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A Single Meal Containing Raw, Crushed Garlic Influences Expression of Immunity- and Cancer-Related Genes in Whole Blood of Humans[1–4]

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Abstract

Background: Preclinical and epidemiologic studies suggest that garlic intake is inversely associated with the progression of cancer and cardiovascular disease.

Objective: We designed a study to probe the mechanisms of garlic action in humans.

Methods: We conducted a randomized crossover feeding trial in which 17 volunteers consumed a garlic-containing meal (100 g white bread, 15 g butter, and 5 g raw, crushed garlic) or a garlic-free control meal (100 g white bread and 15 g butter) after 10 d of consuming a controlled, garlic-free diet. Blood was collected before and 3 h after test meal consumption for gene expression analysis in whole blood. Illumina BeadArray was used to screen for genes of interest, followed by real-time quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) on selected genes. To augment human study findings, Mono Mac 6 cells were treated with a purified garlic extract (0.5 μL/mL), and mRNA was measured by qRT-PCR at 0, 3, 6, and 24 h.

Results: The following 7 genes were found to be upregulated by garlic intake: aryl hydrocarbon receptor (*AHR*), aryl hydrocarbon receptor nuclear translocator (*ARNT*), hypoxia-inducible factor 1α (*HIF1A*), proto-oncogene c-Jun (*JUN*), nuclear factor of activated T cells (NFAT) activating protein with immunoreceptor tyrosine-based activation motif 1 (*NFAM1*), oncostatin M (*OSM*), and V-rel avian reticuloendotheliosis viral oncogene homolog (*REL*). Fold-increases in mRNA transcripts ranged from 1.6 (*HIF1A*) to 3.0 (*NFAM1*) ($P < 0.05$). The mRNA levels of 5 of the 7 genes that were upregulated in the human trial were also upregulated in cell culture at 3 and 6 h: *AHR*, *HIF1A*, *JUN*, *OSM*, and *REL*. Fold-increases in mRNA transcripts in cell culture ranged from 1.7 (*HIF1A*) to 12.1 (*JUN*) ($P < 0.01$). OSM protein was measured by ELISA and was significantly higher than the control at 3, 6, and 24 h (24 h: 19.5 ± 1.4 and 74.8 ± 1.4 pg/mL for control and garlic, respectively). OSM is a pleiotropic cytokine that inhibits several tumor cell lines in culture.

Conclusion: These data indicate that the bioactivity of garlic is multifaceted and includes activation of genes related to immunity, apoptosis, and xenobiotic metabolism in humans and Mono Mac 6 cells. This trial is registered at clinicaltrials.gov as NCT01293591. *J Nutr* 2015;145:2448–55.

Keywords: garlic, cancer, immunity, gene expression, Mono Mac 6

Introduction

Consumer demand for garlic (*Allium sativum* L.) has surged in recent years, with worldwide production almost doubling from 2002 to 2012 (1). This interest in garlic is partly driven by reports attributing various health benefits to garlic consumption. Given the prevalence of chronic diseases such as cancer and cardiovascular disease and their substantial personal, social, and financial impacts, there may be a role for garlic as part of a diet to promote and sustain human health (2, 3).

Preclinical studies have primarily focused on cardiovascular health and on prevention and treatment of cancer. Animal studies suggest that factors related to cardiovascular function such as plasma lipids, blood pressure, and platelet aggregation may be favorably modified by garlic or garlic-derived compounds (4–7). The inhibition of cholesterol synthesis has also been demonstrated in rat hepatoma cells. Of 9 garlic-derived

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[4] Supplemental Tables 1 and 2 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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compounds tested, diallyl disulfide, diallyl trisulfide, and allyl mercaptan were inhibitory, probably by suppression of 4α-methyl oxidase (8). Human clinical trials have produced mixed results with regard to lipid variables. LDL oxidation has been shown in cell studies and a small-scale human study to be suppressed by aged garlic extract (GE)[7] (9, 10), which is high in *S*-allylcysteine in contrast to fresh crushed garlic, which is high in allicin (diallyl thiosulfinate) and γ-glutamyl-*S*-alkylcysteines (11, 12). A recent meta-analysis of 39 randomized, placebo-controlled clinical studies indicated that garlic intake over at least a 2-mo period significantly reduced total serum and LDL cholesterol in humans with very high baseline values (13). However, in humans with moderately high baseline LDL cholesterol, raw garlic, dried garlic powder, and aged GE did not influence total cholesterol, LDL cholesterol, HDL cholesterol, total- to HDL-cholesterol ratio, or TGs during the 6-mo intervention period (14).

