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Garlic Supplementation Modified Enzymatic Omega-6 Polyunsaturated Fatty Oxidation in Mild Hypercholesterolemia

Ho Hang Leung, Yu Fung Yau, Kin Sum Leung, Yiu Yiu Lee, Camille Oger, Thierry Durand, Jean-Marie Galano, Wai Mun Loke, and Jetty Chung-Yung Lee*

Dietary garlic has been shown to alleviate hypercholesterolemia, a condition associated with the development of cardiovascular disease. Reactive oxygen species take part in the disease progression, but it is unknown if antioxidant rich garlic arrests lipid and cholesterol oxidation in hypercholesterolemia. This study provides fermented garlic or non-fermented garlic to mild hypercholesterolemia (total cholesterol: 5.2–6.2 mmol L⁻¹) and determines its ability to relieve in vivo oxidative stress by measuring multiple oxidized lipid products of polyunsaturated fatty acid (PUFA) and cholesterol oxidation products (COPs) in plasma. Plasma fatty acids levels are also analyzed. After 13 weeks supplementation, both groups show a significant increase in α -linolenic acid level ($p < 0.05$). Levels of arachidic acid increase and palmitoleic acid decreases in the group supplemented with non-fermented garlic. Of the oxidized PUFA products, notably those derived from enzymatic oxidation of arachidonic acid, namely hydroxyeicosatetraenoic acid (HETE), show a significant difference. Levels of lipoxygenase mediate 5-HETE and 12-HETE, and CYP-P450 mediates 9-HETE and 11-HETE decreases after non-fermented garlic supplementation only ($p < 0.05$). Non-enzymatic PUFA oxidation and COPs are not altered by garlic supplementation. Overall, only non-fermented garlic intake shows selective antioxidant properties in mild hypercholesterolemia where limited effect is found in reducing lipid oxidation.

Practical Applications: Current research provides a new insight for the use of garlic extract as a nutraceutical to reduce in vivo oxidative stress. The modified enzymatic oxidation of arachidonic acid in mild hypercholesterolemia after non-fermented garlic extract intake potentially prevents the progression of chronic inflammation that can lead to complicated pathologies such as arthritis, cardiovascular diseases, and non-alcoholic fatty liver disease.

1. Introduction

Garlic (*Allium Sativum*) is a perennial plant composed of cloves enveloped by dry skin. Early studies showed garlic intake exhibits hypolipidemic, antiplatelet, anticancer, and pro-circulatory properties.^[1] To date, S-allylcysteine an organosulfur compound present in garlic has been extensively studied for its anticancer property. However, fermented garlic extract has attracted considerable interest due to high antioxidant capacity attributed by the rich polyphenols content than non-fermented ones.^[2] The fermented garlic was shown to contain significantly higher total flavonoids ($1.27 \pm 0.03 \mu\text{g mL}^{-1}$) compared to the non-fermented garlic ($0.29 \pm 0.01 \mu\text{g mL}^{-1}$).^[3] Also, the amount of polyphenols in the fermented garlic ($6.39 \pm 0.27 \text{ mg gallic acid equivalent g}^{-1}$) was significantly higher than the non-fermented garlic ($0.22 \pm 0.06 \text{ mg gallic acid equivalent g}^{-1}$).^[3] However, detailed polyphenol composition of the two garlic variants are not reported.

Fermented garlic is produced by exposing whole bulbs of raw garlic to high temperature (70 °C) under controlled humidity (90% RH) for more than one month^[4] whereas those of non-fermented garlic is simply in the raw form. Supplementation of fermented garlic showed decreased total cholesterol and blood

H. H. Leung, Y. F. Yau, Dr. K. S. Leung, Dr. Y. Y. Lee, Prof. J. C.-Y. Lee
School of Biological Sciences
The University of Hong Kong
Hong Kong SAR
E-mail: jettylee@hku.hk

Dr. C. Oger, Dr. T. Durand, Dr. J.-M. Galano
Institut des Biomolécules Max Mousseron
Université de Montpellier
UMR 5247 CNRS, ENSCM, France

Dr. W. M. Loke
School of Chemical and Life Sciences
Centre for Functional Food & Human Nutrition
Nanyang Polytechnic
Singapore 569830, Singapore

pressure, and increased HDL of patients with moderate hypercholesterolemia^[5] whereas no improvement in the lipid profile was observed when supplemented with non-fermented garlic powder. In a double-blinded randomized trial, daily fermented garlic intake significantly reduced systolic pressure in patients with uncontrolled systolic hypertension when compared with placebo.^[6] However, extensive evidences indicate that garlic supplementation increased antioxidant activity and reduced oxidative stress in animal^[7] and human,^[8] especially F₂-isoprostanes levels^[9] and pro-inflammatory enzymes.^[10]

Human hypercholesterolemia is an asymptomatic condition contributed by multiple factors such as unhealthy diet, pathological inheritance, obesity, pre-existing diabetes mellitus, and stress.^[11] It is associated with atherosclerosis and considered to be a risk factor for coronary heart disease (CHD) and stroke.^[11] Recent study revealed that oxidative stress takes a role in hypercholesterolemia in *ApoE*^{-/-} mice and was reduced in hypercholesterolemia subjects and in rabbits with high cholesterol diet after antioxidant administration.^[12] However, the method of assessment for oxidative stress were non-specific in these studies, and to date, a robust evaluation in plasma samples of mild hypercholesterolemia patients to determine the potential benefits of antioxidants are not well assessed.

Free radical or reactive oxygen species (ROS) or oxidative enzymes (lipoxygenase [LOX], cyclooxygenase) activates polyunsaturated fatty acids (PUFA) peroxidation and cholesterol oxidation, and the oxidized products released are related to various pathophysiological conditions that involves diverse cellular signalling.^[13] Notably, 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) derived from arachidonic acid (AA, C20:4n-6) is not only a biomarker for oxidative stress measurement, it is a potent vasoconstrictor and contributor to the pathogenesis of cardiovascular diseases (CVD).^[13-15] Other isoprostanes namely F₂-dihomo-isoprostanes (F₂-dihomo-IsoPs), F₃-isoprostanes (F₃-IsoPs), and F₄-neuroprostanes (F₄-NeuroPs) derived from adrenic acid (AdA, C22:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), respectively are considered tissue-specific oxidative stress biomarkers and take part in biological pathways such as cardio-protection.^[13,16] Aside from these free radical/ROS mediated oxidized products, enzymatic oxidation such as LOX, for example, releases hydroxyeicosatetraenoic acid (HETE) and hydroxydocosahexaenoic acid (HDHA) from AA and DHA, respectively. Numerous types of HETEs are associated to inflammation and platelet activation while HDHA are proposed to be anti-inflammatory that have pro-resolving properties.^[17]

In this study, we examined whether acute and sub-acute exposure to antioxidant rich fermented garlic or non-fermented garlic extract supplementation can alleviate in vivo oxidative stress in hypercholesterolemia subjects using targeted lipidomics approach.

2. Experimental Section

2.1. Chemicals and Materials

All organic solvents used were HPLC grade. Isoprostanooids, HDHA, HETEs, and resolvin standards were purchased from

Cayman Chemical Co. (Ann Arbor, MI, USA) and dihomoisoprostanes, dihomo-isofurans, neuroprostanes and neurofurans were synthesized by Institut des Biomolécules Max Mousseron (IBMM, Montpellier, France) as described.^[18-21] The fermented and non-fermented garlic extracts were prepared and encapsulated by Defu Foodstuff Pte Ltd (Singapore).

