

Effect of garlic (*Allium sativum*) powder tablets on serum lipids, blood pressure and arterial stiffness in normo-lipidaemic volunteers: a randomised, double-blind, placebo-controlled trial

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Recent studies have cast doubt on the proposed lipid-lowering and blood pressure-lowering effects of garlic. We tested the effect of dried garlic (*Allium sativum*) powder on blood lipids, blood pressure and arterial stiffness in a 12-week randomised, double-blind, placebo-controlled trial. Seventy-five healthy, normo-lipidaemic volunteers (men and women aged 40–60 years) were assigned to dried garlic powder tablets (10.8 mg alliin (3-(2-propenylsulfinyl)-L-alanine)/d, corresponding to about three garlic cloves) or placebo. Sixty-two subjects were eligible for the per-protocol analysis. The primary outcome measure was serum total cholesterol concentration. Secondary outcome measures were LDL-cholesterol, HDL-cholesterol and triacylglycerol concentrations, blood pressure and arterial stiffness (assessed by pulse wave velocity). No significant differences between the garlic and placebo groups were detected for any of the outcome measures. However, garlic powder was associated with a near-significant decrease (12%) in triacylglycerol concentration ($P=0.07$). In conclusion, garlic powder tablets have no clinically relevant lipid-lowering and blood pressure-lowering effects in middle-aged, normo-lipidaemic individuals. The putative anti-atherosclerotic effect of garlic may be linked to risk markers other than blood lipids.

Alliin: Risk factor: Pulse wave velocity: Coronary heart disease

Several trials, mainly from before 1996, have suggested that garlic (*Allium sativum*) could lower total cholesterol and triacylglycerol concentrations and other risk markers of CVD (Auer *et al.* 1990; Mader, 1990; Vorberg & Schneider, 1990; Kiesewetter *et al.* 1991; Holzgartner *et al.* 1992; De Santos & Grunwald, 1993; Jain *et al.* 1993; Adler & Holub, 1997; Kannar *et al.* 2001). These studies employed varied designs, study populations, garlic preparations and doses. Most studies did not control or assess dietary intakes during intervention and their statistical analyses have some limitations.

Recently, a number of well-designed studies have cast doubt on the efficacy of garlic for treatment of hypercholesterolaemia and hypertension (Simons *et al.* 1995; Berthold *et al.* 1998; Isaacsohn *et al.* 1998; Superko & Krauss, 2000; Gardner *et al.* 2001). A meta-analysis including old as well as recent trials with various garlic preparations reported a modest effect, but questioned the usefulness of garlic for hypercholesterolaemia (Stevinson *et al.* 2000).

Hitherto, dried garlic powder is the most widely tested among the available garlic preparations on the market. We conducted a 12-week randomised, double-blind,

placebo-controlled trial to further clarify the effect of dried garlic powder tablets on blood lipids and blood pressure. We also monitored arterial stiffness as assessed by pulse wave velocity (PWV). To our knowledge, the effect of garlic powder on PWV has not been studied in other intervention trials.

Methods

The study was a randomised, double-blind, placebo-controlled, 12-week parallel trial. The participants were mainly recruited from university employees in Copenhagen, Denmark, but also from the general public in May and June 1999. The trial was carried out at the Department of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark. The study was approved by the local ethical committee (J no. KF 01-071/99) and written consent was obtained from all participants.

The study included healthy, normolipidaemic volunteers, non-smoking and smoking men and women aged between 40 and 60 years. Exclusion criteria were: use of lipid or blood pressure lowering medication, anticoagulation medication, use of contraceptives, more than 4 h of intensive

physical exercise per week, in-study infectious disease (plasma C-reactive protein > 10 mg/l). The participants were not allowed to consume other dietary supplements apart from one daily multivitamin tablet and were not allowed to consume more than two garlic cloves per week. The participants were asked not to change dietary, smoking or physical activity habits during the study.

Based on a power of 0.8 to detect a significant difference in total serum cholesterol concentration of 10% ($P=0.05$, two-sided), a sample size of thirty-five in each study group was required. To allow for subjects dropping out, seventy-five participants were recruited for the study.

