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	アラプラス 糖ダウン
機能性関与成分名	５－アミノレブリン酸リン酸塩
表示しようとする機能性	本品は５－アミノレブリン酸リン酸塩を含み、高めの空腹時血糖値を正常に近づけることをサポートし、食後血糖値の上昇を穏やかにする機能があります。血糖値が高めの方に適しています。

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	アラプラス 糖ダウン
機能性関与成分名	５－アミノレブリン酸リン酸塩
表示しようとする機能性	本品は５－アミノレブリン酸リン酸塩を含み、高めの空腹時血糖値を正常に近づけることをサポートし、食後血糖値の上昇を穏やかにする機能があります。血糖値が高めの方に適しています。

２．補足説明

本最終製品の機能性の科学的根拠に関する根拠とした臨床試験は、査読付き論文として公表されている。

Higashikawa et al. 5-aminolevulinic acid, a precursor of heme, reduces both fasting and postprandial glucose levels in mildly hyperglycemic subjects. Nutrition 29: 1030-1036, 2013

臨床試験に用いた製品と販売しようとする製品の間に同一性が失われていない事に関して、次のように考察した。
臨床試験に使用したALA5mgカプセル､販売を予定しているALA15mgカプセルは、共にハードカプセル(カプセル素材HPMC)である。両製剤ではALA配合量が異なるものの、崩壊性試験の規格「日本薬局方 崩壊試験法(補助盤有) 水 20分以内)」は同一であり、其々規格に適合している。このことから、両者は製品として同一であると考える。

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Applied nutritional investigation

5-aminolevulinic acid, a precursor of heme, reduces both fasting and postprandial glucose levels in mildly hyperglycemic subjects

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ABSTRACT

Objective: The aim of this study was to evaluate the combined effects of 5-aminolevulinic acid phosphate (ALA-P) and iron on the glycemic index in mildly hyperglycemic adults.
Methods: This double-blind, randomized placebo-controlled trial comprised 212 subjects (ages 35–70 y, fasting plasma glucose 105–125 mg/dL or hemoglobin (Hb)A_{1c} 6.1%–7.1%). These participants were randomly assigned to four groups receiving either one of three doses of ALA-P and iron as sodium ferrous citrate (5 mg and 0.6 mg, 5 mg and 1.8 mg, or 15 mg and 1.8 mg, respectively) or a placebo, administered orally once a day over a 12-wk period.
Results: Fifteen mg ALA-P plus 1.8 mg iron decreased the fasting plasma glucose level (2.32 mg/dL, 95% confidence interval [CI], 0.24–4.42, $P = 0.029$), serum glycoalbumin (0.22%, 95% CI, 0.02–0.42; $P = 0.031$), and 2h-oral glucose tolerance test levels (14.2 mg/dL, 95% CI, 1.8–26.6; $P = 0.025$) more than the placebo. However, the levels of HbA_{1c}, fasting insulin, serum 1,5-anhydro-D-glucitol, and Homeostasis Model of Assessment-Insulin Resistance showed no appreciable changes. The participant numbers with impaired glucose tolerance and impaired fasting glucose decreased in the highest dosage group of ALA-P plus iron compared with the placebo group.
Conclusion: An oral intake of ALA would be a novel approach to prevent type 2 diabetes mellitus.

Introduction

Diabetes mellitus (DM) is rapidly increasing worldwide with a prevalence of 6.4% (285 million adults) in 2010. It is estimated that it will increase to 7.7% (439 million adults) by 2030 [1]. Several studies have indicated that individuals with prediabetes also may suffer with diabetic complications, such as retinopathy and peripheral neuropathy [2,3]. According to the current treatment guidelines, borderline diabetes becomes scarcely a target of the medical treatment of the medicines for diabetes treatment. Early care is important for individuals with prediabetes to prevent diabetes-related complications and/or the onset of DM.