2.2. Measurement of Antioxidant Capacity in Garlic

The doses selected for the in vitro study was based on S-allylcysteine levels reported in human blood after garlic extract intake. The level reported is $\approx 100-140$ ppb ($100-140 \mu\text{g L}^{-1}$) to reduce cardiovascular risk factors^[22] therefore, a range of upper and lower concentrations ($10-200 \mu\text{g L}^{-1}$) were selected for the test.

The in vitro effects of fermented and non-fermented garlic extract on superoxide dismutase (SOD) activity were measured using a commercially available SOD activity assay kit (Abcam, MA, USA). Briefly, The extracts (final concentrations, 10, 20, 50, 100, and $200 \mu\text{g L}^{-1}$) were incubated with the provided SOD enzyme solution ($20 \mu\text{L}$) and WST substrate (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium salt) solution ($200 \mu\text{L}$) at 37°C for 20 min. At the end of incubation, the absorbance was read at a wavelength of 450 nm. The treatment compounds were added using PBS as vehicle. Control experiments were carried out without the treatment compounds and blank measurements were taken without SOD enzyme. The SOD activity (change%) was expressed as the percentage change in absorbance relative to the control after blank adjustment.

The in vitro radical scavenging activity of fermented and non-fermented garlic extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) method. The ethanolic DPPH solution (48 mg L^{-1}) was freshly prepared. Fermented or non-fermented garlic extracts was mixed into the freshly prepared DPPH solution (1:9 volume ratio) and the change in absorbance at 517 nm was measured using a spectrophotometer. A calibration curve was prepared with Trolox ($0-5 \text{ mmol L}^{-1}$). The radical scavenging results were expressed in mmol L^{-1} Trolox.

2.3. Study Design

Detailed description of the study design has been published previously by Ho et al.^[23] Briefly, a randomized, double-blinded study was conducted, and fermented garlic and non-fermented garlic extracts were given to mild hypercholesterolemia subjects ($5.2-6.2 \text{ mmol L}^{-1}$ total blood cholesterol) of Chinese ethnicity. A total of 41 subjects were recruited. Parametric biomarkers were not significantly different between the two groups of subjects (Table S1, Supporting Information). Supplementations comprised of a daily dose of 1080 mg organic fermented or non-fermented garlic extracts in capsule. This dose was calculated based on S-allylcysteine concentration to reduce cardiovascular risk factors.^[22] Our previous report^[23] showed the concentration of S-allylcysteine

to be $0.37 \pm 0.03 \text{ mg g}^{-1}$ in fermented garlic and $0.21 \pm 0.02 \text{ mg g}^{-1}$ in non-fermented garlic extracts. The study protocol was approved by the Institutional Review Board, Nanyang Polytechnic, (Singapore; NYP IRB No.: SCL-2013-003). Written informed consent was received by each subject in accordance with the ethical standards of the responsible committee on human experimentation (IRB) and with the Helsinki Declaration of 1975. On each test day, the subjects attended the clinic after 12 h overnight fast. Blood pressure measurements and whole blood samples were collected at three time-points: baseline, 3 h after supplementation (acute), and 13 weeks later (sub-acute). All samples were processed and stored at -80°C . All 41 subjects completed the study and showed no significant adverse effects.

2.4. Extraction and Analysis of Plasma Fatty Acids

Plasma fatty acids were extracted and quantified using gas chromatography mass spectrometry (GC-MS) method as described by Quehenberger et al. with modification.^[24] Briefly, the plasma samples were thawed on ice and centrifuged at $3000 \times g$ for 5 min at 4°C to remove the denatured proteins. For each sample, 200 μL of plasma was diluted with phosphate-buffered saline and methanol. Nonadecanoic acid (C19:0) ($1 \mu\text{g } \mu\text{L}^{-1}$ in CH_2Cl_2) was also added to the sample as the internal standard. Alkaline hydrolysis by 1N potassium hydroxide in methanol was performed to release the esterified fatty acids. Total fatty acids were collected by liquid-liquid extraction with iso-octane. After the evaporation of solvent, boron trifluoride derivatization was immediately carried out at 60°C . Fatty acid methyl esters (FAMES) were extracted twice with hexane/diethyl ether (80/20:v/v), then dried completely at 37°C under a stream of nitrogen. The samples were re-suspended in iso-octane and analyzed immediately.

The derivatized samples were analyzed by an Agilent 5977A mass selective detector interfaced with an Agilent 7890B gas chromatograph, equipped with an automatic sampler and a computer workstation. The injection port and GC-MS interface were kept at 240 and 250°C , respectively. The mass spectrometer was used in the negative electrical ionization (NEI) mode. Separation of the FAMES were carried out using a SP⁻2560 capillary column ($100 \text{ m} \times 0.25 \text{ mm}$, d_f $0.20 \mu\text{m}$, Sigma-Aldrich, USA). Helium was the carrier gas with a flow rate of 1 mL min^{-1} . Derivatized samples ($1 \mu\text{L}$) were injected into the GC injection port with a split ratio 1:100. The column temperature was maintained at 140°C for 5 min, then increased to 180°C at 8°C min^{-1} , and then raised to 210°C at 4°C min^{-1} , and further increased to 250°C at $20^\circ\text{C min}^{-1}$, then held at 250°C for 10 min. Selected ion monitoring (SIM) and full scan mode were performed to monitor the FAMES ions. Quantitation was achieved by relating the peak area of the individual FAME with the C19:0 internal standard peak. Calibration curves were set up over a concentration range of $2\text{--}400 \mu\text{g mL}^{-1}$ with Supelco 37 Component FAME Mix (Sigma-Aldrich). Each concentration point was determined in triplicate and the regression coefficient ranged between 0.889–0.994.

2.5. Extraction and Analysis of Plasma Lipid Oxidation Products

The plasma samples were prepared and quantified using liquid chromatography quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) method as previously described with modification.^[25] Briefly, the plasma samples were thawed on ice and centrifuged at $3000 \times g$ for 10 min at 4°C to remove the denatured protein. For each sample, 200 μL of plasma was added to 10 mL Folch solution for lipid extraction. After phase separation, the lipid-containing lower phase chloroform was completely dried. The samples were re-suspended with deuterated internal standard mix ($0.1 \text{ ng } \mu\text{L}^{-1}$ in MeOH). Thereafter, 1N potassium hydroxide in methanol was added for alkaline hydrolysis and then 5N hydrochloric acid was added. The oxidized PUFA products were extracted using mixed anionic solid phase extraction (SPE, MAX Waters, USA). In brief, the SPE cartridges were preconditioned with methanol and 20 mM formic acid. After loading the samples, it was washed with 2% ammonium hydroxide and 20 mM formic acid. The final elute was collected with hexane and then hexane/ethanol/acetic acid (v/v/v;70/29.4/0.6). The elute was dried at 37°C under a stream of nitrogen, and then re-suspended in 100 μL methanol and stored at -20°C before analysis.