The effects of garlic and garlic-derived compounds on the prevention and treatment of cancer have also been reported in a large number of preclinical studies and involve modulation of xenobiotic metabolism, cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, and histone modification (15–23). Results from epidemiologic and clinical intervention studies are inconsistent. In the Iowa Women's Health Study of 41,837 women over 5 y, an RR of 0.68 was reported for colon cancer for the uppermost compared with the lowermost consumption levels of garlic (24). The EPIC (European Prospective Investigation into Cancer and Nutrition) study of >500,000 individuals showed a weak inverse association between garlic and onion intake and risk of intestinal gastric cancer after an average 6.5-y follow-up, but when 477,312 of these participants were assessed after 11 y, this association was no longer present (25, 26). In a randomized double-blind study, supplementation with a combination of steam-distilled garlic oil and aged GE did not influence the incidence of precancerous gastric lesions or gastric cancer incidence after a 7.3-y intervention (27). However, in a double-blind intervention study of participants at high risk of gastric cancer, men (but not women) consuming 200 mg synthetic allitridum (diallyl trisulfide) with 100 μg selenium had a decreased cancer risk after 5 y (28).

There is substantial evidence for a role of garlic in reducing cancer risk and cardiovascular disease, but the inconsistent findings prevent a clear understanding of the role of garlic in health promotion. One reason for the inconsistent findings may be the need for additional biomarkers of biological activity. Gene expression in vivo is a biomarker at the most basic level of biological response and may provide important clues to the biological activity of bioactive food components. Therefore, we conducted a human clinical trial and follow-up in vitro studies to measure the influence of garlic intake on mRNA gene expression in whole blood. Gene expression in the leukocyte population of whole blood is responsive to dietary interventions and may correlate with tissue-specific gene expression (29–31). Whole blood is relatively accessible compared with other human tissues and thus is advantageous for obtaining and measuring mRNA. We chose to focus on genes related to immunity and cancer and studied their response to a single meal containing raw, crushed garlic (RCG).

Methods

Study design, diet, and treatments. The study protocol was approved by the MedStar Health Research Institute (Hyattsville, Maryland). Written, informed consent was obtained from each study participant. A randomized crossover design with two 11-d treatment periods separated by a 17-d washout period was used. Subjects consumed a basal garlic-free diet for 10 d. On the 11th day, participants receiving the control treatment ate a breakfast consisting of 100 g white bread with 15 g butter. Participants receiving the garlic treatment consumed 100 g white bread with 15 g butter and 5 g RCG. The basal diet consisted of adequate protein,~35% of calories from fat, and 3 servings of fruits and vegetables daily to be in accord with average intakes in the United States (32, 33). Participants were instructed to consume all foods and only foods that were provided by the Beltsville Human Nutrition Research Center. Breakfast and dinner on weekdays were consumed at the Beltsville Human Nutrition Research Center, and lunches and weekend meals were packed for carry-out. Study participants were asked to abstain from vitamin and mineral supplements beginning 2 wk before the study and continuing throughout the study.

One week before the garlic meal of the first period, raw cloves of California Early garlic were minced and homogenized. Five-gram portions in sealed plastic containers were stored at −20°C until used. A subsample was reserved for the measurement of organosulfur compounds by using previously published HPLC methods (Silliker) (34).

Study participants. Participants were recruited from Beltsville, Maryland. Eighteen participants began the study. One participant left the study due to a scheduling conflict and 17 participants completed the study. Participant characteristics are reported in **Supplemental Table 1**.

Whole-blood collection and mRNA gene expression. Whole blood was collected into PAXgene blood RNA tubes (Qiagen) immediately before the day 11 breakfast (consisting of a control or garlic meal) and at 3 h after breakfast. Total RNA was isolated according to the manufacturer's instructions. Global mRNA gene expression by Illumina HumanHT-12 v4 BeadChip was used to screen for genes of interest in 12 randomly selected participants (Expression Analysis). Differentially expressed genes ($P < 0.01$) were considered for analysis by qRT-PCR. We selected genes related to immunity and genes potentially involved in cancer-related processes (**Table 1**). All probes and primers for qRT-PCR were designed by using the Primer Express (Applied Biosystems) software package and nucleotide sequences obtained from GenBank.

