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Garlic extract favorably modifies markers of endothelial function in obese patients –randomized double blind placebo-controlled nutritional intervention

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ABSTRACT

Objective: Garlic exerts a range of effects relevant to human health. However, its influence on the endothelium in obese individuals remains unknown. We aimed to determine the effects of garlic extract (GE) on arterial stiffness and markers of endothelial function.

Methods: Ninety-two subjects were enrolled in this study. The participants were randomly assigned to receive 400 mg of GE or placebo daily for 3 months. The arterial stiffness index (SI) and markers of endothelial function such as high-sensitivity C-reactive protein (hsCRP), cholesterol (total, LDL, HDL), triglycerides, and plasminogen activator inhibitor 1 (PAI-1), as well as total antioxidant status (TAS) were quantified at baseline and the end of study.

Results: At the end of study SI ($p = 0.01$), hsCRP ($p < 0.001$, PAI-1 ($p < 0.001$), LDL cholesterol ($p < 0.001$), and TAS ($p < 0.01$) were reduced in the GE-supplemented group, but not in the placebo group.

Conclusion: This randomized, double-blind, placebo-controlled trial demonstrates that supplementation with GE favorably modifies endothelial biomarkers associated with cardiovascular risk and suggests that GE can be used to suppress chronic inflammation in obese individuals.

1. Introduction

Obesity is a common condition that creates a heavy social burden worldwide. The World Health Organization (WHO) reports that 39% of adults aged 18 and over were overweight in 2014, and 13% were obese [1]. Excessive body fat is linked to diseases such as hypertension [2], diabetes mellitus [3], cardiovascular events [4], and certain types of cancer [5]. Obesity-associated oxidative stress and chronic inflammation [6,7] are linked to an increased risk of hypertension and cardiovascular events [8,9]. The chronic inflammation underlying obesity is manifested by increased levels of C-reactive protein (CRP) or by upregulation of proinflammatory cytokines. These factors are also capable of raising the level of endothelium-derived products, such as plasminogen activator inhibitor 1(PAI-1). The blood fibrinolytic system is activated by tissue-type plasminogen activator (t-PA), which converts plasminogen to plasmin. Plasmin degrades fibrin, the main component of thrombus, which in this case is called fibrinolysis. This system is also inhibited by PAI-1, which plays an essential role in the pathogenesis of excess blood coagulability in obese. [10]. Higher levels of PAI-1 are also associated with a progressive decline in insulin clearance; this could therefore be useful in predicting diabetes [11]. Some evidence indicates that PAI-1 influences vascular remodeling and is positively correlated with intima media thickness and arterial stiffness index (SI) [12,13].

Arterial stiffness measurement is a method that has the potential to diagnose the subclinical endothelial changes that co-occur with hypertension and obesity [14]. The early detection of subclinical

Abbreviations: GE, Garlic extract; PAI-1, plasminogen activator inhibitor -1; SI, stiffness index; TAS, total antioxidant status; DVP, digital volume pulse; MDA, malondialdehyde; TNF-α, tumor necrosis factor alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TAC, total antioxidant capacity; t-PA, tissue type plasminogen activator

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endothelial changes is important for a successful therapeutic intervention.

The first line of defense against obesity and its comorbidities is lifestyle modification. A diet rich in fruit, vegetables and in spices is widely held to decrease the risk of cardiovascular disease, cardiometabolic disorders, cancer, and other disorders [15,16,17]. One of the most recognizable functional foodstuffs from this group is garlic (*Allium sativum*), one of a few plants that contain allyl-substituted sulfur compounds, which has served as a remedy for various ailments since the antiquity. [18] Some evidence suggests that garlic and garlic supplements could be beneficial in the prevention and treatment of hypertension [19] and hyperlipidemia [20]. Garlic has also antithrombotic and anti-inflammatory properties [21,22]. The cardioprotective effects of garlic are due to garlic-derived organic polysulfides with allyl moieties, which react with membrane thiols and glutathione to produce hydrogen sulfide, which causes vasorelaxation *via* vascular smooth muscle cell K_{ATP} channel hyperpolarization [23]. Other studies suggest that garlic can also modulate the production and function of nitric oxide (NO) by releasing NO from S-nitrosothiol species and by inhibiting angiotensin-converting enzyme [24], thus lowering blood pressure. The lipid-lowering effect of garlic potentially results from the inhibition of enzymes responsible for cholesterol and fatty acid biosynthesis [25].

To determine how garlic affects the endothelial function in patients with body mass index (BMI) $\geq 25\ kg/m^2$ the present study examined the effects of treatment with garlic extract on markers of arterial stiffness and endothelial function in these patients.

2. Methods and materials

2.1. Participants

The Research Ethics Committee at Poznań University of Medical Sciences, Poland approved the study protocol (approval 544/14). The study conformed to the Declaration of Helsinki. Each patient gave his or her informed consent. The work was registered in the National Court Register under the number DRKS00010533.