For oxidized PUFA products, Sciex X500R QTOF System (Sciex Applied Biosystems, MA, USA) consisting of ExionLC AC liquid chromatograph with a C₁₈ column ($150 \times 2.1 \text{ mm}$, $2.6 \mu\text{m}$ particle size, Phenomenex, USA) maintained at 40°C was used for the analysis. The flow rate was set to $300 \mu\text{L min}^{-1}$ and the injection volume was 10 μL . A mobile phase consisting of 0.1% acetic acid in water (A) and 0.1% acetic acid in methanol (B) was used for the LC. The gradient conditions were held at 20% of solvent B for 2 min and from 20 to 98% in 8 min and held for 5 min. The contribution of solvent B was decreased to 20% over 1 min and maintained for another 5 min re-equilibration to initial conditions. The X500R QTOF System was operated at negative electrospray ionization (ESI) mode. The spray voltage was set to -4500 V and nitrogen gas was used as the curtain gas. The ionization chamber temperature was set at 350°C , and ion source gas 1 and 2 were 35 and 45 ps, respectively. Declustering potential (DP) was set to -80 V and collision energy (CE) -10 V in TOF MS. The scan mode was set as multiple reaction monitoring (MRM).

For cholesterol oxidation products (COPs), same LC-QTOF-MS/MS system was used but the column was maintained at 30°C for the analysis. An isocratic mobile phase consisting of methanol/water (90:10 v/v) with 0.1% acetic acid was used and the total run time was 10 min. The X500R QTOF System was operated at positive ESI mode. The spray voltage was set to $+3000 \text{ V}$ and nitrogen gas was used as the curtain gas. The ionization chamber temperature was set at 350°C , and ion source gas 1 and 2 were 35 and 45 psi, respectively. DP and CE in TOF MS were 100 and 10 V, respectively. The scan mode was set as MRM.

All data acquired by X500R QTOF System were analyzed by Sciex Operating System (version 1.2.0.4122). Quantitation of each analyte was determined by relating the peak area with its corresponding deuterated internal standard peak. For the analytes without its corresponding deuterated internal

standards, quantitation was performed by using the following deuterated internal standards, 7 β -hydroxycholesterol-d₇, 5(S)-HETE-d₈, 15-F_{2t}-IsoP-d₄, and 4-(RS)-4-F_{4t}-NeuroP-d₄. In this study, a total of 49 oxidized PUFA products and five COPs were determined based on the mass ion (*m/z*) presented in Table S2, Supporting Information. As an example, a typical mass spectrum of dihomog-IsoPs is presented in Figure S1, Supporting Information. The *m/z* are extracted from the standards that are available commercially and by those synthesized by IBMM, and the others (12-F_{2t}-IsoP, isofurans and some F_{3t}-IsoPs) from LIPID MAPS[®] database and literature reports.

2.6. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 7.04 for Windows (GraphPad Software, CA). All values are expressed as mean \pm SD. Unpaired Student's *t*-test was performed to compare the basal characteristics of mild hypercholesterolemia subjects. Paired one-way ANOVA was performed for clinical parameters, plasma fatty acids, and cholesterol and lipid oxidation products at different time points. A heat map was constructed to compare the clustering of fatty acid and oxidized PUFA levels. A *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Antioxidant Capacity

The enzymatic and non-enzymatic antioxidant capacity of the fermented and non-fermented garlic extracts were assessed in *in vitro*. Both garlic extracts showed strong free radical scavenging ability from 50 $\mu\text{g L}^{-1}$ concentration and subsequently had a dose-dependent relationship. The fermented garlic extract had higher free radical scavenging capacity than non-fermented one.

On the other hand, the non-fermented garlic extract showed a strong enzymatic antioxidant capacity than fermented ones. Even at lower concentration (20 $\mu\text{g/L}$), non-fermented garlic extract was more effective in up-regulating the enzyme activity of SOD enzyme than fermented garlic extract (**Figure 1**). Non-fermented garlic extract had a dose-dependent relationship from 20 $\mu\text{g L}^{-1}$ and fermented garlic extract from 50 $\mu\text{g L}^{-1}$ onward in SOD enzyme uptake.

3.2. Effect of Garlic Supplementation on Fatty Acid Profile

All subjects recruited in this study showed comparable baseline levels of plasma fatty acids and was not significantly different between those supplemented with non-fermented garlic and fermented garlic extracts. Further, there was no gender difference within and between the groups during the supplementation period. Out of 37 fatty acids measured, 18 were detectable and subsequently quantified (**Table 1**). Of the saturated fatty acids, only arachidic acid (C20:0) significantly increased ($p < 0.05$) after 13 weeks of non-fermented garlic intake. However, palmoic acid (C16:1) of the monounsaturated

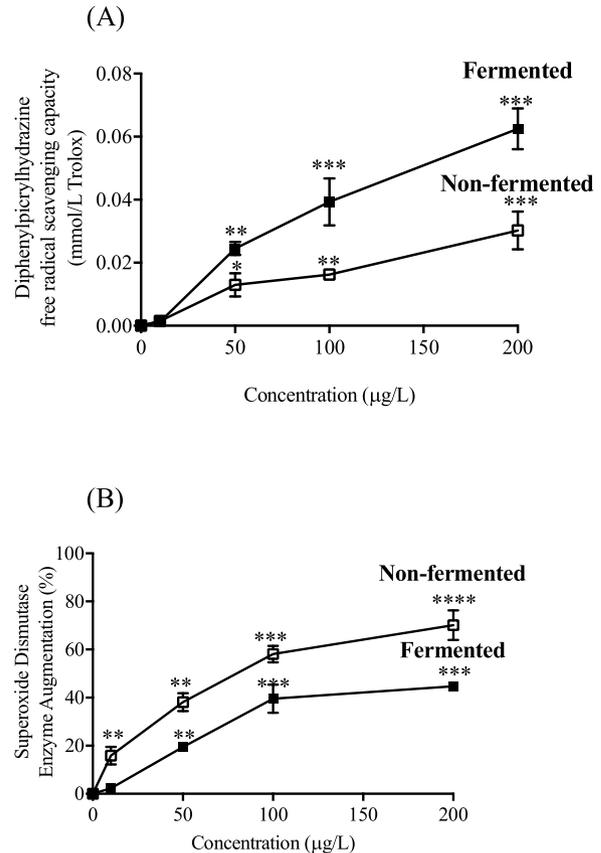


Figure 1. Antioxidant properties of fermented and non-fermented garlic extracts. A) free-radical scavenging capacity and (B) superoxide dismutase up-regulation. Data is presented as mean \pm SD. Change in antioxidant effect from baseline (0 $\mu\text{g L}^{-1}$) was determined by AUC. Significant differences are annotated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ compared to baseline.

fatty acid decreased ($p < 0.05$) 13 weeks of non-fermented garlic intake while oleic acid (C18:1) increased ($p < 0.05$) temporarily after 3 h of fermented garlic intake.

Among the PUFA measured, only the level of α -linolenic acid (ALA, C18:3n-3) was elevated ($p < 0.05$) at 13 weeks for both garlic extract intake (**Figure 2**). All the other fatty acids namely linoleic acid (C18:2n-6), eicosdienoic acid (C20:2 n-6), dihomog- γ -linolenic acid (C20:3n-6), AA, AdA, eicosatrienoic acid (C20:3 n-3), EPA, docosapentaenoic acid (C22:5n-3), and DHA showed no change after both garlic intake and remained at baseline levels throughout the study.