The RNA of the 12 participants used to assess global mRNA gene expression was also used to synthesize cDNA for qRT-PCR. Total RNA from the whole blood of the remaining 5 participants was extracted from PAXgene blood RNA tubes by using the BioRobot Universal system (Qiagen) according to the manufacturer's instructions. RNA quality was assessed by using Experion RNA gel electrophoresis analysis chips (Bio-Rad Laboratories), and the concentration was determined by using a Nanodrop spectrophotometer (Thermo Scientific). cDNA was prepared from 1.35 μg total RNA by using SuperScript II reverse transcriptase according to the manufacturer's protocol (Life Technologies). Quantitative real-time PCR was conducted by using iQ Supermix and a CFX96 real-time PCR system (both, Bio-Rad Laboratories). Data were adjusted for the housekeeping gene cyclophilin A (*PPIA*). Quantitative mRNA fold-changes were derived by using the ΔΔCt (threshold cycle) method (35) and are presented as the fold-change due to the

[7] Abbreviations used: *AHR*, aryl hydrocarbon receptor; AP-1, activator protein 1; *Apc*, adenomatosis polyposis coli; *ARNT*, aryl hydrocarbon receptor nuclear translocator; c-FOS, FBJ murine osteosarcoma viral oncogene homolog; c-JUN, proto-oncogene c-Jun; c-REL, V-Rel avian reticuloendotheliosis viral oncogene homolog; Ct, threshold cycle; CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2, cytochrome P450, family 1, subfamily A, polypeptide 2; GE, garlic extract; GSTA1, glutathione *S*-transferase α 1; HIF-1, hypoxia-inducible factor 1; *HIF1A*, hypoxia-inducible factor 1; INK4a, inhibitor of cyclin-dependent kinase 4a; *JUN*, proto-oncogene c-Jun; *LIF*, leukemia inhibitory factor; LSmean, least squares mean; *NFAM1*, NFAT activating protein with immunoreceptor tyrosine-based activation motif 1; NFAT, nuclear factor of activated T cells; OSM, oncostatin M; *PPIA*, cyclophilin A; RCG, raw, crushed garlic; *REL*, V-rel avian reticuloendotheliosis viral oncogene homolog; *RPL32*, ribosomal protein L32; SIM, single-minded; *TNF*, tumor necrosis factor; UGT1A1, uridine diphosphate glucuronosyltransferase 1 family, polypeptide A1; UGT1A6, uridine diphosphate glucuronosyltransferase 1 family, polypeptide A6; XRE, xenobiotic response element.

TABLE 1 Genes measured in human whole blood by qRT-PCR[1]

Gene	Symbol
AHR pathway genes	
Aryl hydrocarbon receptor	*AHR*
Aryl hydrocarbon receptor nuclear translocator	*ARNT*
Aryl hydrocarbon receptor nuclear translocator 2	*ARNT2*
Hypoxia-inducible factor 1, α subunit	*HIF1A*
Cancer-related genes	
Excision repair cross-complementation group 1	*ERCC1*
IL 6	*IL6*
Proto-oncogene c-Jun	*JUN*
Leukemia inhibitory factor	*LIF*
Oncostatin M	*OSM*
V-rel avian reticuloendotheliosis viral oncogene homolog	*REL*
Tumor necrosis factor receptor superfamily, member 21	*TNFRSF21*
Immunity-related genes	
Calcium/calmodulin-dependent protein kinase IIγ	*CAMK2G*
Chemokine (C-X-C motif) ligand 14	*CXCL14*
IL 2	*IL2*
Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 3	*NFATC3*
NFAT activating protein with immunoreceptor tyrosine-based activation motif 1	*NFAM1*
Housekeeping gene	
Cyclophilin A	*PPIA*

[1] Several genes could be assigned to more than one classification, but for ease of reference are placed within only one group.

garlic treatment relative to that of the control = $2^{-\Delta C't}$, where $\Delta C't = (Ct_{garlic,\ hour\ 3} - Ct_{garlic,\ hour\ 0}) - (Ct_{control,\ hour\ 3} - Ct_{control,\ hour\ 0})$.

Preparation of GE for in vitro studies. Garlic from a local grocery store was processed with a juicer to produce a crude garlic homogenate, which was centrifuged at 4500 *g* for 10 min at 20°C. The supernatant was filtered through a 0.45-μm syringe filter (Millipore) followed by filtration with a 0.22-μm centrifugal filter (Millipore) and centrifuged at 4500 *g* for 5 min at 20°C. The GE was placed into 1.5-mL microcentrifuge tubes and frozen at −80°C. The endotoxin concentration in the extract was below detection (0.01 ng endotoxin/mL) according to the *Limulus* amebocyte lysate assay (ThermoScientific). Organosulfur compounds were measured by HPLC.