The inclusion criteria were met by 92 patients out of a total of 122 with BMI $\geq 25\ kg/m^2$ presenting at the Outpatient Clinic, Department of Obesity Treatment and Education and Metabolic Disorders. These were randomized and their data were analyzed. After the three-month period, four subjects failed to complete the study: two subjects from the garlic group and one from the placebo group withdrew consent, and one from the placebo has had a cardiac event. Finally, the data of 88 subjects was analyzed.

The inclusion criteria were a body mass index (BMI) equal to or greater than $25\ kg/m^2$, age 25–60 years, stable body weight (< 3 kg self-reported change during the previous three months), and no drug-treated hypertension (meaning systolic blood pressure (SBP) less than 160 mmHg and diastolic blood pressure (DBP) less than 100 mmHg) with stable treatment for at least 6 months (diet, physical activity, life style). The treatment scheme was comparable for both the placebo and the garlic group. The exclusion criteria were secondary obesity or secondary hypertension; diabetes; a history of coronary artery disease, stroke, congestive heart failure, or malignancy; a history of use of any dietary supplements within the three months prior to the study; a current need for modification of antihypertensive therapy; abnormal liver or kidney function; any clinically significant process; a history of infection in the month prior to the study; nicotine or alcohol abuse.

2.2. Experimental design

The study has been designed as a randomized double-blind placebo-controlled nutritional intervention trial with two parallel groups. The patients were assigned to receive garlic or placebo accordingly with randomization list and the group allocation was blinded for participants and investigators during the whole study.

An independent statistician randomly assigned participants, with equal odds, to take either a placebo or two capsules of Garlicin (Olimp Laboratories, Poland) with their breakfast each day for 3 months. Both Garlicin and placebo capsules were provided in blister packs without labels. The Garlicin capsule contained odorless extract of garlic (2% allicin) and was dosed at 400 mg/d. The placebo consisted of pure microcrystalline cellulose. The intervention was specially formulated in capsules and kindly provided by pharmaceutical company (Olimp Laboratories, Dębica, Poland). Patients were asked to continue their habitual exercise and diet regimes unchanged for the duration of the study. All patients were fasted for 10–12 h before undergoing laboratory tests and had their blood pressure and anthropometric parameters assessed. Measuremnets of anthropometric, biochemical, and stiffness index parameters were carried out on the same day at baseline and after 3 months of treatment.

Primary objectives in subjects with BMI $\geq 25\ kg/m^2$: to assess the effect of garlic extract on PAI-1 and hsCRP level in the serum blood.

Secondary objectives in subjects with BMI $\geq 25\ kg/m^2$: to assess the effect of garlic extract on SI (arterial stiffness index), SBP (systolic blood pressure), DBP (diastolic blood pressure), TAS (total antioxidant status) and lipid profile.

The sample size of n = 44 per group was calculated assuming a parameter difference of 20%, the standard deviation of 30%, the significance level of 5%, power of the test of 80%, and a drop-out rate of 20%. The standard deviation of 30% is greater than found in previous studies for the primary endpoint (PAI-1) in healthy subjects [26], but was chosen to also accommodate for potentially greater variations in values of the parameters designated as secondary endpoints.

2.3. Anthropometry and physiological measurements

Anthropometric measurements were carried out on patients wearing no shoes and light clothing. Height and weight were recorded to the nearest 0.1 cm and 0.1 kg, respectively. Following the guidelines of the European Society of Hypertension, blood pressure was measured with patients seated [27] using a digital electronic tensiometer (705IT, Omron Corporation, Kyoto, Japan).

2.4. Arterial stiffness measurement

A photoplethysmograph (Pulse Trace Micro Medical, Rochester, UK) transmitting infrared light at 940 nm was positioned on the patient's right-hand index finger. Recommendations for ensuring standard conditions were taken into account and the measurements were taken following ten or more minutes of recumbent rest. All measurements were carried out at a similar time of day. Subjects were acclimatized to a temperature of $22 \pm 1\ ^{\circ}C$ for 30 min or more prior to the recording. The photoplethysmograph's digital volume pulse (DVP) wave output was recorded; the downslope of this pulse has a characteristic point of inflection. The peak-to-peak time was taken as the time elapsed from the first to the second peak. The SI factor was calculated thus: SI = patient's hright (m)/peak to peak time (s) [28,29].

2.5. Biochemical analysis of metabolic parameters

TAS was measured by spectrophotometry (Specord M40, Carl Zeiss, Jena, Germany) using a TAS kit (Randox Laboratories, Crumlin, UK) [30]. Serum PAI-1 was measured by enzyme-linked immunoabsorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA) [31]. High-sensitivity C-reactive Protein (hsCRP) was quantified by an ELISA assay (Diazyme Laboratories, Poway, CA, USA). The assay has a linear range of 0.20–20 mg/L that extends below the measurement range typical of most conventional CRP assays [32].

Plasma total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL), and triglycerides (TG) were