0.21 \pm 0.03 ^a (11)	0.2 \pm 0.05 ^a (7)	0.57 \pm 0.1 ^b (8)	0.21 \pm 0.039 ^a (10)

In parenthesis is the number of observations.

a: Significant by LSD at P < 0.05 from control rats.

b: Significant by LSD at P < 0.05 from L-NAME-treated rats for 2w.

c: Significant by LSD at P < 0.05 between L-NAME-treated rats for 1 and 4 w.

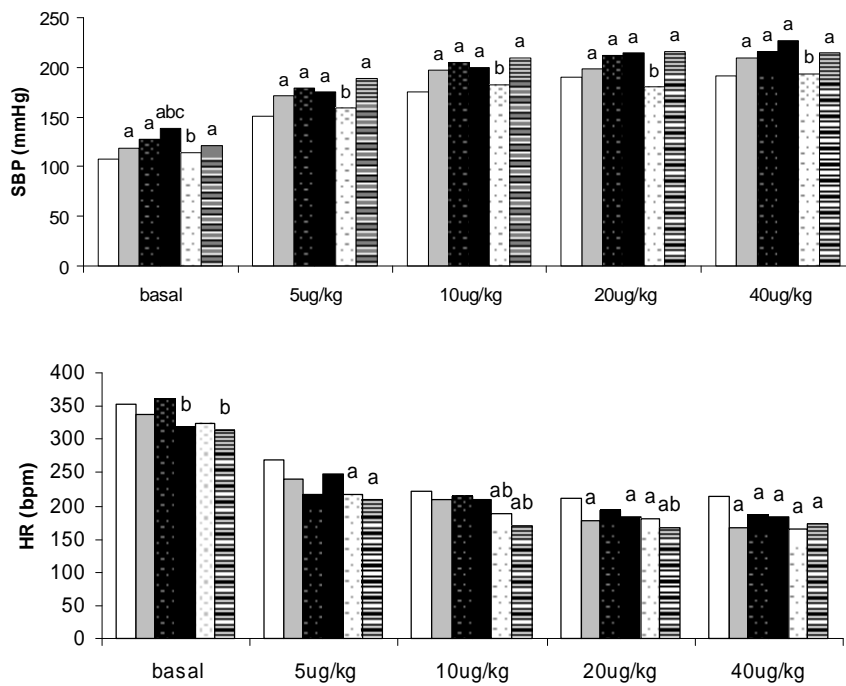


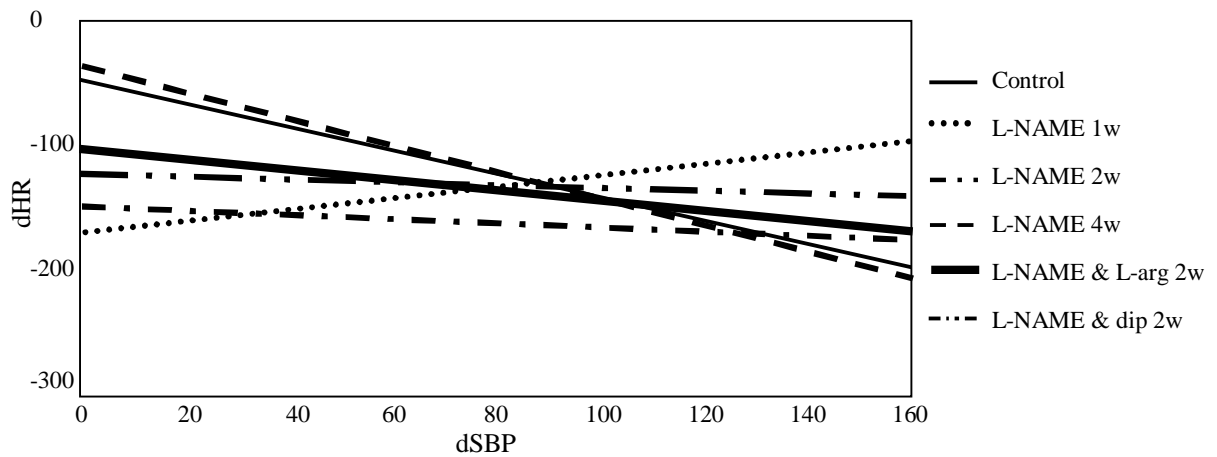
Figure (1) : Basal systolic blood pressure (mmHg) and heart rate (HR, bpm) and their maximal responses to phenylephrine in control rats and L-NAME-treated rats.