Cell culture and sample analysis of Mono Mac 6 cells. Human study gene expression findings were confirmed in vitro by using the Mono Mac 6 cell line. The Mono Mac 6 cell line was selected because it expresses all of the genes that are significantly expressed in humans. In addition, Mono Mac 6 cells are monocytic and therefore model an important class of leukocytes. Cells were obtained from the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and were cultured according to the supplier's instructions. Cells were maintained at a density of 1.0×10^6 cells/mL.

At the beginning of each experiment, 1 mL medium was transferred to the wells of a cell culture plate and combined with 1 mL of cell-free medium with or without GE, resulting in concentrations of 0.0 or 0.5 μL GE/mL medium (n = 3). Viability was determined by flow cytometric analysis on an Attune flow cytometer (Life Technologies) by using an Annexin-fluorescein isothiocyanate/propidium iodide staining kit (Trevigen). Cell gating was performed by using forward and side scatter, and the percentage of live, apoptotic, and necrotic cells was determined from 10,000 cells. The percentage of viable, early apoptotic, late apoptotic, and necrotic cells averaged 93.3%, 3.6%, 1.9%, and 1.1%, respectively, in control cells, and averaged 91.7%, 4.8%, 2.2%, and 1.2%, respectively, in garlic-treated cells.

Samples were collected at 0, 3, 6, and 24 h after adding the GE. Total RNA was isolated from cells by using the RNeasy Plus Mini Kit (Qiagen). RNA quality and concentration were determined as for the human samples. One microgram of total RNA was transcribed to cDNA by using the Quantitect reverse transcription kit (Qiagen). Genes that were significant (P < 0.05) in the qRT-PCR analysis of the human samples were selected for measurement in Mono Mac 6 cells. qRT-PCR was performed by using iQ Supermix and a CFX96 real-time PCR system, with ribosomal protein L32 (*RPL32*) used as the housekeeping gene. Ct data were subtracted from 45 (total number of PCR cycles) so that increasing levels of mRNA are represented by increasing values of the difference between 45 and Ct.

Oncostatin M (OSM) protein was measured in cell culture supernatant by ELISA (R&D Biosystems) following the manufacturer's instructions. Samples were centrifuged at 14,000 *g* for 15 min at 4°C before being added to the plate. In vitro experiments were performed twice, with similar results.

Statistical analysis. ANOVAs of the gene expression data from the human study were performed by using the MIXED procedure in SAS (version 9.3; SAS Institute). Data were tested for normality with the Shapiro-Wilk statistic and by inspection of stem-leaf plots and normal probability plots of residuals. The data for nuclear factor of activated T cells (NFAT) activating protein with immunoreceptor tyrosine-based activation motif 1 (*NFAM1*) and *OSM* were skewed and therefore were ln-transformed. To account for the serial correlation of repeated measures on the same experimental unit (participant), covariance structures were fit to the data by using Akaike's and Bayesian information criteria. The models for aryl hydrocarbon receptor (*AHR*), proto-oncogene c-Jun (*JUN*), leukemia inhibitory factor (*LIF*), and *OSM* were fit with the compound symmetry covariance structure, and the models for hypoxia-inducible factor 1α (*HIF1A*),

TABLE 2 Measurement by qRT-PCR of mRNA gene expression in human whole blood 3 h after garlic consumption relative to the control meal[1]

Gene	Fold of control	P
$P \leq 0.05$		
AHR	2.6	0.017
ARNT	1.8	0.020
HIF1A	1.6	0.027
JUN	1.7	0.045
NFAM1[2]	3.0	<0.001
OSM[3]	1.8	0.001
REL	1.7	0.016
$P > 0.05$		
ARNT2	1.0	0.97
CAMK2G	1.2	0.35
CXCL14	1.3	0.74
ERCC1	1.3	0.11
IL2	1.1	0.61
IL6	1.3	0.14
LIF	1.4	0.08
NFATC3	1.4	0.11
TNFRSF21	1.6	0.09

[1] Fold of control = $2^{-\Delta C't}$, where $\Delta C't = (Ct_{garlic,\ hour\ 3} - Ct_{garlic,\ hour\ 0}) - (Ct_{control,\ hour\ 3} - Ct_{control,\ hour\ 0})$. *AHR*, aryl hydrocarbon receptor; *ARNT*, aryl hydrocarbon receptor nuclear translocator; *ARNT2*, aryl hydrocarbon receptor nuclear translocator 2; *CAMK2G*, calcium/calmodulin-dependent protein kinase IIγ; Ct, threshold cycle; *CXCL14*, chemokine (C-X-C motif) ligand 14; *ERCC1*, excision repair cross-complementation group 1; *HIF1A*, hypoxia-inducible factor 1α; *JUN*, proto-oncogene c-Jun; *LIF*, leukemia inhibitory factor; *NFAM1*, NFAT activating protein with immunoreceptor tyrosine-based activation motif 1; *NFATC3*, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 3; *OSM*, oncostatin M; *REL*, V-rel avian reticuloendotheliosis viral oncogene homolog; *TNFRSF21*, tumor necrosis factor receptor superfamily, member 21.