3.3. Effect of Garlic Supplementation on Oxidative Stress

Out of 49 oxidized PUFA products measured, 27 were in detectable range (15 from omega-6 PUFA and 12 from omega-3 PUFA) and subsequently quantified (Tables 2 and 3). The remaining 22 oxidized PUFA products including 7-, 10-, 13-, 16-, and 20-HDHA Resolvin E1 and D1, Neuro-Protectin D1, Protectin DX, 8-F_{2t}- and 12-F_{2t}-IsoP, 11-, 12-, and 15-F_{3t}-IsoP, 7-, 11-, 13-, 17-, and 20-F_{4t}-IsoP, isofurans, 4(RS)-ST- Δ^8 -8-NeuroF

Table 1. Plasma fatty acids in mild hypercholesterolemia subjects after garlic supplementation.

Fatty acid [mg mL ⁻¹]	Time		
	0 h	3 h	13 weeks
Palmitic acid (C16:0)			
NFG	240.86 ± 68.33 ^a	251.93 ± 76.54 ^a	221.43 ± 55.66 ^a
FG	254.52 ± 71.53 ^a	281.03 ± 79.05 ^a	250.02 ± 54.04 ^a
Margaric acid (C17:0)			
NFG	7.93 ± 3.95 ^a	5.43 ± 1.95 ^a	5.54 ± 1.61 ^a
FG	4.62 ± 0.71 ^a	6.04 ± 1.76 ^a	5.84 ± 2.25 ^a
Stearic acid (C18:0)			
NFG	307.24 ± 166.23 ^a	318.24 ± 166.43 ^a	272.53 ± 48.31 ^a
FG	282.64 ± 100.61 ^a	309.91 ± 106.4 ^a	285.64 ± 58.86 ^a
Arachidic acid (C20:0)			
NFG	2.74 ± 2.21 ^{ab}	2.44 ± 2.17 ^a	4.34 ± 2.56 ^b
FG	4.23 ± 3.44 ^a	3.34 ± 3.13 ^a	5.53 ± 4.51 ^a
Palmoleic acid (C16:1)			
NFG	10.53 ± 8.03 ^a	13.24 ± 6.84 ^a	4.04 ± 4.63 ^b
FG	11.31 ± 9.84 ^a	10.51 ± 10.63 ^a	6.84 ± 7.21 ^a
Oleic acid (C18:1)			
NFG	354.27 ± 146.13 ^a	324.53 ± 165.94 ^a	323.64 ± 95.56 ^a
FG	346.09 ± 104.64 ^a	405.14 ± 134.42 ^b	337.45 ± 81.52 ^{ab}
Gondoic acid (C20:1)			
NFG	13.02 ± 13.04 ^a	13.63 ± 9.04 ^a	10.44 ± 8.52 ^a
FG	12.12 ± 8.67 ^a	8.72 ± 8.44 ^a	8.52 ± 10.04 ^a
Erucic acid (C22:1)			
NFG	1.33 ± 1.36 ^a	1.33 ± 1.37 ^a	0.92 ± 1.24 ^a
FG	1.34 ± 1.32 ^a	1.84 ± 1.84 ^a	1.69 ± 1.62 ^a
Linoleic acid (C18:2n-6)			
NFG	327.23 ± 72.52 ^a	340.04 ± 106.68 ^a	314.79 ± 101.52 ^a
FG	334.44 ± 82.62 ^a	336.32 ± 80.63 ^a	332.73 ± 68.06 ^a
Eicosadienoic acid (C20:2n-6)			
NFG	18.94 ± 12.11 ^a	21.73 ± 10.14 ^a	16.22 ± 10.13 ^a
FG	17.43 ± 10.43 ^a	18.02 ± 9.55 ^a	22.63 ± 9.14 ^a
Dihomo-g-linolenic acid (C20:3n-6)			
NFG	11.85 ± 6.32 ^a	12.13 ± 7.11 ^a	11.01 ± 4.34 ^a
FG	10.24 ± 5.23 ^a	11.32 ± 7.43 ^a	12.15 ± 5.91 ^a
Arachidonic acid (C20:4n-6)			
NFG	226.76 ± 101.11 ^a	235.25 ± 112.83 ^a	225.22 ± 65.48 ^a
FG	184.32 ± 65.83 ^a	191.9 ± 89.3 ^a	216.5 ± 64.1 ^a
Adrenic acid (C22:4n-6)			
NFG	2.62 ± 2.14 ^a	3.24 ± 2.93 ^a	2.53 ± 1.72 ^a
FG	1.73 ± 1.43 ^a	1.36 ± 1.01 ^a	2.14 ± 1.06 ^a
Eicosatrienoic acid (C20:3n-3)			
NFG	9.43 ± 6.84 ^a	11.0 ± 7.31 ^a	8.95 ± 5.47 ^a
FG	9.32 ± 5.35 ^a	8.4 ± 6.02 ^a	11.15 ± 6.84 ^a
Eicosapentaenoic acid (C20:5n-3)			

(Continued)

Table 1. (Continued)

Fatty acid [mg mL ⁻¹]	Time		
	0 h	3 h	13 weeks
NFG	8.78 ± 6.69 ^a	8.64 ± 6.17 ^a	6.83 ± 5.76 ^a
FG	7.94 ± 4.87 ^a	6.23 ± 3.52 ^a	8.34 ± 6.14 ^a
Docosapentaenoic acid (C22:5n-3)			
NFG	4.63 ± 1.93 ^a	5.34 ± 3.14 ^a	4.76 ± 2.34 ^a
FG	3.75 ± 1.52 ^a	4.43 ± 2.63 ^a	4.45 ± 2.23 ^a
Docosahexaenoic acid (C22:6n-3)			
NFG	32.34 ± 18.63 ^a	34.83 ± 23.86 ^a	34.34 ± 22.46 ^a
FG	45.85 ± 15.52 ^a	50.14 ± 16.23 ^a	59.02 ± 18.14 ^a

Values are expressed as mean ± SD, *n* = 20 (FG) and *n* = 21 (NFG). Different alphabets indicate a significant difference by paired one-way ANOVA (*p* < 0.05). NFG, non-fermented garlic; FG, fermented garlic.

and neurofurans were below our limit of detection (<1 pg) therefore were not quantitated. There was no gender difference within and between the groups during the supplementation period.

In this study, only non-fermented garlic extract intake altered the systemic PUFA oxidation. It was effective in reducing products of AA oxidized enzymatically, especially the levels of LOX mediated 5-HETE (*p* < 0.05) after 13 weeks intake, and 12-HETE after 3 h intake (*p* < 0.05) where the reduced effect remained even after 13 weeks (*p* < 0.01). It also lowered the levels of cytochrome P450 mediated 9-HETE (*p* < 0.01) and 11-HETE (*p* < 0.05) after 13 weeks of intake (Figure 3). Surprisingly, levels of 17-dihomo-F_{2t}-isofuran derived from AdA increased (*p* < 0.05) by approximately seven and twofolds after intake of non-fermented and fermented garlic extracts, respectively at the end of 13 weeks.

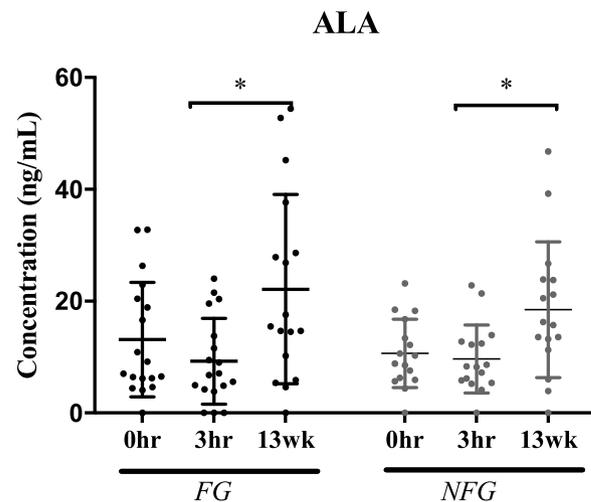


Figure 2. Plasma α -linolenic acid (C18:3n-3) profile in mild hypercholesterolemia subjects after garlic supplementation. **p* < 0.05 and **p* < 0.01 by paired one-way ANOVA. NFG, non-fermented garlic; FG, fermented garlic.