Control L-NAME 1w L-NAME 2w L-NAME 4w
 L-NAME & L-arg 2w L-NAME & dip 2w

a: Significant by LSD at $P < 0.05$ from control rats

b: Significant by LSD at $P < 0.05$ from L-NAME-treated rats for 2 weeks.

c: Significant by LSD at $P < 0.05$ between L-NAME-treated rats for 1 and 4 weeks.



		Correlation coefficient (r)	Intercept (a)	Slope (b)
Control	(43)	-0.47**	-47.2	-0.96
L-NAME 1w	(48)	0.16	-165	0.4
L-NAME 2w	(72)	-0.14	-139	-0.23
L-NAME 4w	(62)	-0.67**	-34.8	-1.07
L-NAME & L-arg 2w	(48)		-107	-0.35
		-0.23		
L-NAME & dip 2w	(41)	-0.06	-123	-0.13

Figure (2): Diagrammatic representation of regression lines and tabulated data of the relationships between Δ SBP and Δ HR in the groups studied.

In parenthesis is the number of observations

** : $P < 0.01$

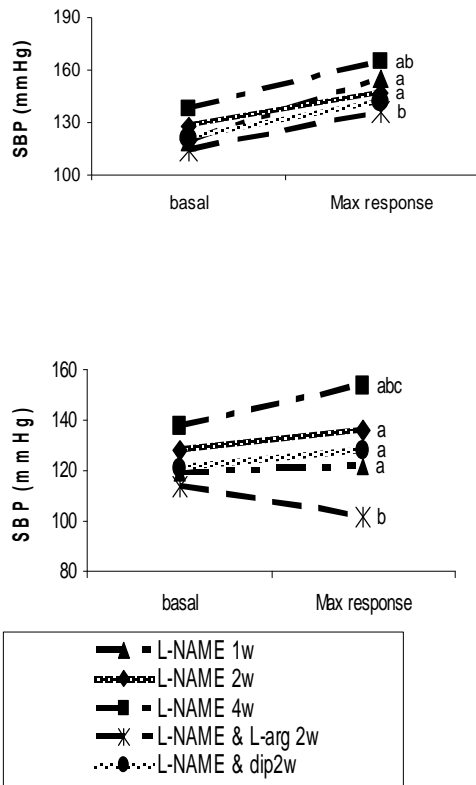


Figure (3): Responses of systolic blood pressure (SBP, mmHg) to angiotensin II (50 ng/kg) (above) and bradykinin (0.5 μ g/kg) (below) in control rats and L-NAME-treated rats.

a: Significant by LSD at $P < 0.05$ from control rats

b: Significant by LSD at $P < 0.05$ from L-NAME-treated rats for 2 weeks.

c: Significant by LSD at $P < 0.05$ between L-NAME-treated rats for 1 and 4 weeks.

Discussion

The results of the present work have demonstrated that rats treated with L-NAME for 1, 2 & 4 weeks, developed significant increase in SBP & DBP than age-matched control rats. Combined administration of L-arginine with L-NAME 2w, L-arg could prevent the induction of L-NAME hypertension, contrary to the L-NAME & dip 2 w group which was still hypertensive and not different from the L-NAME 2 w hypertensive model.

