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Effect of Watermelon Juice Consumption on Brachial Blood Pressure among Sudanese Hypertensive Patients

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Abstract:

Watermelon is a rich source of L-arginine and L-citrulline. L-arginine is the sole substrate of endothelial nitric oxide synthase (eNOS) producing nitric oxide (NO) and L-citrulline. NO is a vasodilator and muscle relaxant. L-arginine is also catabolized by arginase into L-ornithine and urea.The ratio of arginine to its catabolic products, L- citrulline and L-ornithine, accounts to its bioavailability. It has been reported that diminished global arginine bioavailability ratio (GABR) increases the risk of cardiovascular diseases and has been considered as a biomarker for coronary artery diseases. Encouraged by the seek of alternative non-pharmacological hypotensive agents, the effect of watermelon juice supplement was studied among 31 stage 1 hypertensive Sudanese patients. Exclusion criteria included pregnancy, metabolic diseases, diabetes, liver, kidney and cardiovascular diseases. Participants received one liter of freshly prepared watermelon juice daily for three weeks (L-citrulline/L-arginine:1g/0.5g per day). Results showed that watermelon juice supplement significantly increases plasma L-arginine, L-citrulline and L-ornithine levels and reduced brachial blood pressure and global arginine bioavailability ratio. In conclusion, we suggest that watermelon juice could be used as a potential natural hypotensive agent even at low doses. Although watermelon juice increases plasma arginine level, GABR may not be a useful biomarker to assess the progression of hypertension.

Keywords: Global arginine bioavailability ratio; Hypertension; L-arginine; L-citrulline; Watermelon supplement

Introduction:

Hypertension has become a huge public health problem in both developed and developing countries causing serious morbidity and mortality rates [(1)]. According to the 2012 World Health Statistics report, hypertension affects approximately 24.8% of the global population with the range from 19.7% to 35.5% indifferent regions [(2)].In Khartoum, capital of Sudan, the prevalence in 2008 was approximately 18.2% [(3)].It is a major riskfactor for stroke, congestive heart failure, myocardial infarction, peripheral vascular disease, and over all mortality.

It is believed that non-pharmacological approach such as dietary modification plays an important role in controlling blood pressure (BP). The Dietary Approaches to Stop Hypertension (DASH) trial has demonstrated that a diet rich in vegetables, fruits and low in saturated and total fat can substantially lower BP [(4)]. The Dash diet also contained more protein than control diet and hence, it has been hypothesized that arginine-rich protein contributed to the BP-lowering effect of this diet [(4)].

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L-arginine, a semi essential amino acid, serves as the sole nitrogen source for endothelial nitric oxide synthase (eNOS). Nitric oxide (NO) is synthesized from arginine in a multistep reaction carried out by NO synthase, producing NO and L-citrulline [5]. NO is a widespread biological mediator implicated in many physiological and pathophysiological processes. It activates guanylatecyclase in smooth muscle cells [6]. This activation increases the intracellular synthesis of cyclic GMP, which acts as a second messenger, mediating many of the biological effects of NO such as relaxation of smooth muscle and inhibition of platelets aggregation.L-citrulline formed in this reaction can be effectively converted into L-arginine [7, 8]. Of interest, a clinical trial reported that oral intake of L-citrulline increases the plasma L-arginine level more effectively than an equivalent dose of L-arginine in healthy human volunteers [9].

On the other hand, L-arginine is also a substrate of arginase [10] which converts L-arginine to L-ornithine and urea. Therefore, the production of NO is dependent on the relative expression and activities of arginase and eNOS. This has led to the proposal of the concept of "global arginine bioavailability ratio" (GABR) to account for levels of the substrate (L-arginine) and its major catabolic products (L-ornithine and L-citrulline) in vivo [5]. Several studies concluded that diminished GABR is associated with the development and progression of cardiovascular diseases [5, 11, and 12]. Although hypertension is one of the major risk factors of cardiovascular diseases, no studies correlate GABR to the progression of hypertension.

Several studies and clinical trials have attempted to evaluate the role of L-arginine supplementation on regulating BP. A randomized, double blind, placebo-controlled trials study concluded that oral L-arginine supplementation ranging from 4 to 24 g/d significantly lowers both systolic and diastolic blood pressure [13]. In contrast, Neri *et a l*reported that L-arginine supplementation in pregnant women with mild chronic hypertension does not significantly affect overall BP [14].The high cost and poor availability of chemical drugs in developing countries encouraged the seek of alternative hypotensive agents. Watermelon juice, being a rich source of L-arginine and L-citrulline increases plasma arginine concentrations [15] and hence, could be a putative agent to prevent and treat hypertension.

Recently, the effect of watermelon supplementation was investigated on obese postmenopausal women with hypertension and concluded that it reduces arterial stiffness and aortic systolic blood pressure (SBP) by reducing pressure wave reflection amplitude [16]. Another study demonstrated that watermelon extract supplementation(L-citrulline/L-arginine, 6 g daily) reduces ankle BP, brachial BP, and carotid wave reflection in obese middle-aged adults with pre hypertension or stage one hypertension [17].