In the present clinical trial, we evaluated 5-aminolevulinic acid (ALA), which is dissimilar to any of current antidiabetic agents in clinical use. ALA is endogenous to both animals and plants and is the first compound produced in the heme biosynthetic pathway. The responsible enzyme is ALA synthase, which is rate limiting in this pathway. Iron is ultimately incorporated into protoporphyrin to form heme in mitochondria. Heme is a major component of hemoglobin, and of other hemoproteins including myoglobin and cytochrome. Cytochromes play an important role in the electron transport chain in mitochondria, and cytochrome P450s function as metabolic enzymes involved in the oxidation and detoxification of many xenobiotics and endogenous compounds, and in fatty acid desaturation. Hence, as the precursor of heme, ALA is an essential molecule in human and other vertebrates, and may be associated with various metabolic disorders. Indeed, it has been reported that the administration of ALA together with iron stimulates murine hair growth [4].

It has been shown that mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes mellitus (T2DM) [5]. Whether the impaired mitochondrial function is a cause or consequence of insulin resistance is not clear yet. However, the

FH and MS designed the research, and had primary responsibility for the final content. FH, MN, and TA conducted the research. MN and TA were responsible for the data collection and participants. TT provided the capsules used in the study. FH analyzed the data, and wrote the paper.

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defective insulin signaling may promote mitochondrial dysfunction because the mitochondrial function is lower in patients with genetically defective insulin receptors than in healthy controls [6]. Porphyria cutanea tarda is caused by abnormalities in heme biosynthesis and often is accompanied by DM [7]. Moreover, the activity of ALA dehydratase is found to be reduced in a streptozotocin-induced diabetic rat model [8–10]. We thus speculated that the heme biosynthetic pathway may be associated with glucose metabolism, and that mitochondrial activity may be enhanced by the supplementation of ALA whereby the heme biosynthesis is augmented. In the present study, we tried to substantiate the hypothesis by the combined oral administration of 5-aminolevulinic acid phosphate (ALA-P) and iron in a human cohort.

Materials and methods

Participants

Healthy volunteers were recruited from the local community in Hiroshima, Japan through a series of advertisements. The inclusion criteria were as follows: 1) healthy men or women between the ages of 35 and 70 y; and 2) a fasting plasma glucose (FPG) level of 105 mg/dL to 125 mg/dL or a hemoglobin (Hb)A_{1c} of 6.1% to 7.1%. The following exclusion criteria were applied: 1) taking medication for diabetes 2) a body mass index (BMI) <18 kg/m^2 or >30 kg/m^2; 3) pregnant or breastfeeding; 4) renal or hepatic dysfunction; 5) heart disease; 6) history of porphyria, hemochromatosis, or viral hepatitis; 7) functional food intake that may affect plasma glucose level; and 8) participation in any other clinical trial within 90 d of the commencement of this study. The study protocol was approved by the Ethics Committee of Hiroshima University and performed in accordance with the guidelines of the Helsinki Declaration. All participants provided written informed consent before the start of the trial.

Study design

The current trial was a double-blind, randomized, placebo-controlled, parallel-group study conducted at Hiroshima University Hospital, Hiroshima, Japan, from May 2010 to December 2010. The purified ALA-P used in this study is a fermentation product of the photosynthetic bacterium *Rhodobacter sphaeroides*. The capsules used to administer the ALA-P and iron in the form of sodium ferrous citrate were provided by SBI Pharmaceuticals Co., Ltd., Tokyo, Japan.

The 212 eligible participants assessed in this trial were enrolled and stratified according to sex and the HbA_{1c} level (of $\geq$6.3% or less) at baseline by an investigator, and assigned to one of four treatment groups (placebo; 5 mg ALA-P plus 0.6 mg iron; 5 mg ALA-P plus 1.8 mg iron; or 15 mg ALA-P plus 1.8 mg iron) by means of blocked randomization with a block size of 4 and an allocation ratio of 1:1:1:1 using computer-generated random numbers. Randomization assignments were carried out by non-clinical staff who had no subsequent involvement with the trial. The volunteers and outcome assessors were kept blinded to the allocation. The participants were instructed to maintain their ordinary dietary habits during the study period and to take three capsules per day after dinner for 12 wk. They were also asked not to donate blood during the trial. Clinical visits were scheduled every 4 wk at Hiroshima University Hospital, at which time physical examinations, hematologic assessments, serum biochemical measurements, and urinalyses were performed. The oral glucose tolerance test (OGTT), in addition to the fasting insulin, serum adiponectin, leptin, and resistin level tests, was performed at weeks 0 and 12. Blood samples were taken after an overnight fast (of at least 9 h). The body fat percentage was measured using a body composition analyzer (BC-118E; Tanita, Tokyo, Japan). HbA_{1c} values were converted from Japan Diabetes Society values to those of the National Glycohemoglobin Standardization Program by adding 0.4% [11]. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) $\times$ fasting glucose (mg/dL)/405. The participants were provided daily and dated record forms throughout the study period, including 2 wk of run-in period and 4 wk of follow-up period, to make a note of their capsule consumption and health conditions. Adverse events (AEs) that newly emerged or worsened after the intervention were assessed as grade 1 (mild), 2 (moderate), or 3 (severe), as according to the Common Terminology Criteria for Adverse Events version 3.0. Compliance with the treatment regimen was assessed using participants' daily records.