Basal HR was not significantly different in L-NAME 1, 2 & 4 weeks groups compared to control group, although heart rate was significantly decreased in L-NAME 4w and L-NAME & dip 2w groups compared to L-NAME 2w. In contrast, other studies reported significant bradycardia following treatment

with N^G-monomethyl L-arginine⁽¹⁴⁾, and this may reflect differences regarding specific action and selectivity of different NOS inhibitors as well as dose and species differences. However, basal HR was significantly decreased in the group of rats subjected to combined treatment with L-NAME & dip 2 w compared to that treated with L-NAME alone for the same duration, and this could be ascribed to a specific effect of dipyridamole on heart rate. Actions of dipyridamole are largely mediated through adenosine by inhibiting its cellular uptake and this leads to accumulation of adenosine⁽¹⁵⁾ and long-term accumulation of adenosine markedly decreases HR and prolongs atrioventricular (AV)-nodal conduction⁽¹⁶⁾, probably due to a direct adenosine activated K_{ACh} (Potassium-

acetylcholine) channel signal transduction system⁽¹⁷⁾.

In this study, NO metabolic products, estimated as nitrate, in rats treated with L-NAME for 1, 2 & 4 weeks were markedly and significantly reduced in the aorta, and also to a lesser extent, in plasma where it was significant only in L-NAME 2 w group. Such reduction in nitrate was abolished both in aorta and plasma in rats treated with L-NAME combined with L-arginine, but not with dipyridamole.

In view of these data, it can be assumed that NO deficient blood vessels of L-NAME-treated rats are lacking their NO-relaxing effect, thus contributing to the associated rise in arterial blood pressure and development of hypertension. This assumption can be further supported by the finding that hypertension induced by L-NAME was nearly abolished with L-arginine which augments NO production. Thus blood vessels regain their normal tone and their vasorelaxing effect is restored, contributing to the return of arterial blood pressure back to normal.

Responses to Phenylephrine and BRS:

In the L-NAME-treated rats for 1, 2 & 4 weeks, SBP responses to PE sequential doses were significantly higher compared to control rats, and were dose dependent i.e. progressively increasing with the increase of PE dose from a level to a higher level. On the other hand, the maximal responses achieved by each dose of PE were duration non-dependent i.e. reaching to a comparable maximal pressure in the 1 w, 2w & 4w groups of L-NAME treatment, with almost no significant difference in between the groups. The net pressure increments (delta changes) for each dose of PE were significantly increased in L-NAME 1w group compared to control group, some increased and some decreased in L-NAME 2w group and all decreased in 4w group. Also net increments were significantly lower in L-NAME 4w than L-NAME 1 and/or 2w groups. Such progressive fall of net increments from 1 w to 2 w to 4w is clearly related to the almost similar maximal responses in the 3 models to each PE dose minus a progressively higher

basal BP from 1 w to 2w to 4w groups.

In L-NAME & L-arg 2w group, all SBP responses to the 4 doses of PE were significantly lower than L-NAME 2w group, and not different from control group. In L-NAME & dip 2w SBP responses were significantly higher than control group and not different from L-NAME 2w group.

The augmented pressor responses to the α_1 -adrenoceptor agonist PE in L-NAME-treated groups may provide evidence for the important role of the sympathetic nervous system (SNS) as a pathomechanism in such model of hypertension. Such augmented pressor responses in models of L-NAME hypertension could be explained by an increase in the responsiveness of vascular α_1 -adrenoceptors to their agonist PE, probably due to an increase in the number and/or affinity of such receptors upon L-NAME-treatment⁽¹⁸⁾. NO plays an important role in the development and maintenance of vascular α_1 -adrenoceptor desensitization⁽¹⁹⁾, and this may explain how NO released from endothelial cells reduces the α_1 -adrenoceptors-mediated vascular vasoconstriction⁽²⁰⁾.