The study supplements were garlic powder tablets (230 mg per tablet; Futura Hvidløg Forte[®]; Dansk Droge, Ishøj, Denmark) or placebo tablets containing microcrystalline cellulose and calcium phosphate. Two tablets were self-administered twice per d together with morning and evening meals. The daily dose of alliin 3-(2-propenyl-sulfinyl)-L-alanine in the active arm was estimated as 10.8 mg by chemical analysis and corresponded to about three garlic cloves. Compliance was emphasised through administration of sealed tablet-containers combined with counts of left-over pills each month.

The primary outcome measure was serum total cholesterol concentration. Secondary outcome measures were LDL-cholesterol, HDL-cholesterol and triacylglycerol concentrations, systolic and diastolic blood pressures, and arterial stiffness as assessed by PWV. In order to assess any in-study dietary variations, dietary intakes were assessed at baseline and later in the study.

Serum lipids and blood pressure

Fasting (≥ 12 h) blood samples were collected by venepuncture in the right arm after 10 min resting supine and blood pressure was measured on two separate days at baseline and again at the end of the study. All blood samples were analysed for total cholesterol, HDL-cholesterol and triacylglycerol concentrations, and C-reactive protein in the same series after collection of final blood samples to minimise laboratory variability. Serum LDL-cholesterol concentration was calculated by Friedewald's formula: serum LDL cholesterol (mmol/l) = total cholesterol - HDL-cholesterol - (triacylglycerol/2.2) (Friedewald *et al.* 1972).

Blood pressure was measured in left arm with an automated sphygmomanometer (HEM-705CP; Omron Corp., Kyoto, Japan) after 10 min resting supine. Mean values of the duplicate measurements were used for statistical analyses.

Pulse wave velocity

PWV was determined in the arterial segment between the *aorta ascendens* and *arteria radialis* in the left arm after 10 min resting supine, once at baseline and at the end of the study. The pulse transit time from the aortic root to *arteria radialis* was assessed from twenty consecutive measurements of the time difference between the R-peak of the QRS complex of the electrocardiogram and the arrival of the pulse wave at the wrist, as recorded by an

infra-red light probe located over the *arteria radialis* (Pulse 4.0; Southampton University, Southampton, UK). After automatic or if necessary manual marking of the relevant points, the mean transit time was computed with a computer program (Pulse Analysis Program, version 97.1.1; LM Styles, Southampton University). PWV (m/s) was calculated by division of the distance between the sternal notch and the wrist (measured by a tape measure) by mean transit time (Martyn *et al.* 1995).

Diet assessment

Individual 24 h food recalls were collected by the same investigator at baseline and again 1 month before the end of the study. Data were analysed at group level. At the interview, each participant was shown photos of dishes in four different portion sizes, from which the participant could identify the relevant size. If a certain kind of food was not in the photo collection, the amount was estimated in household measures. Subsequently, the amounts of foods and drinks were estimated in g from published material on typical weights of foods and drinks (Andersen *et al.* 1996). The nutrient intake was calculated with a computer program (Dankost, 2000, version 1.4; Dansk Catering Service, Herlev, Denmark).

Statistical methods

Data were analysed with non-parametric methods using SPSS for Windows (version 9.0; SPSS Inc., Chicago, IL, USA). Statistical differences between baseline and end-study data (paired data) were assessed by Wilcoxon signed ranked test, and between-group differences (unpaired data) by Mann-Whitney U test. Significance levels were two-sided. Categorical data at baseline were analysed with a χ^2 test.

Assignment

Participants were stratified into groups based on gender and smoking status. Subsequently, randomisation was performed within the four subgroups by drawing participants' numbers at random and dividing them into groups 1 and 2 alternately. The participants' numbers and their corresponding group numbers were sent to the garlic tablet manufacturer, who randomly assigned groups 1 and 2 to garlic or control tablets. The code was revealed to the researchers by the manufacturer only when recruitment, data collection, laboratory analyses and statistical analyses were completed. The manufacturer had no knowledge of any results at this stage.