Statistical analysis

The sample size was calculated at 45 participants per group with 90% power and a significance level of 0.05 using the two-sided Student's *t* test to detect a 5% difference with an estimated SD of 8% between groups. The baseline characteristics were compared among groups by one-way analysis of variance for continuous variables. The Fisher's exact test was used for all categorical variables to assess differences among the four study groups. Data analysis was carried out as an intention to treat, and the multiple imputation method was applied to missing data. The mean values during the intake (weeks 4, 8, and 12) were used to compare with each baseline for main outcomes. A general linear model of analysis of covariance (ANCOVA) followed by a post hoc multiple *t* test between all paired groups were applied to changes from the baseline of the main study end points including the FPG, glycoalbumin, HbA_{1c}, 2-h glucose concentration after the OGTT (2h-OGTT), fasting insulin, HOMA-IR, and 1,5-anhydro-D-glucitol (1,5-AG) levels, by using each baseline value and the participant's sex as covariates. The 2h-OGTT changes were assessed by subgrouping participants based on baseline values, all participants, participants with $\geq$140 mg/dL, or with $\geq$200 mg/dL, and were calculated as the mean change from the baseline adjusted by ANCOVA as described previously. The Fisher's exact test with a Bonferroni correction was applied to the change in subject number with both an 2h-OGTT $\geq$140 mg/dL and an FPG $\geq$110 mg/dL. Statistical analyses were performed using SPSS (version 17.0, SPSS Japan, Inc.), data are expressed as mean $\pm$ SD (for tables) or SEM (for figure), and $P < 0.05$ was considered significant.

Results

Figure 1 shows the profile of the current study trial. Of the 701 individuals who expressed an interest in participating in the

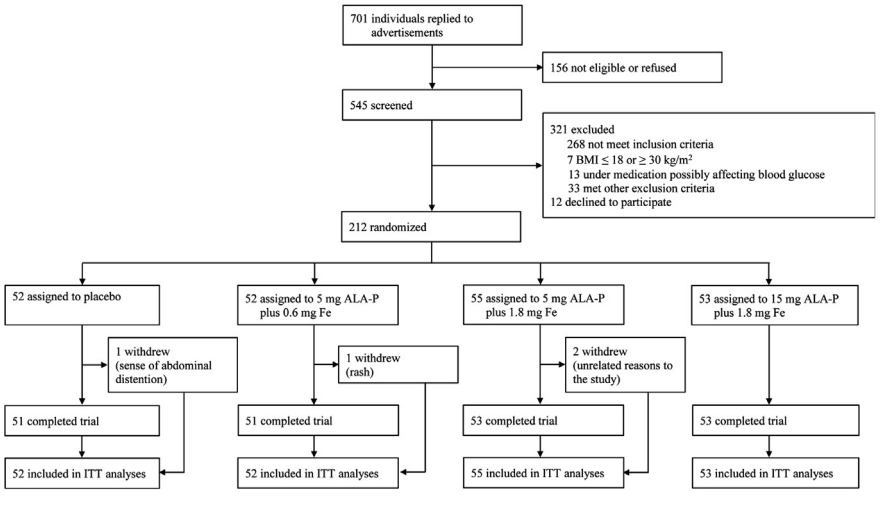

Fig. 1. Trial profile.