[2] Significant sex × treatment interaction, P = 0.010. Fold-change for women = 5.6 (P < 0.001) and for men = 1.5 (P = 0.32).

[3] Significant sex × treatment interaction, P = 0.007. Fold-change for women = 2.5 (P < 0.001) and for men = 1.2 (P = 0.29).

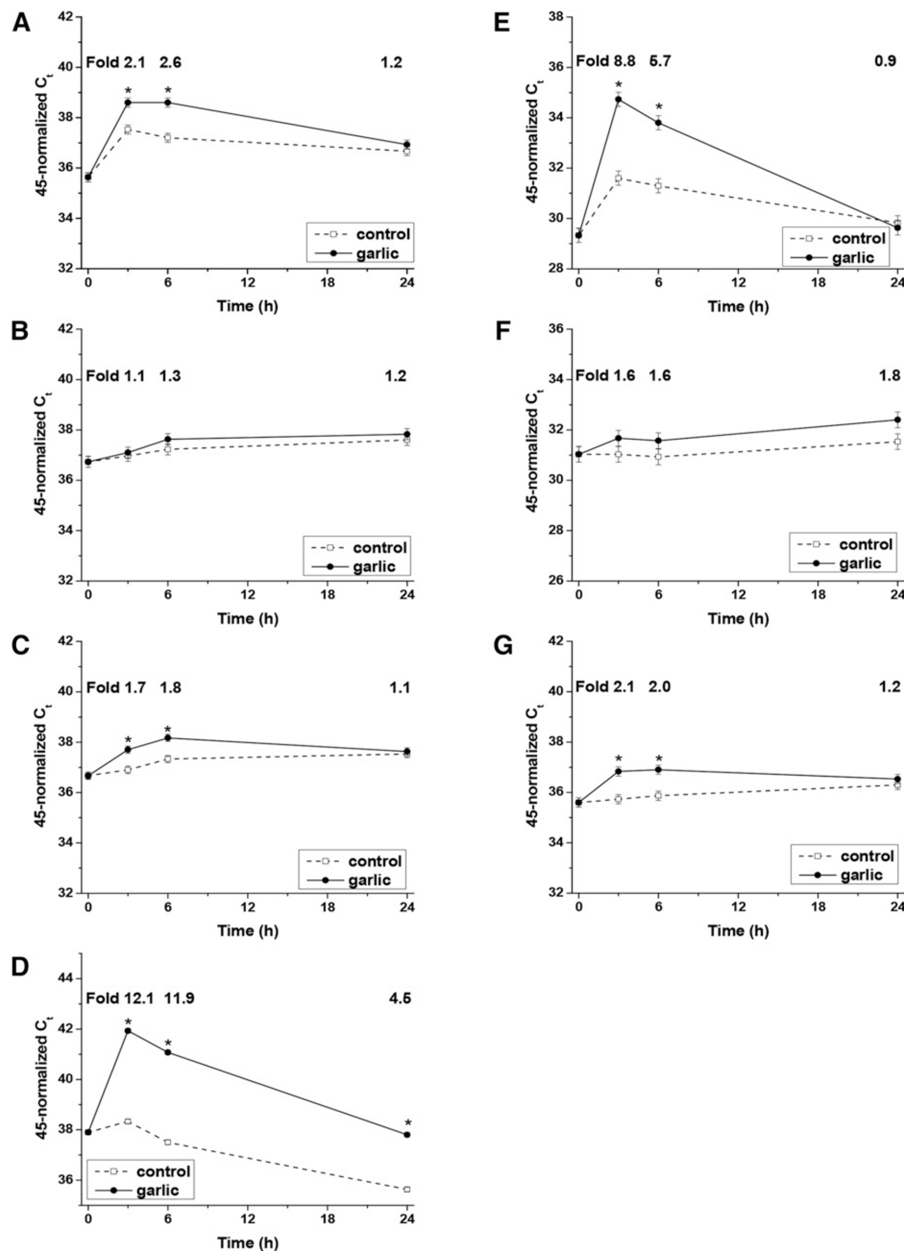

FIGURE 1 Expression of *AHR* (A), *ARNT* (B), *HIF1A* (C), *JUN* (D), *OSM* (E), *NFAM1* (F), and *REL* (G) in Mono Mac 6 cells in response to garlic extract (0.5 μL/mL). Values are least squares means ± SEs; $n = 3$. *Different from control at that time, $P < 0.01$ (Fisher's protected least significant different test). The Ct data were normalized to *RPL32* and are subtracted from 45 (total number of PCR cycles), so that increasing levels of mRNA are represented by increasing values on the *y*-axis. *AHR*, aryl hydrocarbon receptor; *ARNT*, aryl hydrocarbon receptor nuclear translocator; Ct, threshold cycle; *HIF1A,* hypoxia-inducible factor 1α; *JUN*, proto-oncogene c-Jun; *RPL32*, ribosomal protein L32.

NFAM1, and V-rel avian reticuloendotheliosis viral oncogene homolog (*REL*) were fit with the variance components structure. Study period, sequence, and sex were treated as fixed effects, whereas study participant was treated as a random effect. Model effects are reported as least squares means (LSmeans; $P < 0.05$). LSmeans for *NFAM1* and *OSM* were inverse transformed to their original scale. The statistical model for the in vitro gene expression data was a treatment × hour 2-way factorial mixed-effects model, with repeated measures observed on each treatment replication at 0, 3, 6, and 24 h. Fisher's protected least significant difference tests were applied to compare LSmeans at each hour in the in vitro experiment. Data are reported as LSmeans and SEMs.

Results

Composition of the garlic products

The concentrations of the organosulfur compounds in RCG and GE are given in **Supplemental Table 2**. The concentrations of the compounds in RCG may be calculated on a dry weight basis by multiplying the values by 2.42 (mg/g dry wt). The RCG contained >3 times the concentration of γ-glutamyl-*S*-alkylcysteines (14.5 mg/g) compared with thiosulfinates (4.5 mg/g), in contrast to the GE in which the concentrations of γ-glutamyl-*S*-alkylcysteines (3.7 mg/g) and thiosulfinates (3.5 mg/g) were similar. In both products, the concentrations of allyl sulfides were much lower than those of the other organosulfur compounds.