The level of all the other oxidized PUFA products namely 5-F_{2t}- and 15-F_{2t}-IsoP, PGF_{2a}; 8-, 15-, and 20-HETE, 4-F_{3t}-IsoP; 7- and 17-F_{2t}-Dihomo-IsoP, 7-(RS)-ST-Δ⁸-11-Dihomo-IsoF; 5-, 8-,

Table 2. Omega-6 oxidized polyunsaturated fatty acid products in plasma of mild hypercholesterolemia subjects after garlic supplementation.

	Time		
	0 h	3 h	13 weeks
Derived from AA [ng mL ⁻¹]			
5-F _{2t} -IsoP			
NFG	25.97 ± 23.08 ^a	31.03 ± 27.82 ^a	41.65 ± 37.14 ^a
FG	26.45 ± 24.56 ^a	25.84 ± 26.43 ^a	14.83 ± 15.13 ^a
15-F _{2t} -IsoP			
NFG	26.26 ± 6.84 ^a	25.65 ± 6.44 ^a	23.26 ± 3.93 ^a
FG	22.23 ± 9.35 ^a	22.02 ± 8.47 ^a	20.24 ± 6.15 ^a
PGF _{2a}			
NFG	4.96 ± 4.66 ^a	3.73 ± 3.03 ^a	4.92 ± 4.24 ^a
FG	4.46 ± 3.84 ^a	5.45 ± 5.04 ^a	4.24 ± 4.83 ^a
8-HETE			
NFG	16.11 ± 7.82 ^a	15.95 ± 6.34 ^a	14.73 ± 7.33 ^a
FG	15.15 ± 5.55 ^a	15.46 ± 6.27 ^a	14.27 ± 5.07 ^a
15-HETE			
NFG	63.15 ± 46.24 ^a	50.65 ± 27.43 ^a	73.66 ± 47.05 ^a
FG	48.36 ± 25.71 ^a	47.63 ± 24.74 ^a	52.65 ± 35.47 ^a
20-HETE			
NFG	5.25 ± 3.24 ^a	5.03 ± 4.25 ^a	3.04 ± 1.65 ^a
FG	6.64 ± 4.63 ^a	6.61 ± 4.54 ^a	5.02 ± 3.14 ^a
Derived from Omega-6 DPA [ng mL ⁻¹]			
4-F _{3t} -IsoP			
NFG	13.22 ± 16.01 ^a	8.21 ± 7.32 ^a	16.23 ± 13.21 ^a
FG	7.42 ± 8.33 ^a	15.35 ± 15.82 ^a	5.74 ± 7.22 ^a
Derived from AdA [ng mL ⁻¹]			
7-F _{2t} -Dihomo-IsoP			
NFG	10.66 ± 7.63 ^a	8.19 ± 7.24 ^a	9.82 ± 9.56 ^a
FG	8.54 ± 7.04 ^a	4.57 ± 2.45 ^a	9.75 ± 9.45 ^a
17-F _{2t} -Dihomo-IsoP			
NFG	3.75 ± 4.15 ^a	2.83 ± 2.56 ^a	3.53 ± 3.32 ^a
FG	2.22 ± 2.13 ^a	1.62 ± 1.71 ^a	1.42 ± 1.23 ^a
7-(RS)-ST-Δ ⁸ -11-Dihomo-IsoF			
NFG	48.68 ± 38.06 ^a	41.63 ± 35.46 ^a	41.65 ± 36.05 ^a
FG	29.84 ± 20.77 ^a	30.27 ± 19.31 ^a	17.24 ± 12.23 ^a
17-(RS)-10-epi-SC-Δ ¹⁵ -11-Dihomo-IsoF			
NFG	5.47 ± 7.15 ^a	1.02 ± 1.54 ^a	33.33 ± 30.52 ^b
FG	8.78 ± 12.43 ^{ab}	6.53 ± 7.32 ^a	20.82 ± 18.85 ^b

Values are expressed as mean ± SD, n=20 (FG) and n=21 (NFG). Different alphabets indicate a significant difference by paired one-way ANOVA (p < 0.05). NFG, non-fermented garlic; FG, fermented garlic; AA, arachidonic acid; DPA, docosapentaenoic acid; AdA, adrenic acid; IsoP, isoprostane; IsoF, isofuran; HETE, hydroxyeicosatetraenoic acid.

Table 3. Omega-3 oxidized polyunsaturated fatty acid products in plasma of mild hypercholesterolemia subjects after garlic supplementation.

	Time		
	0 h	3 h	13 weeks
Derived from EPA [ng mL ⁻¹]			
5-F _{3t} -IsoP			
NFG	17.01 ± 17.84 ^a	13.56 ± 15.41 ^a	14.72 ± 11.73 ^a
FG	11.02 ± 13.45 ^a	12.52 ± 11.94 ^a	4.81 ± 5.51 ^a
8-F _{3t} -IsoP			
NFG	23.71 ± 17.73 ^a	14.12 ± 8.31 ^a	25.93 ± 25.32 ^a
FG	23.01 ± 21.72 ^a	20.02 ± 14.65 ^a	15.31 ± 13.02 ^a
18-F _{3t} -IsoP			
NFG	21.31 ± 19.02 ^a	20.43 ± 15.65 ^a	27.02 ± 22.83 ^a
FG	19.22 ± 13.05 ^a	22.12 ± 15.36 ^a	10.49 ± 10.10 ^a
Derived from Omega-3 DPA [ng mL ⁻¹]			
14-F _{3t} -IsoP			
NFG	3.41 ± 4.16 ^a	1.22 ± 1.72 ^a	3.13 ± 3.76 ^a
FG	2.72 ± 3.54 ^a	5.45 ± 5.83 ^a	1.72 ± 2.05 ^a
Derived from DHA [ng mL ⁻¹]			
4-F _{4t} -NeuroP			
NFG	34.51 ± 19.54 ^a	32.84 ± 20.63 ^a	28.82 ± 21.53 ^a
FG	28.83 ± 13.92 ^a	32.03 ± 22.85 ^a	27.01 ± 23.04 ^a
10-F _{4t} -NeuroP			
NFG	33.44 ± 23.02 ^a	30.12 ± 15.15 ^a	54.02 ± 33.92 ^a
FG	38.34 ± 27.73 ^a	35.72 ± 26.06 ^a	44.36 ± 22.53 ^a
14-F _{4t} -NeuroP			
NFG	351.22 ± 288.84 ^a	234.23 ± 144.47 ^a	395.54 ± 297.58 ^a
FG	352.92 ± 299.54 ^a	355.02 ± 250.33 ^a	332.98 ± 332.95 ^a
4-HDHA			
NFG	6.22 ± 3.45 ^a	6.62 ± 4.16 ^a	5.45 ± 2.93 ^a
FG	8.26 ± 5.91 ^a	8.04 ± 4.22 ^a	5.33 ± 4.75 ^a
8-HDHA			
NFG	6.51 ± 3.65 ^a	7.04 ± 3.97 ^a	5.66 ± 2.63 ^a
FG	8.23 ± 5.94 ^a	8.02 ± 4.21 ^a	5.95 ± 4.61 ^a
11-HDHA			
NFG	2.64 ± 1.42 ^a	3.04 ± 2.2 ^a	1.92 ± 1.12 ^a
FG	2.73 ± 2.67 ^a	3.02 ± 2.04 ^a	2.03 ± 1.53 ^a
14-HDHA			
NFG	10.64 ± 8.12 ^a	11.12 ± 12.52 ^a	5.34 ± 5.02 ^a
FG	11.72 ± 11.35 ^a	28.13 ± 26.42 ^a	4.14 ± 5.95 ^a
17-HDHA			
NFG	6.52 ± 4.69 ^a	7.64 ± 7.14 ^a	6.12 ± 4.73 ^a
FG	7.26 ± 4.95 ^a	9.53 ± 10.22 ^a	7.82 ± 7.94 ^a

