別紙様式（Ｖ）－1

機能性の科学的根拠に関する点検表

１．製品概要

商品名	日清健康オイル アマニプラス
機能性関与成分名	α－リノレン酸
表示しようとする機能性	本品は、α-リノレン酸を含んでおり、血圧が高めの方に適した機能を持つ食用油です。

２．科学的根拠

【臨床試験及び研究レビュー共通事項】

□（主観的な指標によってのみ評価可能な機能性を表示しようとする場合）当該指標は日本人において妥当性が得られ、かつ、当該分野において学術的に広くコンセンサスが得られたものである。

☑（最終製品を用いた臨床試験又は研究レビューにおいて、実際に販売しようとする製品の試作品を用いて評価を行った場合）両者の間に同一性が失われていないことについて、届出資料において考察されている。

☑最終製品を用いた臨床試験

（研究計画の事前登録）

□UMIN 臨床試験登録システムに事前登録している[注1]。

□（海外で実施する臨床試験の場合であってUMIN 臨床試験登録システムに事前登録していないとき）WHO の臨床試験登録国際プラットフォームにリンクされているデータベースへの登録をしている。

（臨床試験の実施方法）

☑「特定保健用食品の表示許可等について」（平成 26 年 10 月 30 日消食表第 259 号）の別添２「特定保健用食品申請に係る申請書作成上の留意事項」に示された試験方法に準拠している。

□科学的合理性が担保された別の試験方法を用いている。

→□別紙様式（Ｖ）－２を添付

（臨床試験の結果）

☑国際的にコンセンサスの得られた指針に準拠した形式で査読付き論文として公表されている論文を添付している[注1]。

□（英語以外の外国語で書かれた論文の場合）論文全体を誤りのない日本語に適切に翻訳した資料を添付している。

☑研究計画について事前に倫理審査委員会の承認を受けたこと、並びに当該倫理審査委員会の名称について論文中に記載されている。

□（論文中に倫理審査委員会について記載されていない場合）別紙様式（Ｖ）－３で補足説明している。

☑掲載雑誌は、著者等との間に利益相反による問題が否定できる。

□最終製品に関する研究レビュー
□機能性関与成分に関する研究レビュー

□（サプリメント形状の加工食品の場合）摂取量を踏まえた臨床試験で肯定的な結果が得られている。
□（その他加工食品及び生鮮食品の場合）摂取量を踏まえた臨床試験又は観察研究で肯定的な結果が得られている。
□海外の文献データベースを用いた英語論文の検索のみではなく、国内の文献データベースを用いた日本語論文の検索も行っている。
□（機能性関与成分に関する研究レビューの場合）当該研究レビューに係る成分と最終成分の同等性について考察されている。
□（特定保健用食品の試験方法として記載された範囲内で軽症者等が含まれたデータを使用している場合）疾病に罹患していない者のデータのみを対象とした研究レビューも併せて実施し、その結果を、研究レビュー報告書及び別紙様式（Ⅰ）に報告している。

□表示しようとする機能性の科学的根拠として、査読付き論文として公表されている。

□当該論文を添付している。
□（英語以外の外国語で書かれた論文の場合）論文全体を誤りのない日本語に適切に翻訳した資料を添付している。

□PRISMA 声明（2009 年）に準拠した形式で記載されている。
□（PRISMA 声明（2009 年）に照らして十分に記載できていない事項がある場合）別紙様式（Ⅴ）－３で補足説明している。
□（検索に用いた全ての検索式が文献データベースごとに整理された形で当該論文に記載されていない場合）別紙様式（Ⅴ）－５その他の適切な様式を用いて、全ての検索式を記載している。
□（研究登録データベースを用いて検索した未報告の研究情報についてその記載が当該論文にない場合、任意の取組として）別紙様式（Ⅴ）－９その他の適切な様式を用いて記載している。
□食品表示基準の施行前に査読付き論文として公表されている研究レビュー論文を用いているため、上記の補足説明を省略している。

□各論文の質評価が記載されている[注2]。
□エビデンス総体の質評価が記載されている[注2]。
□研究レビューの結果と表示しようとする機能性の関連性に関する評価が記載されている[注2]。

□表示しようとする機能性の科学的根拠として、査読付き論文として公表されていない。

研究レビューの方法や結果等について、
□別紙様式（Ⅴ）－４を添付している。

□データベース検索結果が記載されている[注3]。
□文献検索フローチャートが記載されている[注3]。
□文献検索リストが記載されている[注3]。
□任意の取組として、未報告研究リストが記載されている[注3]。
□参考文献リストが記載されている[注3]。
□各論文の質評価が記載されている[注3]。
□エビデンス総体の質評価が記載されている[注3]。
□全体サマリーが記載されている[注3]。

□各論文の質評価が記載されている[注3]。
□エビデンス総体の質評価が記載されている[注3]。
□研究レビューの結果と表示しようとする機能性の関連性に関する評価が記載されている[注3]。

注１　食品表示基準の施行後１年を超えない日までに開始（参加者１例目の登録）された研究については、必須としない。
注２　各種別紙様式又はその他の適切な様式を用いて記載（添付の研究レビュー論文において、これらの様式と同等程度に詳しく整理されている場合は、記載を省略することができる。）
注３　各種別紙様式又はその他の適切な様式を用いて記載（別紙様式（Ｖ）－４において、これらの様式と同等程度に詳しく整理されている場合は、記載を省略することができる。）

別紙様式（Ｖ）-３

表示しようとする機能性の科学的根拠に関する補足説明資料

１．製品概要

商品名	日清健康オイル アマニプラス
機能性関与成分名	α－リノレン酸
表示しようとする機能性	本品は、α-リノレン酸を含んでおり、血圧が高めの方に適した機能を持つ食用油です。

２．補足説明

機能性関与成分の定量試験及び臨床試験は試作品を用いて行っている。
試作品は、規格に適合した同一原材料を使用しており、調合以外の操作を伴わないため、量産品と品質は変わらず、同一である。
以上より、機能性関与成分の定量試験及び臨床試験に使用した食品（試作品）は、販売品（量産品）と同一性が失われていないと判断した。

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Antihypertensive Effect and Safety of Dietary α-Linolenic Acid in Subjects with High-Normal Blood Pressure and Mild Hypertension

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Abstract: We investigated the antihypertensive effect and safety of α-linolenic acid (ALA) in human subjects. In Experiment 1, subjects with high-normal blood pressure and mild hypertension ingested bread containing 14 g of common blended oil (control oil) or ALA-enriched oil for 12 weeks. The test oil contained 2.6g/14 g of ALA. The subjects ingested strictly controlled meals during the study period. Systolic blood pressure was significantly lower in the ALA group than in the control group after ingestion of the test diet for 4, 8 and 12 weeks. Diastolic blood pressure was significantly lower in the ALA group than in the control group after ingestion of the test diet for 12 weeks. In Experiment 2, we evaluated the safety of high intake of ALA (7.8g/d), particularly its effects on oxidation in the body and blood coagulation. Normotensive, high-normotensive and mildly hypertensive subjects ate bread that contained 42 g of the control oil or the test oil for 4 weeks. No significant difference was noted in the lipid peroxide level, high-sensitive C-reactive protein level, plasma prothrombin time or activated partial thromboplastin time between the two groups. No abnormal changes were noted after test diet ingestion on blood test or urinalysis, and no adverse event considered to have been induced by the test oil was observed in Experiment 1 and 2. These results suggest that ALA have an antihypertensive effect with no adverse effect in subjects with high-normal blood pressure and mild hypertension.