This study has also indicated that BRS showed an early deterioration in L-NAME 1 & 2w groups followed by progressive improvement as shown in the L-NAME 4 w group which became even better than control group. In L-NAME & L-arg 2w group the BRS showed partial improvement with more linear, though insignificant relationship and more negative slope than L-NAME 2w group. The improved BRS in the L-NAME 4w group may underly the smallest rise in net increments in pressure responses in this model. This is in contrast to a previous report that L-NAME treatment is accompanied by a progressive attenuation in sinoaortic baroreflexes⁽⁴⁾, whereas, others reported that BRS remains normal or even augmented following L-NAME treatment^(5,6). In this context, it seems that L-NAME treatment influences BRS through central and peripheral regulatory actions on SNS, and both actions might be conflicting and different in their time course. Intravenous infusion of L-NAME was found to decrease

the activity of baroreceptor-sensitive neurons in the nucleus of the solitary tract, whereas L-arginine infusion reversed the effect⁽²¹⁾. In addition, NO donors were found to have sympatholytic effects, and NOS inhibitors have sympathoexcitatory effects, when microinjected into pressor or depressor regions of the medullary vasomotor center⁽²²⁾. In contrast, within the peripheral nervous system, NO could potentially increase sympathetic activity via its ability to facilitate neurotransmission in sympathetic ganglia and suppress baroreceptor sensitivity in the carotid sinus⁽²³⁾.

Pressor Responses to Angiotensin II:

Moreover, the role of RAS in the pathogenesis of L-NAME hypertension is well indicated by the exaggerated pressor responses to ang II in L-NAME 1, 2 and 4w groups. Such augmented pressor responses were duration dependent, i.e. they were greater in L-NAME 4 w than 2w than 1w. On the other hand in L-NAME & L-arg 2w group, where the NO release was increased back to normal, the pressor response to ang II was significantly lower than L-NAME 2w and not different from control. Similarly in other studies, the systemic pressor responses evoked by ang II were found to be enhanced during NOS inhibition suggesting that ang II administration, is associated with increased NO release that counteracts its blood pressure rising effect⁽²⁴⁾. Therefore, NO-mediated vascular relaxation has been suggested as an important regulator for total peripheral and organ vascular resistance. Ang II-induced vasoconstriction may increase shear stress and NO production, which in turn acts as a regulation system by restraining the constrictor action of ang II⁽²⁵⁾. In support, endogenous NO normally attenuates the pressor response to low dose of ang II for several days, and protects from ang II-induced target organ damage⁽²⁶⁾. NO appears to be the major endogenous antagonist of the vascular actions of ang II, and a balance between ang II and NO appears pivotal for the maintenance of vascular homeostasis. The changes observed after NO synthesis blockade are due, at least in part, to the fact that

physiological NO buffers the influence of endogenous vasoconstrictor systems.

Besides, NO was found to decrease AT₁ receptor expression and activity in vascular smooth muscle cells. The AT₁ promoter region is responsible for the suppressive effect of NO, suggesting that NO may directly regulate AT₁ receptor expression. Also, the metabolic clearance rate of ang II was significantly lower in L-NAME-treated rats, and this decreased clearance of ang II together with the increase in the number of vascular ang II receptors, may account for the increase in pressor responsiveness to infused ang II⁽²⁷⁾. In support co-treatment with L-arginine prevented the L-NAME-induced increase in ang II receptors⁽²⁸⁾.

On the other hand, combination of L-NAME with dipyridamole for 2 w failed to modify the exaggerated pressure responses to angiotensin II bolus administration. Dipyridamole blocks the cellular uptake and deamination of adenosine, and augments the norepinephrine-mediated renal vasoconstriction⁽²⁹⁾. Renal vasoconstriction actions of ang II and norepinephrine were significantly augmented by dipyridamole⁽³⁰⁾.