Values are expressed as mean ± SD, n=20 (FG) and n=21 (NFG). Different alphabets indicate a significant difference by paired one-way ANOVA (p < 0.05). NFG, non-fermented garlic; FG, fermented garlic; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; IsoP, isoprostane; NeuroP, neuroprostane; HDHA, hydroxy-DHA.

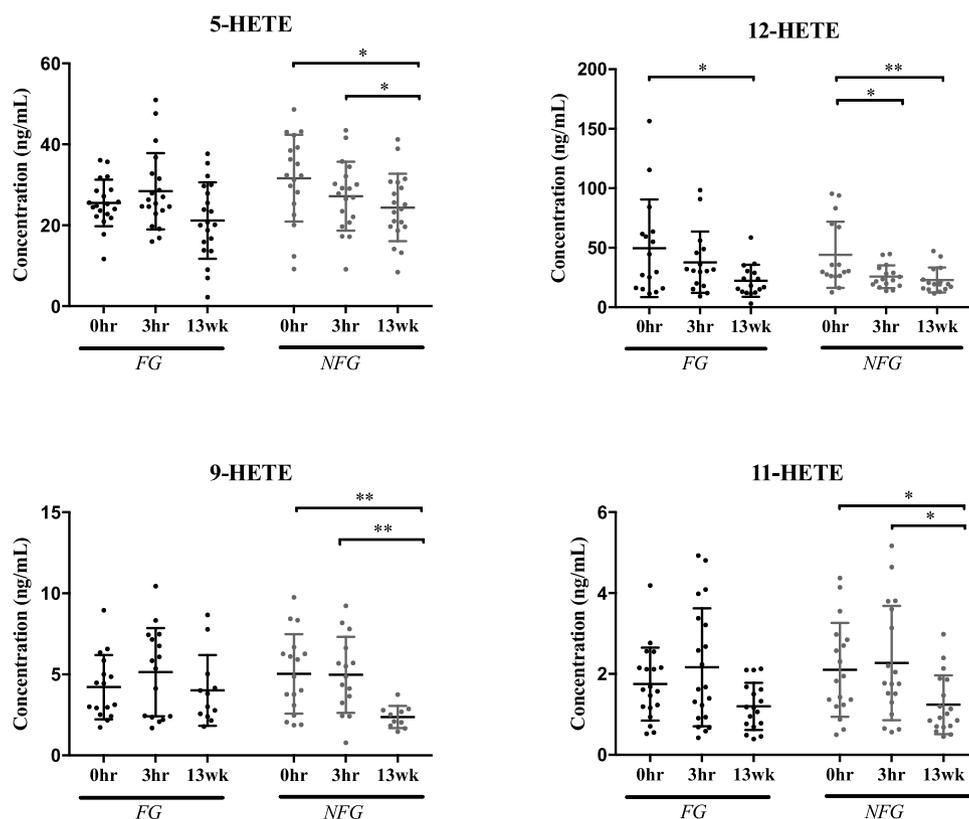


Figure 3. Plasma polyunsaturated fatty acids oxidation products mediated by lipoxygenase (5-HETE and 12-HETE) and cytochrome P450 (9-HETE and 11-HETE) in mild hypercholesterolemia subjects after garlic supplementation. * $p < 0.05$ and ** $p < 0.01$ by paired one-way ANOVA. NFG, non-fermented garlic; FG, fermented garlic; HETE, hydroxyeicosatetraenoic acid.

14-, and 18- F_{3t} -IsoP; 4-, 10-, and 14- F_{4t} -NeuroP; and 4-, 8-, 11-, 14-, and 17-HDHA determined did not alter throughout the supplementation period and was comparable to the baseline levels. In particular, 15- F_{2t} -IsoP derived from AA, a known *in vivo* oxidative stress biomarker were not altered by garlic intake. It should also be noted, none of the enzymatic or non-enzymatic oxidized products of omega-3 PUFA were affected by garlic intake.

Being hypercholesterolemia subjects, it was anticipated that garlic would have an effect on cholesterol oxidation. However, plasma COPs did not show any significant change before and after supplementation. Only 3 (27-hydroxycholesterol, 7 β -hydroxycholesterol, and 7-ketocholesterol) COPs were within the detectable range and thus quantified (Table 4) while 7 α -hydroxycholesterol and 24-hydroxycholesterol were below the detectable range (<1 pg). Nevertheless, the levels of 27-hydroxycholesterol, 7 β -hydroxycholesterol, and 7-ketocholesterol did not show any modification after 13 weeks of supplementation for both groups.

The levels of PUFA and oxidized lipid products were compared to the baseline levels and plotted on a heat map (Figure 4). As noted in our aforementioned results arachidic acid, ALA and 17-dihomo- F_{2t} -isofuran were upregulated (lighter shade of red) and palmoic acid and 5-, 9-, 11-, 12-HETE were down-regulated (lighter shade of green) distinctively in both supplemented groups at 13 weeks. However, clustering of non-

enzymatic PUFA oxidation was also observed, where 4- F_{3t} -IsoP derived from omega-6 docosapentaenoic acid and 14- F_{3t} -IsoP from omega-3 DPA were strongly upregulated at 3 h but not at 13 weeks in the group supplemented with fermented garlic

Table 4. Plasma cholesterol oxidation products (COPs) in mild hypercholesterolemia subjects after garlic supplementation.

COPs [ng mL ⁻¹]	Time		
	0 h	3 h	13 weeks
27-hydroxycholesterol			
NFG	10.62 ± 7.61 ^a	8.13 ± 7.22 ^a	9.85 ± 9.54 ^a
FG	8.54 ± 7.02 ^a	4.55 ± 2.46 ^a	9.78 ± 9.48 ^a
7 β -hydroxycholesterol			
NFG	3.71 ± 4.11 ^a	2.83 ± 2.54 ^a	3.51 ± 3.36 ^a
FG	2.24 ± 2.10 ^a	1.69 ± 1.71 ^a	1.47 ± 1.21 ^a
7-ketocholesterol			
NFG	48.61 ± 38.07 ^a	41.61 ± 35.47 ^a	41.63 ± 36.06 ^a
FG	29.82 ± 20.71 ^a	30.24 ± 19.30 ^a	17.29 ± 12.23 ^a

Values are expressed as mean ± SD, $n = 20$ (FG) and $n = 21$ (NFG). Different alphabets indicate a significant difference by paired one-way ANOVA ($p < 0.05$). NFG, non-fermented garlic; FG, fermented garlic.