Key words: α-linolenic acid, hypertension, blood pressure, human

1 INTRODUCTION

N-3 polyunsaturated fatty acids are attracting attention because of their preventive effects against various diseases including atherosclerosis, coronary heart disease (CHD) and inflammatory disease[1]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are n-3 poly unsaturated fatty acids contained in fish oil, have long been reported to exhibit antithrombotic and blood lipid-reducing effects[2,3]. There have also been many studies on their antihypertensive effects[4-7]. Frenoux *et al.*[4] and Engler *et al.*[5] reported the antihypertensive effects of EPA and DHA using spontaneously hypertensive (SHR) rats. Knapp *et al.*[6] gave fish oil to patients with mild essential hypertension for 4 weeks and reported decreases in the systolic and diastolic blood pressures. α-Linolenic acid (ALA), which is an n-3 fatty acid of plant origin, has also been reported to be effective for the prevention of lifestyle-related diseases[8,9]. Blood pressure was reported to have decreased significantly in SHR administered flax or perilla oil rich in ALA[10-17]. Some epidemiological surveys have indicated negative correlations between ALA intake and blood pressure[18,19]. Djousse *et al.*[20] reported that the incidence of hypertension in a group with a high ALA intake was significantly lower than that in a group with a low intake. There have been many animal and epidemiological studies of the antihypertensive effect of ALA, but only a limited number of

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small clinical studies have been performed in subjects with high-normal blood pressure or mild hypertension[21-23].

Problems such as prolongation of the bleeding time and lipid peroxidation due to the high intake of n-3 fatty acids have been reported[24,25]. A study in Greenland show that the incidences of cerebral infarction and myocardial infarction due to atherosclerosis were lower, but the incidence of stroke was higher, in Inuits, who consume a large quantity of fish oil, than in Danes[26]. There has also been a report that the bleeding time was prolonged by high intakes of EPA and DHA[27]. The excessive intake of ALA as well as EPA and DHA may affect blood coagulation. Since EPA and DHA have 5 or 6 unstable unsaturated bonds, they are very liable to be oxidized. Since ALA also has 3 unsaturated bonds, it is more likely to be oxidized than saturated or monounsaturated fatty acids, though it may not be as oxidizable as EPA or DHA. Excessive intake of ALA may lead to the promotion of lipid peroxide generation or inflammation in the body. However, the effects of a high intake of ALA on blood coagulation, oxidation in the body and inflammatory reactions have not been sufficiently clarified.

ALA is contained in common plant oils such as rapeseed and soybean oils. Among them, the ALA content is particularly high in flax and perilla oils. However, as these oils have a poor taste or stability against oxidation and are not appropriate as common cooking oils, their use is limited. We developed a cooking oil with a high ALA content, excellent taste, and stability against oxidation. In this study, using this newly developed edible oil, the antihypertensive effect (Experiment 1) and safety (Experiment 2) of ALA were investigated in subjects with high-normal blood pressure and mild hypertension.

2 EXPERIMENTAL

2.1 Test diets

The ALA-enriched oil (test oil) is a mixture of rapeseed oil, flax oil and rice oil purified to an edible grade. It has a taste and stability against oxidation similar to those of common cooking oil and is designed to be a general-purpose edible oil that tolerates heat-cooking. Common blended oil, mixture of rapeseed and soybean oils, was used as a control. The fatty acid compositions of the test and control oils were determined by gas chromatography system (6890 series, Agilent Technologies, California, USA) with a capillary column (TC-70, GL-Science, Tokyo, Japan), after methyllation with boron trifluoride[28]. The assay of the ALA content as a free fatty acid was performed using heptadecanoic acid as the internal standard. Table 1 shows the fatty acid compositions of the test and control oils. In Experiment 1, the daily test oil intake was 14 g, which contained 2.6 g of ALA. The daily test oil 42 g, which contained 7.8 g of ALA in Experiment 2. Bread rolls, each containing 7 g of the test or control oil, were prepared. The test and control bread rolls were identical in appearance and taste.

Table 1 Fatty Acid Composition and α-Linolenic Acid (18:3) Content

	Control oil	Test oil
Fatty acid composition	(g/100 g total fatty acids)	
14:0[1]	0.1	0.1
16:0	7.5	6.9
16:1	0.2	0.2
18:0	2.9	2.1
18:1	41.3	46.1
18:2	36.9	22.3
18:3	8.8	19.9
20:0	0.5	0.5
20:1	0.7	0.8
22:0	0.4	0.2
22:1	0.1	0.1
24:0	0.2	0.1
24:1	0.1	0.1
Others	0.6	0.6
Total	100	100
α-Linolenic acid content	(g/100g oil)	
18:3	8.3	18.3

[1]Number of carbon atoms: number of double bonds.

2.2 Subjects

The subjects were adult males and females who were leading healthy social lives and who responded to a recruiting advertisement for paid subjects of clinical trials by Tokyo Heart Center (Tokyo, Japan) and I'rom Co., Ltd. (Tokyo, Japan). In experiment 1, 127 subjects with high-normal blood pressure (130-139 mmHg systolic blood pressure or 85-89 mmHg diastolic blood pressure) and low- and moderate-risk mild hypertension (140-159 mmHg systolic blood pressure or 90-99 mmHg diastolic blood pressure) were selected. In experiment 2, 44 subjects with normal blood pressure (≤130 mmHg systolic and ≤85 mmHg diastolic), high-normal blood pressure and low- and moderate-risk mild hypertension were selected. Subjects with the following conditions were excluded: under antihypertensive treatment, under continuous treatment by a physician, with severe renal dysfunction, severe liver dysfunction, severe anemia, endocrine disorder, sign of cerebrovascular disorder, atrial fibrillation, severe arrhythmia, food allergy, pregnancy and lactation, heart failure, past medical history of myocardial infarction, 30 or higher BMI, and judged ineligible by a physician. Seven subjects with a personal

reason, 3 subjects who ate breakfast on a test day, 2 subjects for whom discontinuation of the study was decided by a physician, and 1 subject who donated blood during the study period were excluded, and thus, data from 114 subjects were analyzed in Experiment 1. One subjects with a personal reason, 2 subjects who ate breakfast on a test day, 1 subject who could not eat six bread rolls per day and 1 subjects for whom discontinuation of the study was decided by a physician were excluded, and 39 subjects completed the study of Experiment 2. Table 2 and 3 show the characteristics of the subjects of experiment 1 and 2. The protocol of this study was approved by the Ethical Review Board, Osaki Hospital Tokyo Heart Center, Kanshin-kai Medical Corporation, and the study was performed in accordance with the Helsinki Declaration. The subjects were sufficiently informed of the contents and methods of the study, and their consent to participate was obtained.

2.3 Study design

Experiment 1: A double-blind controlled study was performed. Before the study, the subjects were divided by a person not directly involved in the study into two groups similar with respect to blood pressure, age, gender, body weight, BMI, and nutritional intake. Another person without access to the data of the groups allocated the groups to the control and ALA. The duration of test diet ingestion was 12 weeks. A 2-week observation period before initiation of test diet ingestion and a 4-week follow-up period after the test period were established. To exclude influences of dietary nutrients, strict dietary control was performed during the study period. The energy intake was set to 2300-2500 kcal/day, lipid intake to 67-70 g/day (including the test or control oil) and salt intake to 12 g/day or lower. Alcohol intake was limited to 25 ml per day. The subjects ate packed meals prepared by accurate measurement for 12 weeks. The menus for the two groups were identical. The subjects were instructed to drink 200 ml of cow milk and eat 150 g of fruits daily. Other than the provided packed meals, cow milk and fruits, food intake was limited to 0-200 kcal energy, 0-3 g of lipid, and 0-3 g of salt. The subjects recorded all foods they ate during the study period. To enable this dietary control, a detailed dietary manual was prepared, and an administrative dietitian explained it to the volunteers before the study. The dietary records were confirmed every week, and the subjects were interviewed every 2 weeks. Similar dietary control was performed for 2 weeks before the study in both groups, and the subjects ate the control diets. No dietary control was performed after completion of the study (4-week follow-up period). Body weight and blood pressure measurements, blood test, urinalysis, survey of subjective symptoms and examination by a physician were performed. The subjects were fasted from 22:00 on the previous day before tests, and blood pressure was measured, and blood and urine

Table 2 Characteristics of Subjects (Experiment 1)[1]

	Control	ALA
Number (man/woman)	53 (34/19)	58 (39/19)
Age (years)	46.2 ± 11.1	46.4 ± 9.1
Body weight (kg)	69.7 ± 10.0	67.9 ± 12.5
Height (cm)	167.6 ± 8.7	166.6 ± 8.4
Body mass index (kg/m^2)	24.8 ± 2.7	24.3 ± 3.1
Systolic blood pressure (mmHg)	135.6 ± 8.6	136.2 ± 8.7
Diastolic blood pressure (mmHg)	88.0 ± 6.8	87.6 ± 6.8
Energy intake (kcal/day)[2]	2275 ± 510	2248 ± 467
Fat intake (g/day)[2]	72.20 ± 18.57	74.00 ± 20.37
n-3 Fatty acid intake (g/day)[2]	2.59 ± 0.88	2.56 ± 1.27
α-Linolenic acid (g/day)[2]	1.75 ± 0.64	1.76 ± 0.73
Eicosapentaenoic acid (g/day)[2]	0.24 ± 0.18	0.23 ± 0.25
Docosahexaenoic acid (g/day)[2]	0.46 ± 0.30	0.43 ± 0.42
Sodium chloride intake (g/day)[2]	12.0 ± 3.7	12.1 ± 3.4
Alcohol intake (g/day)[2]	12.85 ± 23.86	6.61 ± 12.80

[1]Values are mean ± SD.