Table 1
Baseline characteristics of the study participants

	Placebo (n = 52)	Treatment			*P*
		5 mg ALA-P plus 0.6 mg Fe (n = 52)	5 mg ALA-P plus 1.8 mg Fe (n = 55)	15 mg ALA-P plus 1.8 mg Fe (n = 53)	
Age (years)	59.5 ± 7.7	59.3 ± 7.7	58.7 ± 7.2	59.1 ± 8.3	
Sex					0.92
Male	16 (30.8%)	16 (30.8%)	19 (34.5%)	19 (35.8%)	–
Female	36 (69.2%)	36 (69.2%)	36 (65.5%)	34 (64.2%)	–
Current smokers	4 (7.7%)	8 (15.4%)	8 (14.5%)	4 (7.5%)	0.41
Body weight (kg)	58.6 ± 9.3	59.7 ± 11.0	58.1 ± 10.9	58.4 ± 10.3	0.87
BMI (kg/m^2)	23.3 ± 2.7	23.5 ± 3.1	23.1 ± 2.7	22.9 ± 2.7	0.71
Body fat (%)	27.5 ± 6.8	27.9 ± 7.6	27.5 ± 7.3	26.5 ± 7.7	0.79
Abdominal circumference (cm)	85.2 ± 8.0	85.4 ± 8.7	84.0 ± 7.6	83.8 ± 7.5	0.67
Systolic blood pressure (mmHg)	133.0 ± 17.6	134.1 ± 17.8	129.1 ± 14.5	131.8 ± 15.6	0.46
Diastolic blood pressure (mmHg)	80.3 ± 9.7	81.2 ± 10.8	78.1 ± 8.2	80.1 ± 9.7	0.43
Heart rate (beats per min)	75.8 ± 11.3	74.6 ± 10.6	75.2 ± 12.7	75.8 ± 10.7	0.95
Total cholesterol (mg/dL)	221.3 ± 31.1	225.4 ± 35.2	230.3 ± 36.2	216.9 ± 33.7	0.21
LDL cholesterol (mg/dL)	136.7 ± 30.5	148.0 ± 34.8	143.1 ± 35.6	134.2 ± 30.3	0.13
HDL cholesterol (mg/dL)	68.7 ± 15.1	63.9 ± 12.1	71.8 ± 17.4	68.5 ± 13.0	0.049
LDL/HDL ratio	2.11 ± 0.75	2.40 ± 0.71	2.13 ± 0.81	2.02 ± 0.56	0.037
Triglyceride (mg/dL)	110.6 ± 74.7	112.7 ± 52.9	109.8 ± 54.2	98.5 ± 48.3	0.62
Free fatty acids (mEq/L)	0.679 ± 0.214	0.605 ± 0.203	0.659 ± 0.226	0.611 ± 0.240	0.32
Serum adiponectin (μg/mL)	6.88 ± 4.10	6.69 ± 3.79	7.36 ± 3.76	7.09 ± 4.01	0.84
Serum leptin (ng/mL)	7.75 ± 5.39	8.86 ± 5.93	7.89 ± 4.72	7.63 ± 4.75	0.68
Serum resistin (ng/mL)	4.06 ± 4.13	3.75 ± 2.93	3.78 ± 4.90	3.42 ± 2.74	0.85
AST (U/L)	21.3 ± 4.7	23.3 ± 6.9	23.9 ± 5.7	22.0 ± 6.7	0.11
ALT (U/L)	19.1 ± 7.0	22.1 ± 11.3	21.3 ± 10.2	20.0 ± 10.5	0.42
γ-GTP (U/L)	31.9 ± 23.7	30.1 ± 18.7	38.7 ± 35.1	40.6 ± 83.8	0.63
Serum total bilirubin (mg/dL)	0.91 ± 0.28	0.87 ± 0.39	0.94 ± 0.28	0.93 ± 0.26	0.70
Serum creatinine (mg/dL)	0.743 ± 0.144	0.717 ± 0.120	0.716 ± 0.149	0.718 ± 0.129	0.86