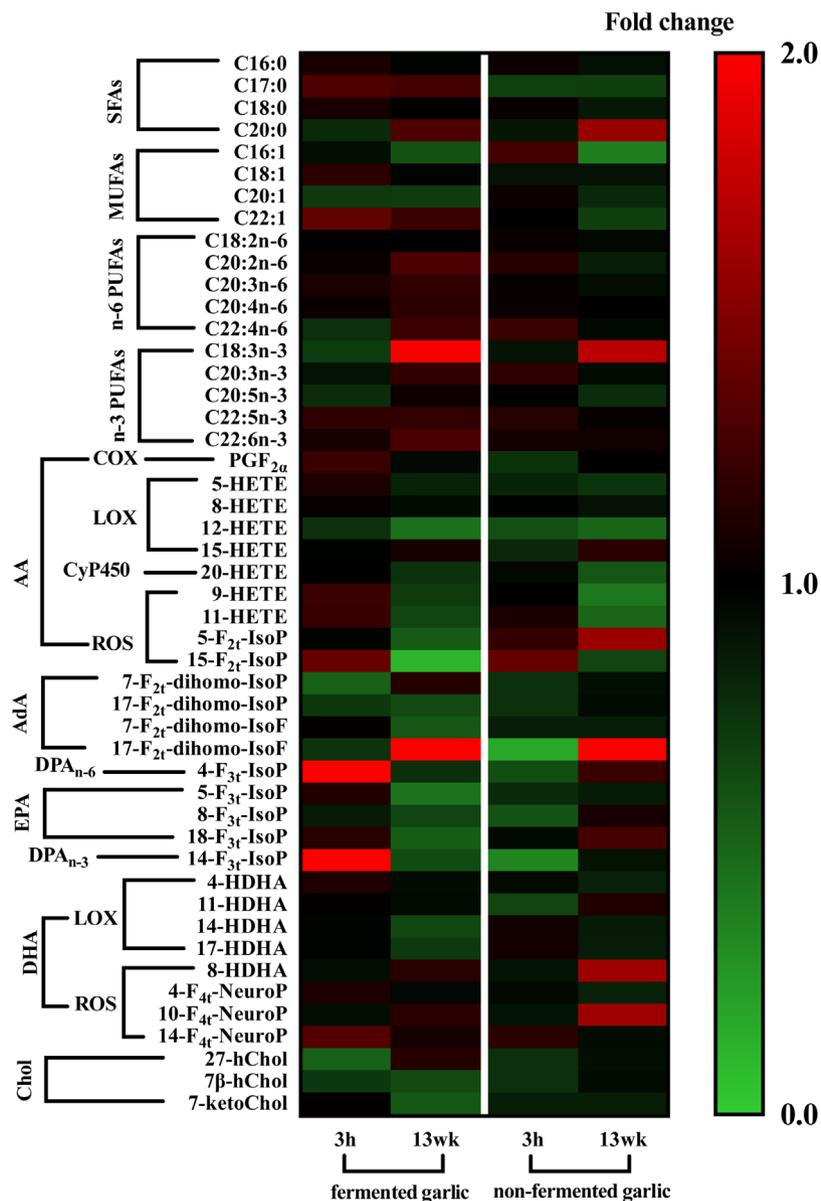


Figure 4. Heat map of plasma fatty acids, polyunsaturated fatty acid oxidation products and cholesterol oxidation products in mild hypercholesterolemia subjects after garlic supplementation. The changes are clustered according to the fatty acid type and the precursors of the oxidized lipids. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Chol, cholesterol; AA, arachidonic acid; AdA, adrenic acid; DPA, docosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HDHA, hydroxy-DHA; hChol, hydroxycholesterol; IsoP, isoprostane; IsoF, isofuran; NeuroP, neuroprostane.

whereas at 13 weeks, non-fermented garlic group had enhanced 8-HDHA and 10-F_{4t}-NeuroP derived from DHA.

4. Discussion

Several studies have been conducted on health benefits of garlic supplements for hypercholesterolemia^[26–29] but the findings have been inconclusive. In the current study, we investigated the role of garlic supplements in regulating lipid and cholesterol oxidation, and oxidative stress. A positive

correlation between plasma lipid levels and lipid peroxide concentrations was reported and suggests that garlic intake regulates *in vivo* lipid peroxidation by modulating plasma lipid levels including fatty acids.^[30] To date, few reports are available on the effects of garlic supplementation in human systemics, and only a handful focused on plasma fatty acids. In an animal study, garlic supplementation was found to increase whole body EPA and DHA levels in juvenile Sturgeon fish.^[31] In essence, such study has not been properly studied in humans on the regulation of fatty acid, including the precursor of pro-inflammatory eicosanoids from omega-6

PUFAs and those of anti-inflammatory omega-3 PUFAs. Contrary to the findings in animal model,^[31] fermented and non-fermented garlic supplements had no effect on plasma EPA and DHA. The difference perhaps is due to dissimilar rate of PUFA metabolism between human and fish or the dose of the garlic extract may be insufficient to instigate EPA and DHA synthesis. Moreover, it is long known fatty acid metabolism in hypercholesterolemia is atypical and the subjects in the study may have limited ability to synthesize long-chain PUFA such as EPA and DHA.^[32,33]

Nevertheless, plasma ALA was augmented after garlic intake, and apparent that it was not metabolized to EPA and DHA. Human body is relatively inefficient in promoting fatty acid desaturation and elongation from ALA or linoleic acid compared to plants and marine animals. Moreover, a higher plasma level of ALA is associated to reduced CVDs risk in hypercholesterolemia patients by lowering plasma lipids, and inhibiting vascular inflammation and endothelial activation.^[34] We also showed systemic arachidic acid was upregulated and palmitoleic acid down regulated after non-fermented garlic intake. Dietary palmitoleic acid is suggested to act like saturated fatty acid and increase serum low-density lipoproteins thus, elevation by garlic intake to an extent may be disadvantageous for hypercholesterolemia patients.^[35]

Polyphenols from garlic extracts are natural antioxidants and the antioxidant activity of garlic in the human body may alleviate oxidative stress-related symptoms such as hypertension.^[30] Fermented garlic is proposed to have stronger antioxidant capacity due to higher polyphenol levels than non-fermented ones.^[2] Despite having higher antioxidant capacity in vitro, our observation did not suggest fermented garlic to be a stronger in vivo antioxidant in mild hypercholesterolemia. Products of non-enzymatic PUFA oxidation are measured in biological specimens to assess oxidative stress, in particular 15-F_{2t}-IsoP derived from AA is widely used to determine in vivo oxidative stress. Previous studies reported garlic supplementation to reduce plasma and urine levels of 15-F_{2t}-IsoP. However, the dose supplemented in current study (1.04 g garlic extract day⁻¹) suggest to be inadequate for an impactful effect where Durak et al.^[38] found about 1 mL of garlic extract per kilogram of body weight per day (10 g garlic/day) supplementation reduced oxidative stress in subjects with high blood cholesterol (>5.98 mmol L⁻¹).^[23,36]

To the best of our knowledge, this study is the first to investigate the effects of garlic extracts on a panel of oxidized PUFA products and COPs by applying a robust targeted lipidomics approach. Contrast with previous findings,^[23,36] supplementation with garlic extracts for 13 weeks did not reduce 15-F_{2t}-IsoP in our observation. This difference between our study and previous reports may be due to the type of analytical method applied (GC-MS vs. LC-MS/MS) where the use of MS/MS is valuable in detecting target of interest in complex matrices such as plasma at low levels; by monitoring precursor and daughter pair ions additionally confirms the target identity among other analytes that might share the same precursor mass ion.^[37]