[2]Based on data collected from 3 days food intake records.

There were no significant differences between the groups.

Table 3 Characteristics of Subjects (Experiment 2)[1]

	Normal blood pressure		High-normal blood pressure and mild hypertension	
	Control	ALA	Control	ALA
Number (man/woman)	10 (4/6)	8(3/5)	10 (7/3)	11 (8/3)
Age (years)	36.3 ± 12.0	39.5 ± 13.3	43.3 ± 14.6	36.7 ± 10.9
Body weight (kg)	57.6 ± 10.2	57.2 ± 9.1	69.0 ± 11.0	69.9 ± 12.2
Height (cm)	165.4 ± 11.4	162.1 ± 8.0	164.8 ± 10.4	167.6 ± 10.8
Body mass index (kg/m^2)	20.9 ± 1.9	21.7 ± 2.7	25.5 ± 4.0	24.9 ± 3.6
Systolic blood pressure (mmHg)	112.2 ± 13.3	111.0 ± 9.5	134.8 ± 5.5	133.7 ± 12.2
Diastolic blood pressure (mmHg)	71.8 ± 7.9	67.1 ± 11.9	85.5 ± 9.0	85.1 ± 6.5
Energy intake (kcal/day)[2]	2159 ± 602	2118 ± 378	2385 ± 451	2266 ± 590
Fat intake (g/day)[2]	69.19 ± 16.19	66.15 ± 5.77	82.32 ± 17.36	72.41 ± 24.03
n-3 Fatty acid intake (g/day)[2]	2.54 ± 0.97	2.36 ± 0.98	3.20 ± 1.16	2.50 ± 1.54
α-Linolenic acid (g/day)[2]	1.85 ± 1.27	1.36 ± 0.22	2.07 ± 0.47	1.75 ± 0.55
Eicosapentaenoic acid (g/day)[2]	0.19 ± 0.13	0.31 ± 0.29	0.31 ± 0.25	0.19 ± 0.29
Docosahexaenoic acid (g/day)[2]	0.37 ± 0.22	0.50 ± 0.39	0.58 ± 0.45	0.38 ± 0.55
Sodium chloride intake (g/day)[2]	10.8 ± 4.2	9.8 ± 1.2	12.7 ± 3.6	10.5 ± 3.8
Alcohol intake (g/day)[2]	9.66 ± 20.01	7.93 ± 10.3	5.10 ± 7.47	5.14 ± 8.38

[1]Values are mean ± SD.

[2]Based on data collected from 3 days food intake records.

There were no significant differences between the groups.

were collected in the fasting state in the morning of the test day.

Experiment 2: A double-blind controlled study was performed. The 44 subjects were divided into two groups same as Experiment 1. The test meal was eaten for 4 weeks. The total energy intake was controlled to 2,300-2,600 kcal/day, total lipid intake (including the test or control oil) to 70-80 g/day, and total salt intake to 14 g/day or less. Alcohol intake was permitted to 25 ml/day as ethanol. The intake of foods other than the provided meals, cow milk and fruits was controlled to an energy intake of 0-400 kcal, lipid intake of 0-3 g, and salt intake of 0-3 g. The subjects were instructed to record all foods they ate during the study period. For 2 weeks prior to the study, the subjects of both groups were given the control diet under dietary control conditions similar to those during the study. The body weight and blood pressure were measured, and blood tests, urinalysis, inquiry of subjective symptoms and physical examinations by a physician were performed.

2.4 Measurements

After arriving at the hospital, the subjects rested in a seated position for at least 5 minutes and underwent measurement of the blood pressure using a mercury sphygmomanometer by the same nurse throughout the study. Blood pressure was measured 3 times at each examination, and the mean value of the 3 measurements was adopted as the value on that day. The heart rate at the first measurement of the blood pressure was recorded.

Blood tests and urinalysis were entrusted to a commercial laboratory (Sanritsu, Chiba, Japan) except for the serum lipid peroxide, ketone body and high-sensitivity C-reactive protein (CRP) concentrations. Mitsubishi Kagaku Bio-Clinical Laboratories, Inc (Tokyo, Japan) performed measurements of the serum lipid peroxide and ketone body concentrations. Measurement of the high-sensitivity CRP was performed by SRL, Inc (Tokyo, Japan).

2.5 Statistical analysis

The measured values are presented as the means ± standard deviation excluding values in Figures. The measured values in Figures are presented as the means ± standard error. After the Smirnov-Grubbs' outlier test, the blood pressure data during the study period were subjected to two-way analysis of variance (ANOVA), and the main effects and interaction of the diet and duration of ingestion were analyzed in Experiment 1. Three outliers were identified statistically by Smirnov-Grubbs' outlier test. For comparison with Week 0 and between-group comparison, the Dunnett's test and unpaired t-test were used, respectively.

For comparison of the blood test data between groups and between Weeks 0 and 12, unpaired or paired *t*-test was used. In Experiment 2, statistical procedures were performed by dividing the subjects into normotensive persons and others (high-normotensive subjects and mildly hypertensive subjects). The blood pressure, heart rate, body weight and blood test results were compared between the two groups using the unpaired *t*-test, and their changes within each group were examined by the paired *t*-test. For comparison of the urinalysis findings between groups and between Weeks 0 and the end of test period, the Wilcoxon rank sum test and the Wilcoxon signed-ranks test were used, respectively. Excel Toukei (SSRI, Tokyo, Japan) was used for the Smirnov-Grubbs' outlier test, and SPSS version 13.0 J (SPSS Japan, Tokyo, Japan) was used for other tests. A significance level less than 5% was regarded as significant.

3 RESULTS

3.1 Dietary survey in experiment 1

No significant differences were noted in the energy, total fat, n-3 fatty acids, sodium chloride or alcohol intake between the two groups during the prior observation or test period (Table 4). The energy and salt intakes during the 12-week test period were consistent with the set values in both groups. There were the periods that an intake of fat did not reach quantity of setting, but the lack was under 1g at the maximum. The total ALA intake in the test group during the test period was 3.4 g/day, which was significantly higher than that in the control group.

3.2 Blood pressure in experiment 1

Time-course changes in systolic blood pressure are shown in Fig 1-A. No interaction between the diet and duration of ingestion was detected by two-way ANOVA, but significant effects of the diet and time were noted. Systolic blood pressure was significantly lower in the ALA group than in the control group after test diet ingestion for 4, 8, and 12 weeks. Compared to Week 0, systolic blood pressure significantly decreased at Week 4, 8 and 12 in the ALA group, and at Week 8 in the control group. At Week 16 (follow-up period), systolic blood pressure of the ALA group increased gently, compared to that in Week 12. No significant difference was noted in systolic blood pressure between the two groups at Week 16.