Masking

Participants and all study personnel (including the data analyst) were blinded to the treatment assignment throughout the study. Placebo tablets were similar in appearance and size to garlic tablets. Blinding of participants was evaluated by asking the participants which treatment they believed that they had received ('garlic', 'placebo' or 'don't know').

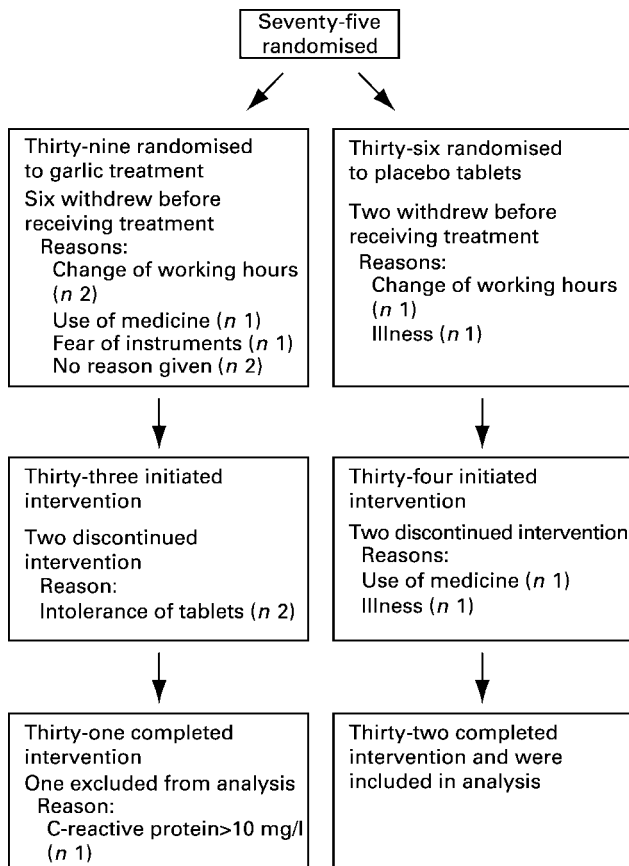


Fig. 1. Flow diagram of the subjects through each stage of the study.

Results

Thirty-eight subjects were randomised to receive garlic powder tablets and thirty-seven to receive placebo tablets (Fig. 1). By mistake, one participant in the placebo group received the wrong intervention. Consequently, at baseline the garlic treatment group and placebo group comprised thirty-nine and thirty-six subjects, respectively. A total of twelve participants withdrew from the study and one participant was excluded. Thus, sixty-two participants

remained for the per-protocol analyses. Reasons for discontinuation are given in Fig. 1.

Baseline characteristics of all participants included in analysis are shown in Table 1. Baseline values, absolute values and changes after 12 weeks for all outcome variables are shown in Table 2. There were no significant differences between garlic and placebo groups at baseline and no significant treatment effects on any outcome variable. However, there was a borderline significant decrease in triacylglycerol concentration in the garlic group (garlic group -0.08 , placebo group $+0.03$ mmol/l; $P=0.07$). HDL-cholesterol concentration increased significantly in the garlic group ($P=0.03$). However, no significant treatment difference was demonstrated.

Post hoc subgroup analysis of participants with baseline total cholesterol concentration >5.2 and <5.2 mmol/l revealed that baseline total cholesterol concentration did not seem to affect the effect of garlic powder tablets (results not shown). No significant changes within groups or differences between groups were seen in any of the subgroups. Similarly, *post hoc* regression analysis of changes in total cholesterol concentration versus baseline values showed no dependency of effect of garlic on baseline value ($r = -0.22$, $P=0.25$; $n 29$). In contrast, the effect of garlic (i.e. change within the garlic group) on triacylglycerol concentration was found to be dependent on baseline triacylglycerol concentration ($r = -0.41$, $P=0.03$; $n 29$).

During the intervention period there was a tendency towards a slight decrease in body weight in the garlic group ($P=0.08$), but not in the placebo group ($P=0.73$) (results not shown). We observed no significant inter-treatment group differences in dietary intakes during the study (results not shown).