In the heat map clustering, we identified 8-HDHA and 10-F_{4t}-Neuroprostane (10-F_{4t}-NeuroP) to be upregulated by non-fermented garlic intake. Insofar, it is known 10-F_{4t}-NeuroP is

associated to neurological diseases.^[39] In addition, we showed 17-dihomo-F_{2t}-isofuran derived from AdA to be augmented after 13 weeks by both supplemental groups. Isofuranoids are products released from PUFA under high oxygen tension (above 21% or 760 mmHg). Although its role in in vivo is poorly understood, 17-dihomo-F_{2t}-isofuran was found to be predominant among the isofuranoids in pig brain tissue.^[19] Whether an increase in dihydro-F_{2t}-isofuran after garlic supplementation is related to potential neural damage is therefore unclear and require further investigation. Nonetheless, AdA is the key PUFA found in the brain white matter and oxidative damage (<21% oxygen tension) resulting in dihydro-F_{2t}-isoprostanates that is suggested to be an early potential biomarker for neural damages.^[40]

PUFA is conjugated to phospholipid that contributes to the fluidity of the biological membranes. The oxidation of AA by LOX and cyclooxygenase (COX) enzymes leads to the production of an array of eicosanoids and are critical for mediating inflammatory responses. The regiospecific LOX include 5-, 8-, 12-, and 15-LOX that catalyze the respective addition of peroxide group on carbon 5, 8, 12, and 15 of the AA; and give rise to 5-, 8-, 12-, and 15-hydroxyeicosatetraenoic acids (HETEs), respectively. Together with COX-mediated eicosanoids (e.g., prostaglandins and thromboxane), 5-HETE, 12-HETE, and 15-HETE are implicated in diseases involving inflammation such as atherosclerosis. They are shown to be potent platelet activators, leading to aggregation of platelet-leukocyte aggregate that recruits immune cells, which in turn exacerbates the inflammatory responses on the blood vessels.^[41] HETEs also takes part in cancer development and progression, where 5-HETE is shown to proliferate pancreatic adenocarcinoma by participating in certain intracellular signaling pathways.^[42] Also 5- and 12-HETEs are promoted during the mobility of prostate cancer cells, suggesting their role in cancer metastasis.^[43] Leukotriene, another series of eicosanoid produced by 5-LOX in mast cells and basophils, induced the development of allergic diseases such as asthma, allergic rhinitis, and inflammatory arthritis.^[44]

The current study showed after non-fermented garlic supplementation, a repressing effect on pro-inflammatory LOX-mediated 5-HETE and 12-HETE were observed and agrees with previous studies.^[45] For instance, Yoo et al.^[45] reported that 5-LOX activity to be downregulated in allergic cells after pre-incubation with BG10, a phenolic- and flavonoid-rich fraction derived from ethyl acetate extract of garlic. Furthermore, BG10 inhibited the phosphorylation of cytosolic phospholipase A₂, an upstream enzyme for the production of the eicosanoid array.^[45] The authors concluded that the phenolic and flavonoid components in garlic are capable of inhibiting eicosanoid production and hence resolve inflammation. Another study investigated the antiplatelet properties of ajoene, an organosulphur compound found in garlic. It showed that ajoene reduced 12-LOX activity and 12-HETE level, and subsequently platelet activity, suggesting that ajoene exerted antiplatelet effect by mediating eicosanoid production.^[46] Interestingly, one study shed light on the LOX- and COX-inhibitory efficacy of the vast array of organosulfur compounds in raw and fermented garlic. Nordihydroguaiaretic acid (NDGA) was found to be the most potent in inhibiting LOX, and ajoene was capable of inhibiting both LOX and COX in mouse skin tumor cells.^[47]

The plasma levels of 9-HETE and 11-HETE were also found to be suppressed by non-fermented garlic supplementation. However, the ambiguous biosynthesis of 9-HETE and 11-HETE involves both cytochrome P450 dependent oxygenation^[48] and lipid peroxidation.^[49] Insofar, the role of 9- and 11-HETE is suggested to involve in generic inflammation,^[50] atherosclerosis,^[50] ischemia,^[51] and myocardial infarction,^[52] which are conditions highly related to the risk of disease development in patients with hypercholesterolemia.

The plasma levels of 27-hydroxycholesterol, 7 β -hydroxycholesterol, and 7-ketocholesterol did not show any modification upon garlic supplementation. They are of great concern in hypercholesteremic condition because of their association with atherosclerosis.^[53,54] The biosynthesis of these COPs is dependent on cytochrome P450 ω hydroxylase and epoxygenase activity,^[55] which involves multiple regulatory mechanisms. Therefore, it appears 13 weeks of garlic supplementation was insufficient to affect the activities of P450s in mild hypercholesterolemia.

The patients recruited in the present study were mild hypercholesteremic with blood cholesterol level slightly higher than normal range for Asians. Hypercholesterolemia has been shown to be associated with atherosclerosis^[56] and studies demonstrated that in addition to promoting platelet biogenesis from bone marrows, high blood LDL could also activate leukocytes to form platelet-leukocyte aggregate on the blood vessel walls.^[57,58] Although a significant change in plasma cholesterol^[57,58] and COPs levels were not observed after acute and prolonged supplementation with non-fermented and fermented garlic in mild hypercholesterolemia, they did show significant reduction on HETEs content after non-fermented garlic intake, suggesting that there is an alternative pathway which garlic could potentially exert its anti-atherogenic effect induced at least in part by hypercholesterolemia. The results suggest that, regardless to antioxidant capacity and compared to fermented garlic, non-fermented garlic exerts health protective effect in a target-specific manner, possibly by inhibiting oxidative enzymes.

5. Conclusion

The current study indicates that garlic supplements may provide additional health benefits to mild hypercholesterolemia through plasma ALA enrichment and AA-LOX inhibition. The suppressing effect of 5-LOX and 12-LOX activity of the garlic, hypothetically by the phenolic compounds in garlic resolves systemic inflammation that potentially lead to complicated symptoms like CVD. The enriched plasma ALA in mild hypercholesterolemia by the garlic intake further exacerbate the anti-inflammatory effect.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Abbreviations

AA, arachidonic acid; AdA, adrenic acid; CHD, coronary heart diseases; COPs, cholesterol oxidation products; COX, cyclooxygenases; DHA,

docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FG, fermented garlic; HDHA, hydroxydocosahexaenoic acid; HETE, hydroxyeicosatetraenoic acid; IsoF, isofuran; IsoP, isoprostane; LOX, lipoxygenases; NeuroP, neuroprostane; NFG, non-fermented garlic; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species.

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H.H.L, Y.F.Y., J.C.Y.L., and W.M.L conceived, designed and performed the experiments; Y.Y.L. and J.C.Y.L. assisted in technical support for the mass spectrometry analysis; C.O., J.M.G., and T.D. synthesized standards for the study; H.H.L. and J.C.Y.L. wrote the paper. The authors thank Defu Foodstuff (Singapore) Pte Ltd., for their invaluable advice and support. The authors are grateful to Ching Yu Wu, Chun Ho Cheung, Hau Man Mak, and Kei Fung Wong for their technical support with the LC-MS/MS analysis.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

cholesterol oxidation products, garlic, HETEs, isoprostanes, oxidative stress

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