Time-course changes in diastolic blood pressure are shown in Fig 1-B. No interaction between the diet and duration of ingestion was detected by two-way ANOVA, but significant effects of the diet and time were noted. Compared to Week 0, diastolic blood pressure significantly decreased at Week 12 in the only ALA group. Diastolic blood pressure was significantly lower in the ALA group than in the control group after test diet ingestion for 12 weeks. At Week 16 (follow-up period), diastolic blood pressure of the ALA group increased slightly, compared to that in Week 12. No significant difference was noted in diastolic

Table 4 Intake of Energy, Fat, Fatty Acids, Sodium Chloride and Alcohol (Experiment 1)[1]

	Group	Week -2 and -1	Week 0-3	Week 4-7	Week 8-11
Energy (kcal/day)	Control	2307 ± 117	2324 ± 97	2355 ± 130	2377 ± 149
	ALA	2313 ± 94	2322 ± 117	2366 ± 107	2382 ± 156
Fat (g/day)	Control	66.37 ± 2.87	66.31 ± 2.78	66.92 ± 1.53	66.89 ± 2.00
	ALA	67.40 ± 3.22	67.16 ± 1.57	66.65 ± 3.59	67.35 ± 1.58
n-3 Fatty acids (g/day)	Control	2.37 ± 0.14	2.32 ± 0.08	2.35 ± 0.09	2.34 ± 0.09
	ALA	2.35 ± 0.13	3.95 ± 0.09#	3.98 ± 0.07#	3.98 ± 0.07#
α-Linolenic acid (g/day)	Control	1.77 ± 0.09	1.73 ± 0.07	1.74 ± 0.06	1.72 ± 0.07
	ALA	1.77 ± 0.08	3.37 ± 0.07#	3.39 ± 0.06#	3.38 ± 0.05#
Eicosapentaenoic acid (g/day)	Control	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.01
	ALA	0.15 ± 0.03	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01
Docosahexaenoic acid (g/day)	Control	0.33 ± 0.06	0.33 ± 0.03	0.34 ± 0.07	0.33 ± 0.02
	ALA	0.32 ± 0.05	0.32 ± 0.03	0.33 ± 0.02	0.33 ± 0.03
Sodium chloride (g/day)	Control	10.1 ± 0.7	10.0 ± 0.8	10.0 ± 0.8	10.0 ± 0.8
	ALA	10.1 ± 0.7	10.0 ± 0.8	10.0 ± 0.8	10.0 ± 0.6
Alcohol (g/day)	Control	6.2 ± 6.6	6.3 ± 6.5	6.7 ± 6.9	7.2 ± 7.0
	ALA	5.1 ± 5.0	6.5 ± 5.8	6.7 ± 5.5	6.9 ± 5.5

[1]Values are mean ± SD, n = 53 (control group), 58 (ALA group)

Significantly different compared with the value of control group, at #$P < 0.05$ by unpaired *t*-test.

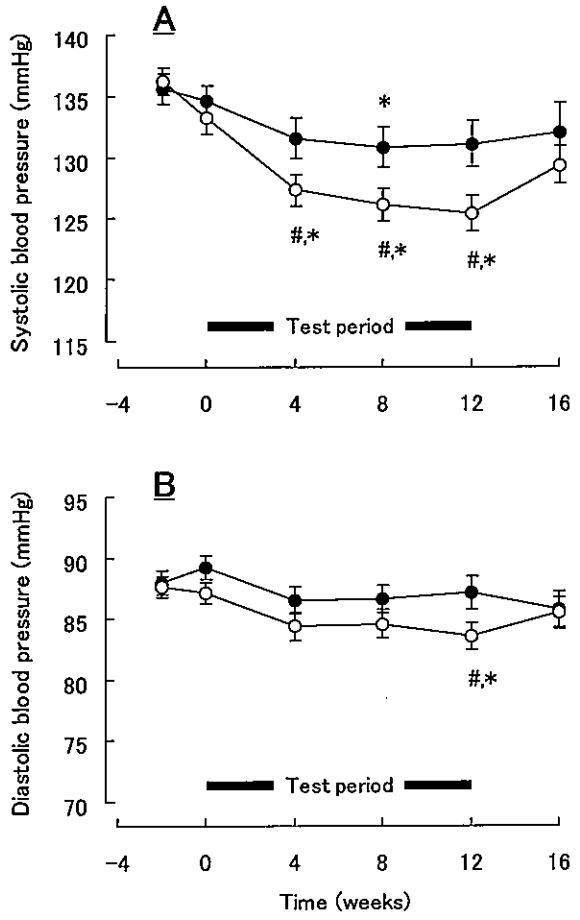

Fig. 1 Changes of Systolic Blood (A) and Diastolic Blood Pressure (B) After Intake the Control (Closed Circles, n = 53) or α-Linolenic Acid Diet (Open Circles, n = 58) in the Subjects with High-Normal Blood Pressure and Mild Hypertension. Each point represents the mean ± standard error. Significantly different from the Week 0, $*P < 0.05$. Significantly different from the control group, $\#P < 0.05$.

blood pressure between the two groups at Week 16.

3.3 Body weight, heart rate and blood test in experiment 1

The results of heart rate, body weight and blood test are shown in Table 5. There were no significant differences in the body weight or heart rate between the two groups. After completion of the study, no significant differences were noted in any blood test item excluding mean corpuscular volume (MCV) between the two groups. The MCV value at Week 12 was significantly higher in the ALA group than in the control group. The MCV value at Week 12 was within the normal range (87-102 fl) in the ALA group, excluding one subject in whom the value was lower than the normal range in Weeks 0 and 12. In this subject, the value was slightly increased by the test diet ingestion (Week 0: 83 fl, Week 12: 84 fl). On intragroup comparison, significant changes were noted in 22 blood test items at Week 12, compared to those at Week 0. However, all of these mean values remained within normal ranges.

3.4 Urinalysis in experiment 1

The urinalysis findings are shown in Table 6. No positive reactions were noted in a urine sugar or urobilinogen test in Week 0 or 12. No significant difference was noted in urinary protein between the two groups in Week 0 or 12. No significant changes were observed in urinary protein test between before and after the study in either group. Urinary protein was positive after the study in 1 subject in the ALA group. No abnormality was noted in the blood test results related to kidney function (creatinine, blood urea nitrogen, inorganic phosphate, calcium, sodium, potassium, chloride) in the subject, and the subject was in the menstrual phase.

3.5 Adverse events and examination findings in experiment 1

Fifty-two episodes of adverse events occurred during the 12-week study period. The major adverse events were 25, 8 and 5 episodes of common cold, headache and pharyngeal pain, respectively, but none of all episodes was related to the test diet. Transient soft stool was noted in 1 subject each in the control and test groups. One case each of unpleasant feeling of the stomach and gastroenteritis were noted in the control group. No adverse events attributable to the test diet were noted in the survey of subjective symptoms or examination by a physician.

3.6 Dietary survey in experiment 2

No significant difference was observed in energy, total fat, n-3 fatty acids, sodium chloride or alcohol intake during the test period between the ALA and control groups (Table 7). The energy, fat and salt intakes during the test period were consistent with the set values in both groups. The total ALA intake during the study in the ALA group was 9.1 g/day, which was significantly higher than that in the control group.