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GTP, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein
Data are mean ± SD or n (%)

study, 156 did not satisfy the inclusion criteria on their application forms or cancelled their involvement. A total of 545 individuals who participated in the study orientation sessions were subsequently screened and 212 individuals who satisfied the inclusion criteria were enrolled in the study. The characteristics of the study participants are summarized in Table 1. Significant differences among the four groups at baseline were observed for only high-density lipoprotein cholesterol (HDL-C) and the low-density lipoprotein (LDL)/HDL ratio. Approximately two-thirds of the participants were women and all were Japanese. Three participants dropped out after the week 4 and one at week 12 visits, respectively, due to a rash (5 mg ALA-P plus 0.6 mg iron group), flatulence (placebo group), and reasons unrelated to the study (5 mg ALA-P plus 1.8 mg iron group). The remaining 208 subjects (98%) therefore completed the study. The compliance rates in taking three capsules per day were 96.5%, 96.4%, 97.0%, and 96.6%, in placebo, 5 mg ALA-P plus 0.6 mg iron, 5 mg ALA-P plus 1.8 mg iron, and 15 mg ALA-P plus 1.8 mg iron groups, respectively, with little change observed throughout the study.

The effects of the administration of ALA-P supplemented with iron at the given amounts on the main study end points and hemoglobin level are summarized in Table 2. The FPG levels declined in all groups but the degrees of reduction in the 5 mg ALA-P plus 1.8 mg iron and 15 mg ALA-P plus 1.8 mg iron groups were higher than those in the placebo group ($P = 0.036$ and $P = 0.029$, respectively, Table 2). Glycoalbumin, which represents an average of the circulating plasma glucose level in the shorter term (2 wk) compared with the HbA_{1c} level (1–2 mo), was reduced in 15 mg ALA-P plus 1.8 mg iron group ($P = 0.031$ versus placebo group). Conversely, the HbA_{1c} level did not change much in any treatment group. Changes in the values for fasting insulin, the serum 1,5-AG level, and the HOMA-IR did not differ among the four study groups. A small increase in the hemoglobin level was observed in 15 mg ALA-P plus 1.8 mg iron group (Table 2).

The 2h-OGTT was also found to decrease in the 15 mg ALA-P plus 1.8 mg iron group ($P = 0.025$ versus placebo; Fig. 2). Although the 2h-OGTT levels were in fact elevated in all groups except for the 15 mg ALA-P plus 1.8 mg iron group following the intervention, these were within the range of a measurement error or individual variations. When we applied subgroup analyses, impaired glucose tolerance (IGT) and "diabetic type" (≥140 mg/dL and ≥200 mg/dL of 2h-OGTT levels, respectively; set by the JDS), as determined by the OGTT, were found to be dramatically improved in the 15 mg ALA-P plus 1.8 mg iron group, and were measured at 12.2 ± 5.5 mg/dL and 22.7 ± 9.2 mg/dL reductions compared with the placebo group, which showed 7.6 ± 5.7 mg/dL and 6.9 ± 9.5 mg/dL increases, respectively. As a consequence of the reduction in both the FPG and 2h-OGTT levels through a combination of ALA-P and iron, the number of participants who met the conditions of a 2h-OGTT ≥140 mg/dL and fasting glucose ≥110 mg/dL that includes IGT, impaired fasting glucose (IFG), both IGT and IFG, or diabetic type, declined in the 15 mg ALA-P plus 1.8 mg iron group ($P = 0.011$; Fig. 3).

Although the effects of a combined consumption of ALA-P and iron on lipid metabolism also were assessed as secondary outcomes, meaningful changes were not detected in any of the following indices, which were originally within the normal range at baseline: total cholesterol; LDL-cholesterol; HDL-C; LDL/HDL ratio; triglyceride; free fatty acids; serum adiponectin; leptin; and resistin (data not shown).

Additional serum indices and the results of physical examinations were monitored to detect possible AEs of ALA-P and iron intake. A slight elevation of the serum total bilirubin (grade 1: >upper limits of normal [ULN] and ≤1.5 × ULN) was detected in