Observed side-effects in the garlic group were eructation 15 min after consumption of garlic tablets ($n 12$), garlic odour ($n 5$) and flatulence ($n 3$). Eructation was experienced in four participants in the placebo group.

Discussion

We observed no significant effect of 12 weeks' dietary supplementation with garlic powder tablets containing 10.8 mg alliin/d on any variables: fasting total cholesterol,

Table 1. Baseline demographics and clinical characteristics (Mean values and standard deviation)

	Garlic ($n 30$)		Placebo ($n 32$)	
	Mean or no.	SD	Mean or no.	SD
Gender (M/F)	12/18		12/20	
Age (years)	49.6	5.5	50.9	4.6
BMI (kg/m^2)	24.2	3.9	24.7	2.8
Waist:hip ratio (M/F)	0.98/0.82	0.07/0.10	0.97/0.81	0.04/0.06
Smokers (n)	6		8	
No. female subjects (pre-/menopausal/post-)	10/2/6		7/6/7	
Hormone replacement therapy or hormone loop (n)	2		1	
Systolic blood pressure (mmHg)	113*	13*	116†	15†
Diastolic blood pressure (mmHg)	74*	8*	76†	10†

M, male; F, female; Pre-, premenopausal; post-, postmenopausal.

* $n 29$.

† $n 30$.

Table 2. Effect of garlic (*Allium sativum*) powder tablets (10.8 mg alliin/d) v. placebo tablets for 12 weeks on serum lipids, blood pressure and pulse wave velocity††
(Median values and interquartile ranges)

		Garlic (n 30)		Placebo (n 32)		Statistical significance of effect: P (garlic v. placebo)
		Median	Interquartile range	Median	Interquartile range	
Total cholesterol (mmol/l)	Before	4.91	4.43–5.95††	5.36	4.75–6.42	0.11
	After	5.18	4.22–6.01††	5.41	4.72–6.41	0.10
	Change*	0.05	–0.18–0.22††	–0.12	–0.29–0.22	0.38
HDL-cholesterol (mmol/l)	Before	1.27	1.02–1.56††	1.24	1.10–1.47	0.93
	After	1.29	1.09–1.63††	1.26	1.12–1.53	0.79
	Change†	0.05	–0.04–0.12††	0.03	–0.02–0.08	0.41
LDL-cholesterol (mmol/l)	Before	3.29	2.68–4.11††	3.64	3.04–4.46	0.08
	After	3.33	2.76–4.09††	3.68	2.87–4.39	0.15
	Change‡	0.01	–0.17–0.21††	–0.15	–0.34–0.17	0.21
Total triacylglycerol (mmol/l)	Before	0.94	0.73–1.34††	0.93	0.78–1.26	0.90
	After	0.80	0.67–1.28††	0.87	0.75–1.43	0.17
	Change§	–0.08	–0.37–0.11††	0.03	–0.13–0.16	0.07
Systolic blood pressure (mmHg)	Before	111.0	106.0–121.8††	115.0	104.9–128.5§§	0.44
	After	114.0	106.5–123.3††	119.0	109.0–131.0§§	0.20
	Change	2.0	–1.0–7.5††	3.0	–4.4–5.5§§	0.90
Diastolic blood pressure (mmHg)	Before	73.5	68.3–78.5††	74.5	69.9–80.6§§	0.56
	After	72.5	69.5–81.8††	77.0	71.9–82.9§§	0.27
	Change¶	0.0	–2.3–3.3††	0.5	–1.6–3.6§§	0.73
PWV (m/s)	Before	3.77	3.54–4.01	3.81	3.39–4.13¶¶	0.70
	After	3.80	3.61–4.21	3.89	3.64–4.17¶¶	0.71
	Change**	0.11	–0.11–0.52	0.15	–0.27–0.42¶¶	0.88

PWV, pulse wave velocity.

* $P=0.41$ and $P=0.41$ for garlic and placebo, respectively.

† $P=0.03$ and $P=0.10$ for garlic and placebo, respectively.

‡ $P=0.61$ and $P=0.22$ for garlic and placebo, respectively.