3.7 Blood pressure, heart rate and body weight in experiment 2

Table 8 shows the blood pressure, heart rate and body weight data. In the normotensive subjects, no significant difference was observed in blood pressure, heart rate and body weight between the ALA and control groups. Compared to Week 0, diastolic blood pressure significantly increased at Week 4 in the ALA group. However, the mean value of diastolic blood pressure remained within optimum range. In the high-normotensive and mildly hypertensive subjects, no significant difference in blood pressure was noted between the two groups. No significant difference

Table 5 Heart Rate, Body Weight and Blood Test Results (Experiment 1)[1]

	Control group		ALA group	
	Week 0	Week 12	Week 0	Week 12
Heart rate (beats/min)	72.3 ± 9.8	70.3 ± 9.0	71.6 ± 9.0	69.3 ± 7.8
Body weight (kg)	68.8 ± 9.8	68.7 ± 10.0	67.2 ± 12.1	67.0 ± 11.7
Lipid peroxides (nmol/mL)	1.10 ± 0.44	0.99 ± 0.24	1.10 ± 0.37	1.00 ± 0.18
High-sensitive C-reactive protein (ng/mL)	2772 ± 11633	1382 ± 2638	906 ± 1947	896 ± 1734
Prothrombin time (activity) (%)	108 ± 8	113 ± 10*	111 ± 9	114 ± 9*
Activated partial thromboplastin time (sec)	30.3 ± 3.1	30.2 ± 3.6	30.0 ± 2.9	29.9 ± 2.8
Asparate aminotransferase (IU/L/37℃)	19.8 ± 5.5	21.0 ± 7.3	22.0 ± 7.5	23.1 ± 7.1
Alanine aminotransferase (IU/L/37℃)	24.8 ± 14.8	24.2 ± 18.1	29.3 ± 20.1	28.5 ± 17.4
γ-Glutamyltranspeptidase (IU/L/37℃)	38.1 ± 30.6	41.4 ± 34.3	38.1 ± 29.5	39.7 ± 40.5
Alkaline phosphatase (IU/L/37℃)	221 ± 58	233 ± 67*	231 ± 72	255 ± 96*
Lactate dehydrogenase (IU/L/37℃)	170 ± 25	174 ± 25*	179 ± 31	184 ± 33
Total bilirubin (mg/100 ml)	0.95 ± 0.35	0.81 ± 0.34*	0.91 ± 0.37	0.72 ± 0.27*
Thymol turbidity test (U)	1.65 ± 1.09	1.72 ± 1.43	1.55 ± 1.23	1.53 ± 1.38
Zinc sulfate turbidity test (U)	7.39 ± 2.38	6.59 ± 2.10*	7.18 ± 2.66	6.82 ± 2.90*
Cholinesterase (IU/L/37℃)	361 ± 67	365 ± 71	350 ± 76	354 ± 80
Leucine aminopeptidase (IU/L/37℃)	50.9 ± 10.1	50.4 ± 11.3	52.4 ± 11.4	53.3 ± 16.6
Total protein (g/100 ml)	7.40 ± 0.45	7.35 ± 0.43	7.25 ± 0.38	7.31 ± 0.37
Albumin (g/100 ml)	4.60 ± 0.23	4.53 ± 0.31*	4.48 ± 0.26#	4.48 ± 0.30
A/G	1.67 ± 0.24	1.62 ± 0.23*	1.65 ± 0.26	1.62 ± 0.26
Creatinine (mg/100 ml)	0.80 ± 0.18	0.77 ± 0.17*	0.80 ± 0.15	0.78 ± 0.15
Blood urea nitrogen (mg/100 ml)	13.9 ± 3.4	13.7 ± 2.7	13.5 ± 3.2	13.2 ± 2.6
Uric acid (mg/100 ml)	6.18 ± 1.54	5.89 ± 1.36*	6.12 ± 1.33	5.68 ± 1.32*
Total cholesterol (mg/100 ml)	202 ± 35	194 ± 32*	195 ± 32	188 ± 28*
LDL cholesterol (mg/100 ml)	125 ± 32	122 ± 29	119 ± 29	117 ± 26
HDL cholesterol (mg/100 ml)	58.2 ± 12.4	62.9 ± 15.6*	59.5 ± 14.3	63.4 ± 17.7*
Triglyceride (mg/100 ml)	124 ± 71	112 ± 61	114 ± 57	101 ± 54*
Phospholipids (mg/100 ml)	216 ± 33	224 ± 35*	211 ± 29	219 ± 31*
Free fatty acid (mEq/L)	0.54 ± 0.14	0.49 ± 0.18	0.55 ± 0.19	0.45 ± 0.17*
Total ketone bodies (μ mol/L)	90.5 ± 62.2	57.3 ± 45.4*	113.9 ± 122.1	59.4 ± 48.6*
Glucose (mg/100 ml)	91.8 ± 8.3	91.2 ± 9.0	92.9 ± 12.2	92.6 ± 11.7
Hemoglobin A1c (%)	5.01 ± 0.39	4.97 ± 0.32	5.04 ± 0.46	5.02 ± 0.45
Glycosylated albumin (%)	14.3 ± 1.2	13.7 ± 1.1*	14.2 ± 1.5	13.8 ± 1.7*
Iinsulin (μU/mL)	4.82 ± 2.65	5.65 ± 2.53*	4.23 ± 2.55	4.81 ± 2.57
Sodium (mEq/L)	140 ± 2	141 ± 2*	140 ± 2	142 ± 2*
Potassium (mEq/L)	4.18 ± 0.28	4.15 ± 0.32	4.16 ± 0.34	4.22 ± 0.32
Chloride (mEq/L)	103 ± 2	103 ± 2	103 ± 2	104 ± 2
Calcium (mg/100 ml)	9.44 ± 0.32	9.56 ± 0.36*	9.27 ± 0.33#	9.47 ± 0.34*
Inorganic phosphate (mg/100 ml)	3.42 ± 0.48	3.48 ± 0.49	3.32 ± 0.45	3.39 ± 0.44
Magnesium (mg/100 ml)	2.30 ± 0.16	2.29 ± 0.15	2.29 ± 0.16	2.28 ± 0.13
White blood cell counts ($\times 10^3/\mu L$)	5.69 ± 1.42	5.49 ± 1.27	5.65 ± 1.42	5.49 ± 1.52
Red blood cell counts ($\times 10^4/\mu L$)	484 ± 40	488 ± 44	483 ± 40	486 ± 42
Hemoglobin (g/100 ml)	14.3 ± 1.5	14.4 ± 1.6	14.6 ± 1.2	14.6 ± 1.2
Hematocrit (%)	43.9 ± 3.7	44.6 ± 4.2*	44.6 ± 3.4	45.4 ± 3.5*
Mean corpuscular volume (fl)	90.8 ± 5.1	91.4 ± 5.2*	92.5 ± 3.7#	93.3 ± 4.0#*
Mean corpuscular hemoglobin (pg)	29.6 ± 2.2	29.4 ± 2.2*	30.2 ± 1.3	30.0 ± 1.3*
Mean corpuscular hemoglobin concentration (%)	32.6 ± 1.1	32.2 ± 1.1*	32.7 ± 0.7	32.1 ± 0.7*
Platelet count ($\times 10^4/\mu L$)	25.2 ± 6.3	24.6 ± 6.0	24.7 ± 5.6	24.6 ± 5.3

[1]Vales are mean ± SD, n = 53 (control group), 58 (ALA group)

Significantly different compared with the value of control group, #$P < 0.05$ by unpaired t-test.

Significantly different compared with the value of Week 0, *$P < 0.05$ by paired t-test.

Table 6 Results of Urinalysis (Experiment 1)

		Week 0					Week 12				
	Group	−	±	+	++	+++	−	±	+	++	+++
Sugar	Control	53	0	0	0	0	53	0	0	0	0
	ALA	58	0	0	0	0	58	0	0	0	0
Protein	Control	49	4	0	0	0	49	4	0	0	0
	ALA	55	2	1	0	0	53	4	0	1	0
Urobilinogen	Control	-	53	0	0	0	-	53	0	0	0
	ALA	-	58	0	0	0	-	58	0	0	0

n = 53 (control group), 58 (ALA group)

There were no significant differences between the two groups, or between Week 0 and 4.

Table 7 Intake of Eergy, Fat, Fatty Acids, Sodium Chloride and Alcohol (Experiment 2)[1]

	Normal blood pressure		High-normal blood pressure and mild hypertension	
	Control (n = 10)	ALA (n = 8)	Control (n = 10)	ALA (n = 11)
Energy (kcal/day)	2371 ± 108	2326 ± 156	2388 ± 153	2337 ± 111
Fat (g/day)	76.97 ± 2.29	76.42 ± 3.76	77.64 ± 2.91	76.15 ± 2.65
n-3 Fatty acids (g/day)	4.79 ± 0.05	9.64 ± 0.18#	4.80 ± 0.05	9.72 ± 0.07#
α-Linolenic acid (g/day)	4.19 ± 0.03	9.06 ± 0.13#	4.18 ± 0.02	9.11 ± 0.04#
Eicosapentaenoic acid (g/day)	0.15 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.02
Docosahexaenoic acid (g/day)	0.33 ± 0.02	0.32 ± 0.04	0.34 ± 0.02	0.34 ± 0.03
Sodium chloride (g/day)	11.1 ± 0.4	11.1 ± 1.0	11.5 ± 1.0	11.2 ± 0.7
Alcohol (g/day)	2.4 ± 4.2	2.6 ± 4.1	4.3 ± 6.8	3.3 ± 6.5

[1]Values are mean ± SD.