Depressor Responses to Bradykinin:

In response to BK, a significant depressor response was obtained in the control group but not in rat models L-NAME 1, 2 & 4 weeks, where the response was pressor and not depressor. In the group of L-NAME combined with L-arginine, but not dipyridamole, bradykinin injection decreased pressure again i.e. the response became depressor and not different from control and significantly lower than the L-NAME 2w group. In accordance, BK infused chronically was found to produce a marked fall in SBP, but when BK was administered concurrently with NOS inhibitor, ABP rose significantly, reaching very high values at the end of treatment⁽³¹⁾. From these data, it appears that NO production is essential for the vasorelaxant effect of BK, otherwise when NO biosynthesis is interfered with by the use of NOS inhibitors, the action of BK becomes vasopressor and induces vasoconstriction instead of vasodilation.

BK induces vasodilation via endothelial

type 2 (B₂) receptors, and this effect can be blocked partly by inhibitors of NOS⁽³²⁾. The relaxant effect of BK that is not blocked by NOS inhibitors is generally attributed to the endothelial-derived hyperpolarizing factor(s) such as prostacyclin, K⁺ and cytochrome P-450 products of arachidonic acid ⁽³³⁾. However, such vasorelaxant factors, in the absence of NO released by activation of bradykinin B₂ receptors can not exceed the BK vasoconstrictor effect which appears following NOS inhibition as evidenced in the present study. The SNS may be involved in mediation of vasoconstrictor action of BK as well as other BK effects within the CVS ⁽³⁴⁾; and sympathoexcitation caused by BK could be attributed to its depolarizing action on sympathetic ganglia⁽³⁵⁾. BK was demonstrated to depolarize superior cervical ganglion neurons of the rat through an inhibition of M-type K⁺ (K_m) channels causing depolarization and increasing action potential

discharge^(36,37). Also, a central neuronal pathway in the BK-mediated pressor response could be involved since BK was found to stimulate functional connection between the small collection of neurons in the dorsal lateral medulla, the paratrigeminal nucleus (Pa5) and the rostroventrolateral reticular nucleus (RVL) ⁽³⁸⁾.

Therefore, the results of the present study are indicating that in L-NAME hypertension, beside the early deterioration in BRS, the activities of both SNS and RAS were exaggerated. This presents strong evidence for the important role of NO- Larginine pathway not only as regard the pathomechanisms of hypertension, but also in the field of control and treatment of hypertension. In this respect, we may recommend that L-arginine or other NO donors be included in combinations of antihypertensive drug protocols for trial and investigation

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Conflicts of Interest: Because the essence of review articles and editorials is selection and interpretation of the literature, the journal expects that the authors of such articles will not have any financial associations with a company (or its competitors) that makes a product discussed in the article.

Review articles also include:

- **Current Concepts** articles focus on clinical topics, including those in specialty areas but of wide interest. The text is limited to 2000 words, with a maximum of four figures and tables plus up to 50 references.
- **Drug Therapy** articles detail the pharmacology and use specific drugs or classes of drugs, or the various drugs used to treat particular diseases. There is a limit of 4000 words.
- **Issues In Clinical Practice** articles are evidence-based reviews of topics relevant to practicing physicians, both primary care providers and specialists. Articles in this series should be no longer than 2500 words and should include the following sections: clinical context, strategies and evidence, areas of uncertainty, guidelines from professional societies, and recommendations from author(s).
- **Mechanisms Of Disease** articles discuss the cellular and molecular mechanisms of diseases or categories of diseases. The text is limited to 4000 words.
- **Medical Progress** articles provide comprehensive, overviews of important clinical subjects, with the principal focus on developments during the past years. Each article details how the perception of a disease, diagnostic approach, therapeutic intervention has evolved in recent years. The text is limited to 4000 words, with a maximum of six tables and figures.

OTHER SUBMISSIONS:

- **Clinical Implications of Basic Research** articles discuss papers from preclinical journals. The purpose is to explain the findings and comment on their possible clinical applications in fewer than 1000 words.
- **Occasional Notes** are accounts of personal experiences or descriptions of material from outside the usual areas of medical research and analysis.