Table 2
Glycemic status and hemoglobin level of the subjects during the study

Variable	Placebo (n = 52)	Treatment		
		5 mg ALA-P plus 0.6 mg Fe (n = 52)	5 mg ALA-P plus 1.8 mg Fe (n = 55)	15 mg ALA-P plus 1.8 mg Fe (n = 53)
Fasting plasma glucose (mg/dL)				
Baseline	108.1 ± 12.8	107.8 ± 11.1	110.9 ± 11.9	105.9 ± 7.4
Intervention	106.6 ± 11.5	106.5 ± 11.4	106.4 ± 9.3*	102.7 ± 7.8*
Glycoalbumin (%)				
Baseline	16.6 ± 1.6	16.8 ± 1.8	17.0 ± 1.7	16.4 ± 1.3
Intervention	16.4 ± 1.7	16.5 ± 1.7	16.6 ± 1.8	16.0 ± 1.3*
HbA1c (%)				
Baseline	6.16 ± 0.36	6.15 ± 0.29	6.13 ± 0.35	6.09 ± 0.30
Intervention	6.07 ± 0.34	6.09 ± 0.27	6.10 ± 0.36	6.05 ± 0.26
Fasting insulin (μU/mL)				
Baseline	6.1 ± 3.1	6.4 ± 3.6	6.0 ± 3.2	6.0 ± 2.7
Intervention	6.0 ± 3.4	6.4 ± 3.4	6.0 ± 3.2	5.8 ± 3.0
HOMA-IR				
Baseline	1.67 ± 1.00	1.74 ± 1.04	1.66 ± 1.00	1.58 ± 0.76
Intervention	1.58 ± 0.95	1.69 ± 0.99	1.58 ± 0.92	1.46 ± 0.83
1,5-AG (μg/dL)				
Baseline	18.1 ± 5.8	17.9 ± 6.4	18.5 ± 7.3	18.2 ± 6.8
Intervention	17.7 ± 5.4	17.7 ± 6.0	18.0 ± 6.9	18.1 ± 6.9
Hemoglobin (g/dL)				
Baseline	13.89 ± 1.49	13.77 ± 0.97	13.84 ± 1.25	13.86 ± 1.16
Intervention	13.73 ± 1.59	13.73 ± 1.02	13.80 ± 1.17	13.97 ± 1.20*

Data are mean ± SD
* $P<0.05$ *vs.* placebo.

more cases in the 5 mg ALA-P plus 1.8 mg iron and 15 mg ALA-P plus 1.8 mg iron groups ($P < 0.05$ versus placebo by Fisher's exact test with Bonferroni correction; Table 3). Bilirubin is a final metabolic product of heme and ALA is a precursor of heme. Six of 9 participants in the 5 mg ALA-P plus 1.8 mg iron group and 9 of 10 individuals in the 15 mg ALA-P plus 1.8 mg iron group experienced a grade 1 AE only once during the intervention.

No differences in the hepatic indices (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transpeptidase, lactate dehydrogenase, choline esterase, and alkaline phosphatase levels), serum mineral compositions (Na^+, Cl^-, and K^+), other serum indices (amylase, total protein, blood urea nitrogen, serum creatinine, and uric acid), or physical characteristics (BMI, body fat, abdominal circumference, and blood pressure), were observed in the ALA-P plus iron treatment groups when compared with the placebo treatment group. In terms of hematologic values, the red blood cell and hematocrit levels were slightly increased in the 15 mg ALA-P plus 1.8 mg iron group compared with the placebo group (red blood cell: $0.02 \pm 0.15 \times 10^6$ /μL versus $-0.07 \pm 0.19 \times 10^6$ /μL, $P < 0.05$; hematocrit: 0.45 ± 1.51 % versus -0.52 ± 1.70 %, $P < 0.01$ by Dunnett's test), like hemoglobin. The frequencies of various other self-reported symptoms were found not to differ among the four groups (Table 4).