Significantly different compared with the value of control group, #$P < 0.05$ by unpaired t-test.

was observed in the heart rate or body weight in the normotensive, high-normotensive, or mildly hypertensive subjects at Week 4 between the two groups. No significant change was observed in body weight between before and after the study in either group.

3.8 Blood tests of normotensive subjects in experiment 2

Table 9 shows the results of blood tests in the normotensive subjects. Serum lipid peroxide, high-sensitivity CRP, plasma prothrombin time (PT) and activated partial thromboplastin time (APTT) showed no significant difference between the ALA and control groups, and no significant difference was observed between Week 0 and 4. The blood test related to liver function, kidney function, carbohydrate metabolism, lipid metabolism, blood cells and electrolytes showed no significant difference between the two groups. Although statistically significant changes were observed between Week 0 and 4 in the blood tests, all of the mean values remained within normal range.

3.9 Blood tests of high-normotensive and mildly hypertensive subjects in experiment 2

Table 10 shows the results of blood tests in the high-normotensive and mildly hypertensive subjects. No significant difference was noted in the serum lipid peroxide or high-sensitivity CRP level between the ALA and control groups. Neither APTT nor PT showed a significant difference between the two groups. Also, no significant difference was noted in the blood test related to liver function, kidney function, carbohydrate metabolism, blood cells, and electrolytes between the two groups except for the serum Na level. The serum Na level showed no significant change between before and after the study in the ALA group. Also, the serum Na level was in the normal range at 0 and 4 weeks in the ALA group.

Table 8 Systolic Blood Pressure, Diastolic Blood Pressure, Heart Rate and Body Weight (Experiment 2)[1]

		Normal blood pressure		High-normal blood pressure and mild hypertension	
	Group	Week 0	Week 4	Week 0	Week 4
Systolic blood pressure (mmHg)	Control	110.2 ± 11.3	106.2 ± 7.7	132.0 ± 5.6	128.0 ± 11.4
	ALA	110.5 ± 12.1	115.4 ± 13.1	125.8 ± 10.5	123.2 ± 12.1
Diastolic blood pressure (mmHg)	Control	69.9 ± 7.5	67.5 ± 5.4	82.3 ± 9.7	84.1 ± 11.7
	ALA	66.0 ± 9.2	73.3 ± 11.3*	84.7 ± 10.5	81.5 ± 13.3
Heart rate (beats/min)	Control	67.0 ± 6.1	66.6 ± 6.7	65.2 ± 6.2	65.4 ± 9.3
	ALA	69.4 ± 7.2	74.0 ± 12.9	74.0 ± 10.6#	68.7 ± 10.0
Body weight (kg)	Control	57.5 ± 9.8	58.2 ± 9.8	68.3 ± 10.8	68.4 ± 10.5
	ALA	56.6 ± 9.1	56.9 ± 8.5	69.4 ± 12.2	69.3 ± 11.8

[1]Values are mean ± SD, n = 10 (normal blood pressure, control group), 8 (normal blood pressure, ALA group), 10 (high-normal blood pressure and mild hypertension, control group), 11(high-normal blood pressure and mild hypertension, ALA group).
Significantly different compared with the value of control group, #$P < 0.05$ by unpaied t-test.
Significantly different compared with the value of Week 0, *$P < 0.05$ by paired t-test.

3.10 Urinalysis in experiment 2

Table 11 shows the results of urinalysis in the normotensive, high-normotensive, and mildly hypertensive subjects. No positive reactions were noted in a urine sugar or urobilinogen test in Week 0 or 4. No significant differences were noted in urinary protein between the two groups in Week 0 or 4. No significant changes were observed in urinary protein between before and after the study in either group. Urinary protein was positive after the study in 1 subject in the ALA group. However, no abnormality was noted in the blood test results related to kidney function (creatinine, blood urea nitrogen, inorganic phosphate, calcium, sodium, potassium, chloride) in the subject.

3.11 Adverse events and examination findings in experiment 2

During the 4-week study, 2 adverse events (bronchitis, left neck pain) were observed in the ALA group, but neither was related to the test diet. Also, no symptom considered to be a side-effect of the test diet was noted on inquiry about symptoms or examination by a physician.

4 DISCUSSION

Systolic and diastolic blood pressure in the ALA group was significantly lower than that before the study and that in the control group, showing that ALA exhibited an antihypertensive effect on systolic and diastolic blood pressure. It was also suggested that ingestion of this ALA-enriched edible oil decreases blood pressure, compared to general edible oil. A decrease in blood pressure reduces risks of cerebral stroke and CHD[29-31]. Daily ingestion of ALA-enriched edible oil might be useful for prevention of these diseases. Many epidemiological studies suggested that ALA ingestion reduces the incidence of CHD, although not all study results were consistent[32]. Systolic blood pressure was significantly decreased, compared to that before the study, in not only the test diet group but also in the control diet group. To exclude the influences of dietary nutrients, particularly ALA, EPA and DHA, diets including alcohol[33] and sodium chloride[34] intake were strictly controlled, and the subjects continuously ingested well-balanced meals. This may have been the cause of the blood pressure reduction[35]. After discontinuation of the test diet ingestion, systolic and diastolic blood pressures slowly increased and returned to the pre-study levels. No rebound due to discontinuation of the test diet occurred.

The antihypertensive effect of ALA has been repeatedly reported by animal and epidemiological studies, but no effect was noted in clinical studies performed in subjects with high-normal blood pressure or mild hypertension[21-23]. Venter *et al.*[22] investigated the antihypertensive effect of ALA in 15 subjects without dietary control. Singer *et al.*[21,23] performed a study in groups consisting of 10-15 subjects with some dietary control. To evaluate the antihypertensive effect of ALA, a large-scale study with strict dietary control might be necessary. We performed this study with strict dietary control in groups each consisting of more than 50 subjects.

ALA is an essential fatty acid. Since it is expected to have a preventive effect against cardiovascular diseases as well as being necessary for the maintenance of brain and nerve functions, its intake is recommended. According to the report of Tsuji *et al.*[36], ALA intake of the Japanese in

Table 9 Results of Bood Test in the Normotensive Subjects (Experiment 2)[1]