In Sudan, It is attractive to use watermelon juice to lower BP and prevent hypertension taking into consideration the availability of this fruit most of the year.The purpose of this study is: First, to test the hypothesis that watermelon juice supplementation as a rich source of L-arginine and L-citrulline reduces systolic and diastolic blood pressure among Sudanese hypertensive patients even at low concentrations (L-citrulline/L-arginine: 1g/0.5g per day). Second, to assess the credibility of GABR as a biomarker for the progression of hypertension.

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Methods:

Participants

Thirty one stage 1 hypertensive patients (male/female ratio 19:12) attending Ahmed Gasim out-patient clinic were recruited for this study. The age group ranged between 21and 70years of age (mean ±SEM= 47.9±2.2). The inclusion criterion was a minimum systolic blood pressure (SBP) of 140 mmHg and/or a minimum diastolic blood pressure (DBP) of 90 mmHg confirmed on two separate days after 20 minutes of rest. All participants were examined by physicians to exclude current pregnancy, metabolic diseases, diabetes, liver, kidney and cardiovascular diseases. Echo cardiograph and electrocardiogram were performed in Ahmed Gasim hospital to ensure normal cardiac functions. All volunteers continued on their regular hypotensive drugs but none had received any lipid-lowering drugs, oral contraceptives or other exogenous ovarian hormones. All participants signed an informed consent form and the study was approved by the Sudan Academy of Sciences Ethics Committee.

Watermelon supplementation

Watermelon juice was prepared from the fleshy part of the fruit. The level of free L-citrulline and free L-arginine in one litre of the juice was estimated using amino acid analyzer (Sykam S 334, Munich, Germany) following standard procedure described by manufacturer. One litre of the freshly prepared juice containing (L-citrulline/L-arginine: 1g/0.5g) was given to each participant daily for three weeks.

Blood samples

Five mls venous blood samples were drawn from each participant in heparinized tubes after an overnight fast and avoidance of caffeinated drinks and intense exercise for at least 24h prior to blood collection. Blood samples were collected at baseline and 3 weeks after watermelon intervention.

Determination of plasma L-arginine, L-citrulline and L-ornithine

Plasma was separated within half an hour after blood collection by centrifugation at 3000 rpm for 5 minutes. Protein was precipitated by 20% sulfosalicylic acid, centrifuged at 4^{o}C for 15 min at 12000 rpm and the clear supernatant was used for analysis. Amino acids were determined by amino acids analyzer (Sykam S 334, Munich, Germany) following standard procedure described by manufacturer.

Calculation of global arginine bioavailability ratio (GABR)

GABR is calculated by dividing the level of plasma L-arginine by the sum of plasma L-citrulline and L-ornithine.

Systolic and diastolic blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) were measured at baseline and three weeks after watermelon intervention using mercurial sphygmomanometer (desk type, SM-300, Osaka. Japan). Participants were asked to avoid caffeinated drinks for 24h before examination. Measurements were taken after an overnight fast and participants were allowed to rest for at least 20 min before data collection. Measurements were all conducted at the same time of the day (7-9 pm) for each subject to reduce possible diurnal variations in vascular parameters at baseline and

at the end of treatment. Echo cardiograph and electro cardiogram were also performed to ensure normal cardiac functions of participants.

Anthropometric parameters

Body weight was measured in kilograms to the nearest 100 g using a Seca® scale, which was regularly calibrated. Height was measured in meters to the nearest 5 mm using a Seca® height gauge. Body mass index (BMI) was calculated as weight divided by height square (kg/m2). Waist was measured with a non-stretchable tape over the unclothed abdomen at the narrowest point between the lowest rib and the iliac crest (18). Hip circumference (H) in centimeters was measured at the level of the greater trochanters using a similar procedure. Two measures were made and the mean was used for analyses. The waist-hip ratio (W/H) was obtained by dividing the waist circumference by the hip circumference.

Statistical Analysis

Kolmogorov- Smirnov test was used to test the normality of data, since the data followed normal distribution, parametric tests were used. The effect of watermelon intervention on the different parameters was assessed using paired t-test. Independent t-test was carried out to assess differences between males and females in the different parameters before and after watermelon supplementation. Differences were considered significant with a 5% significance level ($p<0.05$). Statistical analysis was performed with the SPSS 20.0 for Windows software (SPSS Inc. Chicago, IL, USA).

Results:

Watermelon supplementation was well tolerated by all subjects,and no adverse effects were observed.

Participant characteristics including age, gender, height, weight, hip circumference (H), waist circumference (W), at baseline are presented in Table 1. Values are presented as mean (SEM).

Considering the effect of gender, no significant difference was observed in all parameters between males and females before and after watermelon supplementation.