Human clinical trial

Gene expression in whole blood. Sixteen genes were selected for mRNA gene expression measurement. Of these genes, *AHR*, aryl hydrocarbon receptor nuclear translocator (*ARNT*), *HIF1A*, *JUN*, *NFAM1*, *OSM*, and *REL* were significantly upregulated 3 h after the RCG intervention (**Table 2**). *NFAM1* had the largest fold-increase (3.0) and *HIF1A* had the smallest increase (1.6). For both *NFAM1* and *OSM*, there was a significant treatment × sex interaction ($P < 0.05$) in which the gene expression in men did not change, whereas that in women increased significantly.

In women, the *NFAM1* fold-change was 5.6 ($P < 0.001$) and the *OSM* fold-change was 2.5 ($P < 0.001$)

In vitro experiments with Mono Mac 6 cells

Gene expression in response to GE. Five of the 7 genes that were significantly upregulated in the human clinical trial also were upregulated in Mono Mac 6 cells treated with GE: *AHR*, *HIF1A*, *JUN*, *OSM*, and *REL* (**Figure 1**). The expression of these genes was higher in GE-treated cells than in control cells at 3 and 6 h, and with the exception of *JUN*, expression did not differ between GE-treated and control cells at 24 h. The largest fold-increases occurred for *JUN*, with a fold-increase of 12.1 at 3 h and 11.9 at 6 h, followed by *OSM* with fold increases of 8.8 at 3 h and 5.7 at 6 h. The smallest fold-change that was significant was for *HIF1A*, which increased 1.7-fold at 3 h.

Concentration of OSM protein in response to GE. The concentration of OSM was significantly higher in GE-treated cells than in control cells at 3, 6, and 24 h (**Figure 2**). The most rapid accumulation of OSM occurred from 3 to 6 h, when OSM concentration increased by 157% from 19.0 ± 1.4 to 48.6 ± 1.4 pg/mL. The beginning of this high rate of OSM protein synthesis coincided with the peak concentration of the *OSM* transcript, which occurred at 3 h. The concentration of OSM at 24 h in the GE-treated medium was 74.8 ± 1.4 pg/mL, which was 3.8-fold that of the control cell medium.