	Control group		ALA group	
	Week 0	Week 4	Week 0	Week 4
Lipid peroxides (nmol/mL)	1.14 ± 0.53	0.84 ± 0.13	1.00 ± 0.43	0.93 ± 0.21
High-sensitive C-reactive protein (ng/mL)	884 ± 1842	308 ± 215	1607 ± 2631	2589 ± 6632
Prothrombin time (activity) (%)	103 ± 12	104 ± 12	113 ± 7	113 ± 5
Activated partial thromboplastin time (sec)	33.3 ± 2.5	32.6 ± 1.8	31.3 ± 2.9	31.5 ± 3.9
Asparate aminotransferase (IU/L/37℃)	16.7 ± 3.7	22.9 ± 20.0	25.1 ± 16.0	20.3 ± 6.0
Alanine aminotransferase (IU/L/37℃)	12.5 ± 4.8	22.3 ± 32.0	30.5 ± 39.6	19.1 ± 7.0
γ-Glutamyltranspeptidase (IU/L/37℃)	19.3 ± 9.2	22.4 ± 15.4	35.9 ± 44.2	28.1 ± 23.1
Alkaline phosphatase (IU/L/37℃)	212 ± 36	219 ± 41	188 ± 34	182 ± 42
Lactate dehydrogenase (IU/L/37℃)	161 ± 21	156 ± 19	174 ± 28	164 ± 22
Total bilirubin (mg/100 ml)	0.68 ± 0.29	0.71 ± 0.32	0.59 ± 0.19	0.69 ± 0.28
Thymol turbidity test (U)	1.42 ± 0.67	1.67 ± 0.95	1.60 ± 1.09	1.45 ± 0.77
Zinc sulfate turbidity test (U)	7.27 ± 1.88	7.16 ± 1.81	7.46 ± 2.18	7.64 ± 2.48
Cholinesterase (IU/L/37℃)	302 ± 26	306 ± 36	338 ± 100	341 ± 98
Leucine aminopeptidase (IU/L/37℃)	47.2 ± 6.6	47.4 ± 9.2	51.0 ± 15.0	47.0 ± 8.3
Total protein (g/100 ml)	7.39 ± 0.37	7.31 ± 0.26	7.63 ± 0.75	7.41 ± 0.74
Albumin (g/100 ml)	4.42 ± 0.20	4.36 ± 0.25	4.59 ± 0.30	4.50 ± 0.39
A/G	1.51 ± 0.23	1.50 ± 0.22	1.53 ± 0.15	1.56 ± 0.19
Creatinine (mg/100 ml)	0.68 ± 0.13	0.65 ± 0.16	0.65 ± 0.10	0.64 ± 0.12
Blood urea nitrogen (mg/100 ml)	12.4 ± 2.7	12.0 ± 2.8	11.6 ± 2.2	12.5 ± 0.9
Uric acid (mg/100 ml)	5.04 ± 1.70	4.79 ± 1.31	5.06 ± 1.16	5.13 ± 1.39
Total cholesterol (mg/100 ml)	160 ± 37	160 ± 39	175 ± 22	164 ± 14
LDL cholesterol (mg/100 ml)	84.4 ± 29.1	91.4 ± 30.4*	92.1 ± 26.9	91.6 ± 21.6
HDL cholesterol (mg/100 ml)	61.4 ± 10.7	63.5 ± 13.3	67.9 ± 19.2	67.4 ± 25.2
Triglyceride (mg/100 ml)	68.0 ± 31.0	69.8 ± 24.2	72.9 ± 34.0	72.9 ± 45.2
Phospholipids (mg/100 ml)	185 ± 31	198 ± 40	201 ± 21	202 ± 33
Free fatty acid (mEq/L)	0.39 ± 0.21	0.33 ± 0.16*	0.44 ± 0.18	0.49 ± 0.18
Total ketone bodies (μmol/L)	79.6 ± 42.9	71.9 ± 65.1	135.2 ± 179.2	130.9 ± 77.1
Glucose (mg/100 ml)	83.8 ± 6.5	83.9 ± 6.8	89.5 ± 10.8	85.8 ± 4.6
Hemoglobin A1c (%)	4.74 ± 0.24	4.72 ± 0.23	4.84 ± 0.33	4.89 ± 0.29
Glycosylated albumin (%)	13.4 ± 1.5	13.6 ± 1.4	13.8 ± 1.0	14.2 ± 1.1*
Iinsulin (μU/mL)	2.95 ± 1.12	2.75 ± 1.30	6.61 ± 6.20	4.66 ± 3.72
Sodium (mEq/L)	140 ± 1	140 ± 2	140 ± 1	140 ± 2
Potassium (mEq/L)	4.13 ± 0.29	4.04 ± 0.26	4.23 ± 0.16	4.04 ± 0.11*
Chloride (mEq/L)	103 ± 1	102 ± 2	102 ± 2	102 ± 2
Calcium (mg/100 ml)	9.51 ± 0.33	9.51 ± 0.34	9.78 ± 0.38	9.49 ± 0.33
Inorganic phosphate (mg/100 ml)	3.53 ± 0.57	3.30 ± 0.53	3.49 ± 0.25	3.35 ± 0.60
Magnesium (mg/100 ml)	2.25 ± 0.14	2.18 ± 0.09	2.31 ± 0.08	2.19 ± 0.22
White blood cell counts ($\times 10^3/\mu L$)	5.56 ± 1.56	5.20 ± 1.51	6.16 ± 0.64	6.19 ± 0.78
Red blood cell counts ($\times 10^4/\mu L$)	471 ± 48	476 ± 47	473 ± 34	478 ± 34
Hemoglobin (g/100 ml)	14.1 ± 1.2	14.3 ± 1.3	14.1 ± 0.7	14.1 ± 0.8
Hematocrit (%)	43.6 ± 3.1	44.2 ± 3.5	43.3 ± 2.0	43.8 ± 2.1
Mean corpuscular volume (fl)	93.1 ± 7.4	93.2 ± 6.3	91.6 ± 3.9	91.6 ± 3.7
Mean corpuscular hemoglobin (pg)	30.2 ± 2.2	30.2 ± 2.3	29.8 ± 1.2	29.5 ± 1.2
Mean corpuscular hemoglobin concentration (%)	32.5 ± 0.9	32.4 ± 0.7	32.5 ± 0.6	32.1 ± 0.4*
Platelet count ($\times 10^4/\mu L$)	25.7 ± 5.0	25.5 ± 6.1	25.9 ± 5.2	25.0 ± 4.1

[1]Values are mean ± SD, n = 10 (control group), 8 (ALA group).

Significantly different compared with the value of control group, $^{\#}P < 0.05$ by unpaied *t*-test.

Significantly different compared with the value of Week 0, $^{*}P < 0.05$ by paired *t*-test.

Table 10 Results of Bood Test in the High-Normal Blood Pressure and Mild Hypertensive Subjects (Experiment 2)[1]