§ $P=0.07$ and $P=0.60$ for garlic and placebo, respectively.

|| $P=0.09$ and $P=0.21$ for garlic and placebo, respectively.

¶ $P=0.49$ and $P=0.30$ for garlic and placebo, respectively.

** $P=0.08$ and $P=0.17$ for garlic and placebo, respectively.

†† For details of the subjects, supplements and procedures, see Table 1 and p. 702.

‡‡ n 29.

§§ n 30.

||| n 27.

¶¶ n 29.

HDL-cholesterol, LDL-cholesterol and triacylglycerol concentrations, blood pressure or PWV. A near-significant 12% decrease in triacylglycerol concentration was seen in the garlic group compared with the placebo group ($P=0.07$). The power to detect a 12% decrease in triacylglycerol concentration was very low (16%). Consequently, we cannot rule out a modest triacylglycerol-lowering effect of dried garlic powder based on our present results.

Our per-protocol analyses included sixty-two subjects, which means that the present study is among the larger garlic intervention trials published so far. We selected a dried garlic preparation as the study supplement. The advantage of dried garlic powder is its content of active substances remarkably similar to those found in raw, whole garlic cloves, and that alliin, the precursor of allicin (2-propene-1-sulfinothioic acid *S*-2-propenyl ester) and one of the principal active compounds in garlic, can be standardised in the tablet. Furthermore, dried garlic was the preferred preparation in the majority of earlier trials. The conversion of alliin into allicin is catalysed by the enzyme alliinase only when the tablet is dissolved in the intestinal tract. The existence of alliin as well as alliinase in the present study supplement was verified by laboratory analysis (DB-lab, Odense, Denmark).

The compliance to supplementation in the present trial was very high according to counts of left-over pills. On average, 95 (range 67–100)% of prescribed tablets were taken. Most subjects (95%) took all the tablets allocated to them. A weakness of the trial was an unsuccessful participant blinding. Due to garlic odour, 77% of the garlic-group subjects v. none in the placebo group correctly assumed that they received garlic tablets (χ^2 test, $P<0.001$).

The lack of a significant effect on blood lipids in the present study is in accordance with four other trials that were comparable with our present trial with regard to length of study, garlic preparation, dosage of alliin and dietary assessment (Simons *et al.* 1995; Isaacsohn *et al.* 1998; Superko & Krauss, 2000; Gardner *et al.* 2001). A summary table of previous randomised, double-blind, placebo-controlled trials with dried garlic powder is given in Table 3.

Reviewing earlier randomised, double-blind, placebo-controlled dried garlic powder trials, we found that only six of eighteen studies demonstrated dried garlic powder to be associated with a significantly lower total cholesterol, triacylglycerol or LDL-cholesterol concentration compared with placebo (Mader, 1990; Vorberg & Schneider, 1990;

Table 3. Randomised, double-blind and placebo-controlled trials investigating the effect of dried garlic (*Allium sativum*) powder on serum lipids and blood pressure

Source and year	Design	n	Su	Dose dried powder/d (mg)	Duration (weeks)	Dried garlic effect (%) = active change (%) – placebo change (%)				
						TC	TAG	LDL	HDL	SBP/DBP
Luley <i>et al.</i> (1986)	CO	51	HL	1350	2 × 6	NS	NS	NS	NS	NS
Auer <i>et al.</i> (1990)	P	47	HT	600	12	-5*	-12*	na	na	-6/-9*
Vorberg & Schneider (1990)	P	40	HC	900	16	-18	-19	na	na	-6/-3*
Mader (1990)	P	221	HL	800	16	-9	-15	na	na	na
Kiesewetter <i>et al.</i> (1991)	P	60	HL	800	4	NS	NS	na	na	na
Jain <i>et al.</i> (1993)	P	42	HC	900	12	-5	NS	-8	NS	NS
De Santos & Grunwald (1993)	P	52	HC	900	26	-4	NS	-3†	NS	-17/-11
Simons <i>et al.</i> (1995)	CO, D	28	HC	900	2 × 12	NS	NS	NS	NS	NS
Neil <i>et al.</i> (1996)	P	106	HL	900	26	NS	NS	NS	NS	na
Adler & Holub (1997)	P, D	23	HC	900	12	-12	NS	-13	NS	na
McCrinkle <i>et al.</i> (1998)	P	30	HC	900	8	NS	NS	NS	NS	NS
Isaacsohn <i>et al.</i> (1998)	P, D	42	HC	900	12	NS	NS	NS	NS	NS
Lash <i>et al.</i> (1998)	P	35	HC	1360	12	-7*	NS	-11*	NS	na
Byrne <i>et al.</i> (1999)	P	31	HC	900	26	NS	-3*	NS	NS	na
Superko & Krauss (2000)	P, D	50	HC	900	12	NS	NS	NS	NS	na
Gardner <i>et al.</i> (2001)	P, D	51	HC	500/1000	12	NS	NS	NS	NS	na
Kannar <i>et al.</i> (2001)	P, D	43	HC	880	12	-7	NS	-12	-8	na
Turner <i>et al.</i> (2004)	P, D	62	NL	920	12	NS	NS	NS	NS	NS