The levels of plasma L-arginine, L-citrulline, L-ornithine, GABR, brachial blood pressure, pulse rate, BMI, W/H ratio before and after watermelon juice intervention for 3 weeks are presented in Table 2. Significant increase in the level of L-arginine ($p<0.012$), L-citrulline and L-ornithine ($p<0.001$) was observed post watermelon juice intake compared to baseline levels presented inTable 1. However, the level of GABR significantly decreased post watermelon intervention. SBP and DBP levels significantly decreased after treatment ($p<0.001$) (Table 2). While no significant difference was observed in W/H ratio, BMI was significantly decreased ($p<0.001$) after watermelon intervention. No significant difference was noted in pulse rate.

Table (1): Participants characteristics at baseline (*n*=31)

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Age, y	47.9 (2.2)
M/F	19/12
Height, m	1.70 (0.01)
Weight, kg	85.0 (2.5)
Hip circumference, cm	99.4 (1.5)
Waist circumference, cm	97.9 (1.9)

Values are presented as mean ±(SEM).

Table (2): Statistical differences of measured variables before and after watermelon juice supplementation (n=31)

Variable	**Watermelon supplementation**		***P*-value**
	Pre	**Post**	
L-arginine, µmol/l	82.03 (3.6)	94.64 (4.0)	*P*<0.01*
L-citrulline, µmol/l	38.98 (3.8)	53.14 (5.8)	*P*<0.001*
L- ornithine, µmol/l	84.55 (4.4)	128.84 (6.0)	*P*<0.001*
GABR	0.71 (0.05)	0.5 (0.04)	*P<0.001**
SBP, mm Hg	144.8 (2.3)	128.4 (2.1)	*P*<0.001*
DBP, mm Hg	92.3 (1.2)	83.9 (1.1)	*P*<0.001*
Pulse rate	73.1 (1.7)	73.2 (1.9)	*P*<0.9
BMI	30.0 (0.85)	29.0 (0.86)	*P*<0.001*
W/H	0.98 (0.02)	0.99 (0.01)	*P*<0.5

*Values are presented as mean ±(SEM). * indicate statistically significant difference*

Discussion:

Results obtained clearly demonstrate that regular intake of watermelon juice at the rate of 1l/d (L-citrulline/L-arginine: 1g/0.5g per day) for three weeks lowers brachial blood pressure among a group of stage 1 hypertensive volunteers irrespective of gender. This was concomitant with significant increase in plasma arginine, citrulline and ornithine but reduced global arginine bioavailability ratio. The study is characterized by the use of low level of L-citrulline/L-arginine dose compared to higher doses used in previous reported studies since we only used the juicy fleshy part of the fruit (not the rind).

Watermelon is a known source of L- arginine and L-citrulline, the amount of L-citrulline being double that of L-arginine in the fresh juice. L-citrulline plays an important role in the metabolism and regulation of NO by being effectively recycled to L-argininevia argininosuccinate synthase and argininosuccinatelyase of thecitrulline-NO cycle [8]. Indeed the BP lowering effect of L-arginine supplementation was well documented by several studies and clinical trials [18, 19, 20, and 13].

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In contrast, L-citrulline supplementation did not reduce brachial blood pressure in a study conducted by Ochiai *et al* (2012). This was explained by the fact that Ochiai *et al* (2012) participants were normotensive hence L-citrulline supplementation improved only brachial–ankle pulse wave velocity rather than brachial BP [(22)].

This study, lowering effect of watermelon on SBP among hypertensive volunteers is comparable to recent studies conducted on obese middle-aged adults with prehypertension or stage 1 hypertension [(17)] and postmenopausal obese women with stage 1 hypertension [(16)].

Apart of the BP lowering effect, watermelon seems to reduce body weight among participants as indicated by the decrease in BMI post treatment. This could be attributed to the diuretic effect of watermelon juice.

Interestingly, the global arginine bioavailability ratio (GABR) was significantly decreased among our patients after watermelon intervention. Although several studies correlated GABR to the progression of coronary artery diseases [(5)]and chronic systolic heart failure [(12)], nothing was reported on the effect of GABR on hypertension.A pilot study among Sudanese reported lower level of GABR among CAD patients compared to healthy controls (unpublished data).

Of notice, the increase in plasma L-citrulline (14.16%) and L-ornithine (44.29%) in the study group after three weeks watermelon supplementation was higher than that of L-arginine (17.16%). We suggest that the exogenous L-arginine from the watermelon juice has been effectively consumed by eNOS to produce NO and L-citrulline leading to reduced brachial pressure and increased plasma citrulline level. On the other hand, L-arginine might also be a substrate of arginase which accounts for the increased plasma ornithine level. This could explain the limited increase in plasma arginine compared to citrulline and ornithine. A limitation of our study is that we did not include a placebo control group.

In conclusion, watermelon juice could be a potential natural hypotensive agent even at low doses. Although watermelon juice increases plasma arginine level, GABR may not be a useful biomarker to assess the progression of hypertension.

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