	Control group		ALA group	
	Week 0	Week 4	Week 0	Week 4
Lipid peroxides (nmol/mL)	1.24 ± 0.34	0.99 ± 0.20*	1.33 ± 0.60	1.22 ± 0.37
High-sensitive C-reactive protein (ng/mL)	1109 ± 848	2770 ± 4139	3564 ± 9551	2211 ± 4126
Prothrombin time (activity) (%)	115 ± 11	115 ± 8	114 ± 12	109 ± 10*
Activated partial thromboplastin time (sec)	30.6 ± 3.1	29.9 ± 2.9*	30.4 ± 4.2	30.8 ± 3.6
Asparate aminotransferase (IU/L/37℃)	23.2 ± 8.5	27.8 ± 19.9	19.0 ± 5.9	19.7 ± 7.2
Alanine aminotransferase (IU/L/37℃)	32.7 ± 26.6	41.2 ± 43.3	23.5 ± 17.3	23.1 ± 17.5
γ-Glutamyltranspeptidase (IU/L/37℃)	33.9 ± 20.6	35.5 ± 28.5	48.5 ± 45.5	39.6 ± 35.1
Alkaline phosphatase (IU/L/37℃)	266 ± 107	285 ± 109	245 ± 52	242 ± 53
Lactate dehydrogenase (IU/L/37℃)	166 ± 34	177 ± 35*	180 ± 26	178 ± 27
Total bilirubin (mg/100 ml)	0.90 ± 0.63	0.92 ± 0.59	0.79 ± 0.46	0.71 ± 0.39
Thymol turbidity test (U)	1.49 ± 1.16	1.81 ± 1.07	1.56 ± 1.26	1.69 ± 1.95
Zinc sulfate turbidity test (U)	6.37 ± 2.01	6.32 ± 2.51	6.17 ± 2.24	6.45 ± 2.48
Cholinesterase (IU/L/37℃)	388 ± 46	396 ± 55	374 ± 92	362 ± 101
Leucine aminopeptidase (IU/L/37℃)	52.0 ± 9.0	52.8 ± 11.3	53.0 ± 10.1	50.4 ± 9.0*
Total protein (g/100 ml)	7.52 ± 0.40	7.42 ± 0.40	7.69 ± 0.27	7.44 ± 0.31*
Albumin (g/100 ml)	4.57 ± 0.27	4.66 ± 0.25	4.61 ± 0.26	4.54 ± 0.28
A/G	1.58 ± 0.25	1.72 ± 0.26*	1.51 ± 0.22	1.59 ± 0.25
Creatinine (mg/100 ml)	0.79 ± 0.14	0.79 ± 0.13	0.78 ± 0.16	0.78 ± 0.11
Blood urea nitrogen (mg/100 ml)	15.5 ± 4.5	14.2 ± 3.5	14.8 ± 3.5	14.4 ± 3.8
Uric acid (mg/100 ml)	6.20 ± 1.79	5.69 ± 1.70*	5.86 ± 1.38	5.90 ± 1.30
Total cholesterol (mg/100 ml)	192 ± 32	180 ± 16	194 ± 40	178 ± 42*
LDL cholesterol (mg/100 ml)	114.3 ± 24.1	109.9 ± 14.9	105.7 ± 29.4	106.8 ± 35.1
HDL cholesterol (mg/100 ml)	59.2 ± 14.2	57.4 ± 15.3	65.5 ± 22.1	60.3 ± 15.5
Triglyceride (mg/100 ml)	94.9 ± 47.5	137.9 ± 69.4	134.5 ± 67.2	129.4 ± 85.3
Phospholipids (mg/100 ml)	202 ± 34	212 ± 15	225 ± 55	214 ± 43
Free fatty acid (mEq/L)	0.44 ± 0.17	0.41 ± 0.14	0.57 ± 0.28	0.53 ± 0.24
Total ketone bodies (μmol/L)	82.5 ± 74.4	54.9 ± 36.3	160.1 ± 167.7	179.4 ± 287.6
Glucose (mg/100 ml)	87.7 ± 6.8	87.5 ± 6.5	86.6 ± 7.0	87.0 ± 6.2
Hemoglobin A1c (%)	4.78 ± 0.33	4.87 ± 0.28	4.82 ± 0.25	4.91 ± 0.27*
Glycosylated albumin (%)	12.8 ± 1.4	13.0 ± 1.4	12.7 ± 1.5	13.3 ± 1.6*
Iinsulin (μU/mL)	6.42 ± 4.68	5.62 ± 3.25	6.53 ± 4.29	5.55 ± 3.63
Sodium (mEq/L)	141 ± 1	141 ± 1	140 ± 2	140 ± 1#
Potassium (mEq/L)	4.15 ± 0.30	4.08 ± 0.24	4.25 ± 0.36	4.08 ± 0.17
Chloride (mEq/L)	103 ± 2	102 ± 1	102 ± 2	102 ± 2
Calcium (mg/100 ml)	9.75 ± 0.44	9.66 ± 0.34	9.61 ± 0.24	9.46 ± 0.24*
Inorganic phosphate (mg/100 ml)	3.35 ± 0.29	3.22 ± 0.34	3.55 ± 0.51	3.45 ± 0.48
Magnesium (mg/100 ml)	2.30 ± 0.16	2.30 ± 0.16	2.30 ± 0.14	2.28 ± 0.13
White blood cell counts ($\times 10^3/\mu L$)	5.71 ± 1.42	6.27 ± 2.13	6.50 ± 2.43	5.70 ± 1.77
Red blood cell counts ($\times 10^4/\mu L$)	500 ± 52	500 ± 56	509 ± 70	508 ± 67
Hemoglobin (g/100 ml)	14.9 ± 1.3	14.9 ± 1.5	15.1 ± 1.7	15.0 ± 1.7
Hematocrit (%)	45.5 ± 3.7	45.8 ± 3.9	46.3 ± 4.7	46.1 ± 4.7
Mean corpuscular volume (fl)	91.3 ± 3.2	91.9 ± 3.3	91.3 ± 5.2	91.1 ± 4.5
Mean corpuscular hemoglobin (pg)	29.9 ± 0.9	29.9 ± 0.9	29.7 ± 1.6	29.6 ± 1.5
Mean corpuscular hemoglobin concentration (%)	32.8 ± 0.6	32.5 ± 0.8	32.5 ± 0.6	32.5 ± 0.7
Platelet count ($\times 10^4/\mu L$)	23.8 ± 2.6	23.6 ± 2.7	26.0 ± 4.8	25.6 ± 4.4

[1]Values are mean ± SD, n = 10 (control group), 11 (ALA group).

Significantly different compared with the value of control group, $^{\#}P < 0.05$ by unpaied *t*-test.

Significantly different compared with the value of Week 0, $^{*}P < 0.05$ by paired *t*-test.

Table 11 Results of Urinalysis (Experiment 2)

		Normal blood pressure										High-normal blood pressure and mild hypertension									
		Week 0					Week 4					Week 0					Week 4				
	Group	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Sugar	Control	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0
	ALA	8	0	0	0	0	8	0	0	0	0	11	0	0	0	0	11	0	0	0	0
Protein	Control	9	0	1	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0
	ALA	7	1	0	0	0	8	0	0	0	0	11	0	0	0	0	9	1	1	0	0
Urobilinogen	Control	-	10	0	0	0	-	10	0	0	0	-	10	0	0	0	-	10	0	0	0
	ALA	-	8	0	0	0	-	8	0	0	0	-	11	0	0	0	-	11	0	0	0

n = 10 (normal blood pressure, control group), 8 (normal blood pressure, ALA group), 10 (high-normal blood pressure and mild hypertension, control group), 11(high-normal blood pressure and mild hypertension, ALA group).
There were no significant differences between the two groups, or between Week 0 and 4.

1999 was 1.25 g/day. In this study, we administered a newly developed ALA-enriched oil at 42 g/day for 4 weeks and examined its safety in Experiment 2. The test oil contained 7.8 g of ALA, and this amount was about 6 times the average Japanese ALA intake reported by Tsuji *et al* [36].

Since ALA is likely to be oxidized, its excess intake is expected to promote oxidation in the body. In this study, however, no significant change in the lipid peroxide level was noted in the ALA group compared with the control group. Oxidation in the body may induce tissue inflammation. Therefore, we also measured high-sensitivity CRP, which is an index of inflammation. The intake of ALA had no effect on the high-sensitivity CRP level. These results suggest that intake of the test oil at 42 g/day does not promote oxidation or inflammation in the body.

While EPA has an antithrombotic effect, it has been reported to prolong the bleeding time if ingested excessively. Since ALA is an n-3 PUFA, as is EPA, we examined the effect of a high intake of ALA on blood coagulation. At Week 12 of Experiment 1, no significant differences were noted in the PT or APTT, which are indices of blood coagulation, between the control and ALA groups. In Experiment 2, no significant change was also noted in PT or APTT between the two groups. Kelley *et al.*[37] administered flax oil containing ALA at 20.5 g/day for 56 days and showed that the administration had no effect on not only PT or APTT but also bleeding time. In our study, intake of the test oil at 42 g/day was suggested to exert no adverse effect on blood clotting factors.

No significant differences were noted in any blood test item between the two groups excluding MCV (Experiment 1) and sodium (Experiment 2). The mean MCV value was significantly increased at Week 12, compared to Week 0, in the ALA group, but the change was small and within the normal range, suggesting that this change was not medically problematic. In the high-normotensive and mildly hypertensive subjects, the serum Na level showed a significant difference between the two groups, but it showed no significant change between before and after the study. Also, as the serum Na level was in the normal range in all subjects, the significant difference between the ALA and control groups is not considered to be of clinical significance. Significant changes were noted in 26 blood test items at the end of study, compared to those at Week 0 in Experiment 1 and 2. These changes in blood test items might have been due to dietary control or seasonal variation. No significant differences were noted in urinalysis findings between the two groups. Urinary protein was positive in 2 subjects of the ALA group after the study. However, the blood test results related to kidney function were normal in these subjects, and one of the subjects was in the menstrual phase. Although 54 adverse events were noted during the experiments, neither was related to the test meal. In addition, no symptom considered to be a side-effect of the intake of ALA was noted. These results indicate the safety of dietary ALA and the test oil.

5 CONCLUSION

In this study, ALA ingestion (2.6 g/day) decreased systolic and diastolic blood pressure in subjects with high-normal blood pressure and mild hypertension. ALA-enriched oil ingestion (42 g/day) had no negative influence on oxidation or blood coagulation in subjects with high-normal blood pressure or mild hypertension. These results suggest that ALA have an antihypertensive effect on blood pressure with no adverse effect in subjects with high-normal blood pressure and mild hypertension. These findings also suggest that this ALA-enriched test oil might safely con-

trol blood pressure close to the normal level.

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