Discussion

We chose to use RCG because it is less processed than cooked garlic and other garlic formulations and thus represents garlic in one of its most basic forms. The organosulfur composition of the RCG was similar to that reported in previous studies (34). Concentrations of the organosulfur compounds tended to be lower in GE than in RCG. This difference may be a result of the fact that the garlic from which the GE was produced was purchased several months after harvest and that storage causes degradation of γ-glutamyl-*S*-alkylcysteines by means of γ-glutamyltranspeptidase (36). Also of note is that the GE used in the present study is chemically distinct from the similarly named aged GE, in which thiosulfinates are present at very low concentrations (11).

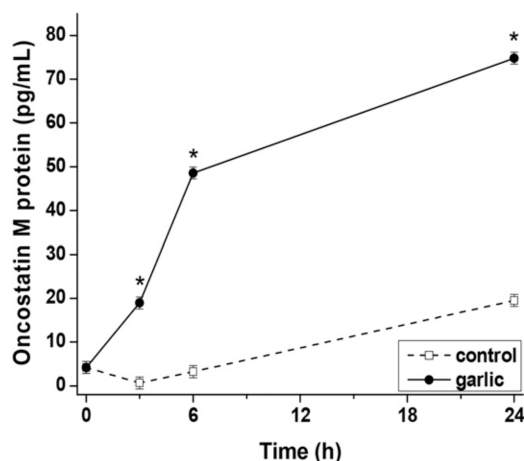

FIGURE 2 Concentration of oncostatin M protein produced by Mono Mac 6 cells treated with garlic extract (0.5 μL/mL). Values are least squares means ± SEs; $n = 3$. *Different from control at that time, $P < 0.01$.

Genes affected by garlic. A single meal with RCG induced the expression of 7 genes involved in immunity and cancer-related processes 3 h after consumption. Remarkably, 5 of these genes were also upregulated in GE-treated Mono Mac 6 cells. *AHR* is a ligand-activated member of the basic helix-loop-helix/Per-ARNT-single-minded (SIM) superfamily of transcription factors (37). When activated by ligand binding, AHR protein moves from the cytoplasm to the nucleus where it dimerizes with ARNT and binds to xenobiotic response elements (XREs) within the promoters of target genes (38). AHR has been classically associated with its activation by halogenated aromatic hydrocarbons and nonhalogenated polycyclic aromatic hydrocarbons and the subsequent induction of xenobiotic metabolizing enzymes such as cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1), cytochrome P450, family 1, subfamily A, polypeptide 2 (CYP1A2), uridine diphosphate glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), uridine diphosphate glucuronosyltransferase 1 family, polypeptide A6 (UGT1A6), and glutathione *S*-transferase α 1 (GSTA1) (39–41). In addition, AHR suppressed intestinal carcinogenesis in adenomatosis polyposis coli $(Apc)^{\text{multiple intestinal neoplasia/+}}$ mice by its participation in a ligand-dependent E3 ubiquitin ligase that degrades β-catenin, a protein whose overexpression is associated with cancers of the colon, skin, liver, ovaries, and prostate (42, 43). AHR was activated by natural ligands derived from dietary tryptophan and glucosinolates, a class of secondary plant metabolites associated with protection from colon cancer (44). These results, together with our finding that the consumption of RCG and GE induced *AHR* expression, suggest a mechanism by which garlic may inhibit intestinal cancer in humans. *AHR* also has a role in the development and function of effector and regulatory T cells, a subpopulation of the blood cells collected in our human clinical trial, supporting a role for RCG in the modulation of immune function (45).

Similar to AHR, HIF1A is a dimeric partner with ARNT, forming the transcription factor hypoxia-inducible factor 1 (HIF-1). Genes activated by HIF-1 increase glucose transport, glycolysis, angiogenesis, and erythropoietin (46). In hypoxic environments such as in solid tumors, the increase in HIF-1 leads to increased oxygen transport and mediates adaptive responses to oxygen deprivation, which may support tumor development (47). In ischemic disorders, upregulation of HIF-1 can counteract the pathologic effects of hypoxic conditions and promote reparative neovascularization (48). Therefore, the RCG-induced expression of *HIF1A* may have different implications depending on the health status of the individual, with benefits for those with ischemic conditions and risks for those with existing tumors.