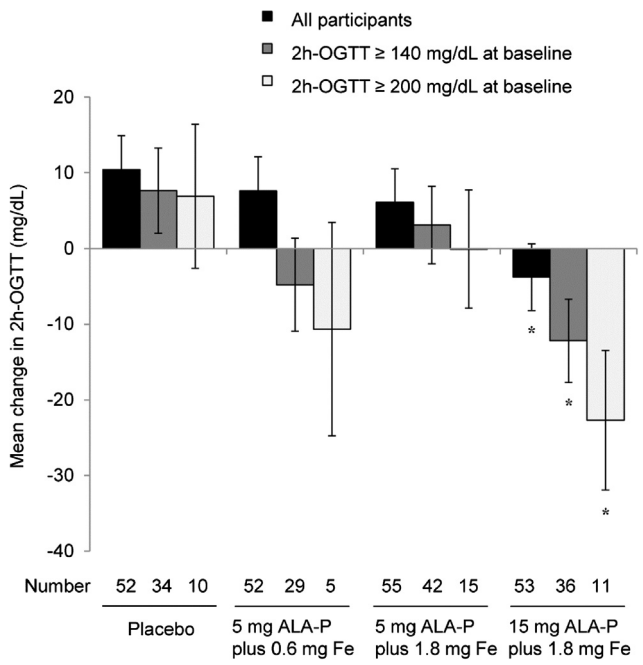

Fig. 2. Mean change from the baseline of the 2-h glucose concentration after the 75 g oral glucose tolerance test (2h-OGTT). Data are mean ± SEM (adjusted for baseline and participant sex by ANCOVA). *$P < 0.05$ versus placebo.

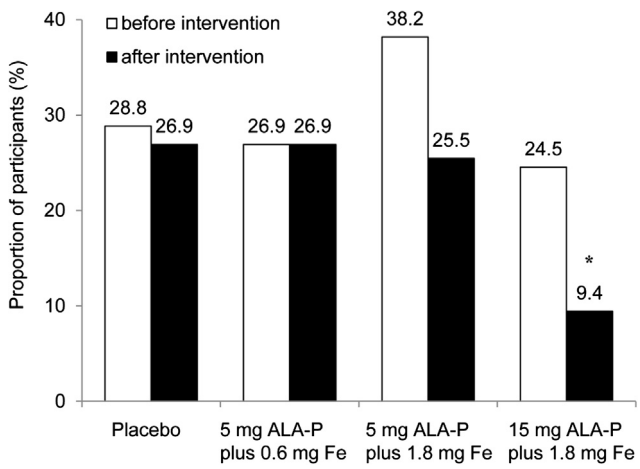

Fig. 3. Proportion of participants with both a 2h-OGTT ≥140 mg/dL and a fasting plasma glucose ≥110 mg/dL before and after the interventions. *$P = 0.011$ versus placebo by Fisher's exact test with a Bonferroni correction.

Table 3
Number of subjects showing adverse events that were possibly related to the study design or treatments

	Placebo (n = 52)	Treatment			*P*
		5 mg ALA-P plus 0.6 mg Fe (n = 52)	5 mg ALA-P plus 1.8 mg Fe (n = 55)	15 mg ALA-P plus 1.8 mg Fe (n = 53)	
White blood cell count					0.79
Grade 1	5 (10%)	8 (15%)	9 (16%)	6 (11%)	
Grade 2	1 (2%)	0	2 (4%)	1 (2%)	
Hemoglobin					0.57
Grade 1	3 (6%)	5 (10%)	2 (4%)	2 (4%)	
Grade 2	1 (2%)	0	0	0	
Platelet					0.37
Grade 1	1 (2%)	0	1 (2%)	0	
AST					0.73
Grade 1	1 (2%)	4 (8%)	2 (4%)	3 (6%)	
Grade 2	0	0	1 (2%)	1 (2%)	
Grade 3	1 (2%)	0	0	1 (2%)	
ALT					0.28
Grade 1	3 (6%)	2 (4%)	4 (7%)	0	
Grade 2	1 (2%)	0	0	1 (2%)	
γ-GTP					0.69
Grade 1	1 (2%)	1 (2%)	3 (5%)	2 (4%)	
Grade 2	1 (2%)	0	0	2 (4%)	
Alkaline phosphatase					0.71
Grade 1	1 (2%)	1 (2%)	0	1 (2%)	
Serum total bilirubin					0.001
Grade 1	1 (2%)	1 (2%)	9 (16%)	10 (19%)	
Grade 2	3 (6%)	0	1 (2%)	1 (2%)	
Grade 3	0	1 (2%)	0	0	
Serum albumin					0.71
Grade 1	1 (2%)	1 (2%)	0	1 (2%)	
Serum amylase					0.30
Grade 1	3 (6%)	0	1 (2%)	2 (4%)	
Grade 2	0	0	0	1 (2%)	
Serum sodium					0.31
Grade 1	8 (15%)	13 (25%)	15 (27%)	16 (30%)	
Serum potassium					0.33
Grade 1	3 (6%)	4 (8%)	2 (4%)	7 (13%)	
Serum creatinine					0.65
Grade 1	4 (8%)	6 (12%)	3 (5%)	3 (6%)	
Serum uric acid					0.16
Grade 1	3 (6%)	4 (8%)	7 (13%)	1 (2%)	