TC, total cholesterol; TAG, triacylglycerol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CO, crossover; P, parallel; D, diet assessment included; HL, hyperlipidaemic; HT, hypertensive; HC, hypercholesterolaemic; NL, normolipidaemic; SU, subject.

* Significant change within the garlic group, but the essential statistical comparison between active and placebo treatment was not reported.

† Difference was reported, but was not significant.

Jain *et al.* 1993; De Santos & Grunwald, 1993; Adler & Holub, 1997; Kannar *et al.* 2001). Four of these six positive studies could be criticised for one or more shortcomings, such as lack of diet control or assessment, lack of compliance assessment or statistical weaknesses (Mader, 1990; Vorberg & Schneider, 1990; Jain *et al.* 1993; De Santos & Grunwald, 1993).

The lack of effect of dried garlic powder on blood pressure in the present trial is in accordance with the results in the majority of earlier placebo-controlled trials. Only one of nine trials (including the present study) reported an effect of garlic powder on blood pressure significantly different from placebo (De Santos & Grunwald, 1993).

Our present study could not confirm the inverse association between garlic powder intake and PWV reported by Breithaupt-Grögler *et al.* (1997). In an age- and gender-matched cross-sectional study of healthy elderly individuals ($n = 101 \times 2$), they found that regular garlic powder consumption (average intake 460 mg; present study 920 mg) was associated with a lower PWV level, suggesting that dried garlic may attenuate the age-related increase in arterial stiffness. The apparent discrepancy could be explained by the widely differing time frames of the two studies. Our present study lasted only 3 months, while cases in the cross-sectional study had consumed garlic powder tablets for an average period of 7 years. PWV is determined by the proportion of elastin and collagen in the vessel, as well as the presence of atherosclerotic plaques and vascular tone. If garlic has any anti-atherogenic effect, it is therefore more probable that a long-term effect on PWV in year-long studies would be found. However, garlic might also have an acute effect on the vascular tone and PWV as indicated by preliminary

findings by our group (B Turner, C Mølgaard and P Marckmann, unpublished results). Any such immediate effect during the postprandial hours would not be observed in our present trial where subjects were studied in the fasted state.

In conclusion, our present trial indicates that garlic (powder) has no clinically relevant impact on fasting serum LDL and HDL concentrations and blood pressure, whereas a modest triacylglycerol-lowering effect cannot be excluded in middle-aged, normo-lipidaemic volunteers. Evidence from at least one important recent study suggests that garlic may indeed have some anti-atherosclerotic effect (Koescielny *et al.* 1999). This could probably be explained by an effect of garlic on endothelial function and arterial stiffness (Breithaupt-Grögler *et al.* 1997; B Turner, C Mølgaard and P Marckmann, unpublished results) or improvement of resistance of LDL to oxidation (Munday *et al.* 1999), rather than lowering of total cholesterol and triacylglycerol concentrations as formerly thought. More research is obviously needed to clarify the effect of garlic on risk markers of CVD other than cholesterol levels.

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