JUN codes for c-JUN, a protein that partners with the proto-oncogene FBJ murine osteosarcoma viral oncogene homolog (c-FOS) to form activator protein 1 (AP-1), an early response transcription factor. Although c-JUN is required for normal development, many studies suggest that c-JUN is associated with cancer development (49, 50). In contrast, other studies indicate that c-JUN interferes with tumorigenesis. In c-JUN–deficient breakpoint cluster region-Abelson+ (BCR-ABL+) tumor cell lines, mRNA and protein of the tumor suppressor and cell cycle inhibitor $p16^{\text{inhibitor of cyclin-dependent kinase 4a (INK4a)}}$ are down-regulated, suggesting that c-JUN inhibits the tumorigenic silencing of $p16^{INK4a}$ (51). In tylophorine-treated carcinoma cells, altered binding of c-JUN to the regulatory regions of the cyclin A2 promoter resulted in decreased expression of cyclin A2 and increased G1 phase arrest (52). These results represent mechanisms by which the induction of *JUN* after RCG consumption may inhibit tumorigenesis.

NFAM1 is expressed in B cells, T cells, and monocytes and is involved in B cell signaling and development (53). Given that NFAM1 was upregulated in whole blood from the human study but not in Mono Mac 6 cells, which are monocytic, garlic intake may primarily influence B and/or T cells. NFAM1 activates the NFAT signaling pathway, leading to activation of tumor necrosis factor (*TNF*) and *IL13* promoters in the human mast cell line 1 (HMC-1) (54). The upregulation of *NFAM1* points to an immunomodulating effect of RCG intake, an effect that has been hypothesized, but not proven, to reduce cancer risk (55).

REL codes for V-Rel avian reticuloendotheliosis viral oncogene homolog (c-REL), which exists as homodimers or heterodimers in the NF-κB family of transcription factors. These transcription factors have many roles in both normal and pathologic processes and activate genes related to apoptosis, development, and immune and inflammatory responses (56). Overexpression of c-REL has been linked with mammary tumorigenesis in Michigan Cancer Foundation-7 (MCF7) cells and in primary human breast cancer tissue samples; in addition, the proliferation of B cell lymphoma cell lines was stimulated by c-REL (57–59). In contrast, 3 of 6 $REL^{-/-}$ mice developed lymphoproliferative lesions after 12 mo of infection with *Helicobacter pylori*, suggesting that c-REL–mediated signaling may reduce the risk of lymphomagenesis in gastric mucosa–associated lymphoid tissue (60). *REL* has many functions, and the effect of its upregulation by RCG may be equally diverse and depend on an individual's health status.

OSM encodes a pleiotropic cytokine belonging to the IL-6 family of cytokines, which is characterized by a common signal transducing receptor component, glycoprotein 130. OSM protein is produced by activated T cells, monocytes, dendritic cells, and neutrophils (61–64). The induction of *OSM* after RCG consumption seems not to be a general inflammatory response because *IL6* and *LIF* (also an IL-6 family gene) did not change. Note that OSM has been shown to inhibit proliferation of Human Tumor Bank 10 (HTB10) neuroblastoma cells, A549 lung carcinoma cells, and A375 and Sloan Kettering Melanoma 28 (SK-MEL-28) melanoma cells and in 4 of 5 chondrosarcoma cell lines, because this indicates that OSM may have a role in reducing cancer risk (65, 66). It may also be significant that OSM upregulated *AHR* and *HIF1A* in hepatoma G2 (HepG2) cells and *JUN* in human fibroblasts and M1 leukemic cells, because these results suggest that the increased expression of *AHR*, *HIF1A*, and *JUN* measured in our human clinical trial and in vitro study may have occurred in response to increased expression of *OSM* (67–69).

Although gene expression is an important determinant of protein abundance, gene expression is not perfectly correlated to amounts of protein present. The abundance of mRNA is correlated to cellular protein concentration, but only approximately half of the variation in cellular protein concentration can be attributed to mRNA levels (70–72). The other half is the result of post-transcriptional modification and protein degradation (71, 72). Therefore, although our results highlight potential pathways that may be influenced by garlic, they should not be considered definitive proof of alterations in metabolism.

Conclusions. Seven genes related to immunity and/or cancer were upregulated in whole blood 3 h after RCG consumption, and 5 of these genes were also upregulated in the monocytic cell line Mono Mac 6 when treated with garlic extract. The upregulated genes have a variety of functions, including roles in xenobiotic metabolism, inflammation, B cell and T cell development, apoptosis, and tumorigenesis. The measurement of gene expression allows insight into early events initiated by RCG intake. It has been used infrequently in preclinical studies, and to our knowledge, this is the first clinical human trial to assess gene expression in response to the consumption of garlic.

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