Data are number (%). *P* values were obtained via the Fisher's exact test
Grades 1–3 were assigned according to the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0)

Discussion

In addition to a diagnosis of DM, a condition of IGT, IFG, or both is also a concern with regards to the pathogenesis of diabetic complications [2,3]. It should be noted that IGT is common in individuals with a normal fasting glucose level (100–109 mg/dL) and indicates a high risk for developing DM in the future [11]. Hence, alleviating glucose intolerance in particular in prediabetic individuals may be an effective prophylactic approach to the onset of DM.

In this study, we evaluated the effects of a combination of ALA-P supplemented with iron on the glycemic status of individuals with prediabetes. The decrease in FPG levels was observed following the oral intake of ALA-P supplemented with iron. The fasting insulin levels were not increased in the four treatment groups under study, indicating that the observed reduction in the FPS level was not caused by increased insulin secretion. It has been suggested in a number of studies that postprandial glucose excursion may be more important than the FPG level for determining cardiovascular events [12–14]. Interestingly, the glucose tolerance levels in our present study participants, as judged by 2h-OGTT, were notably improved by the highest dose intake of ALA-P supplemented with iron (15 mg ALA-P plus 1.8 mg iron group) within 12 wk, particularly in those with a more severe impairment of glucose tolerance (2h-OGTT $\geq$200 mg/dL) at baseline. As described earlier, because IGT is a major risk factor for the development of T2DM and diabetic complications, this improvement in the 2h-OGTT is of central importance for prediabetic individuals. Likewise, the number of participants with both IFG and IGT (or worse) significantly declined in the highest dosage treatment group, suggesting that the oral administration of ALA-P supplemented with iron probably reduces the risks for and prevalence of DM.

The serum glycoalbumin levels also were found to be decreased in the highest ALA-P dosage group, whereas no HbA_{1c} reduction was detected in these subjects. This may be explained by the fact that the HbA_{1c} measurement does not reflect rapid changes in the plasma glucose levels due to the approximately 120-d life span of red blood cells, and/or the fact that glycoalbumin reflects postprandial glucose excursion, whereas HbA_{1c} does not [15,16]. Furthermore, the HbA_{1c} level might be altered by the increase in hemoglobin in the 15 mg ALA-P plus 1.8 mg iron group. Thus, although HbA_{1c} is a convenient index, OGTT and serum glycoalbumin seem to be more valuable than HbA_{1c} to monitor the short-term treatment effects and achievement of glycemic control. No significant differences were observed in the HOMA-IR values among our treatment groups, contrary to the expectation that an improved glucose tolerance, as revealed by the OGTT, should also be reflected in the HOMA-IR. This may be because the baseline HOMA-IR values (1.66 $\pm$ 0.95) were not

Table 4
Frequency of self-reported symptoms that may have been related to the study design or supplements used

	Placebo (n = 52)	Treatment			*P*
		5 mg ALA-P plus 0.6 mg Fe (n = 52)	5 mg ALA-P plus 1.8 mg Fe (n = 55)	15 mg ALA-P plus 1.8 mg Fe (n = 53)	
Gastrointestinal disorders					
Constipation	7 (13%)	5 (10%)	4 (7%)	5 (9%)	0.77
Diarrhea	12 (23%)	14 (27%)	9 (16%)	8 (15%)	0.39
Nausea/vomiting	3 (6%)	3 (6%)	3 (5%)	4 (8%)	0.98
Abdominal pain	5 (10%)	10 (19%)	9 (16%)	10 (19%)	0.51
Flatulence	3 (6%)	2 (4%)	3 (5%)	7 (13%)	0.33
Pain	11 (21%)	7 (13%)	5 (9%)	9 (17%)	0.36
Skin and subcutaneous-tissue disorders	4 (8%)	1 (2%)	5 (9%)	3 (6%)	0.46
General disorders					
Fatigue	6 (12%)	9 (17%)	6 (11%)	7 (13%)	0.78
Edema peripheral	0	0	2 (4%)	0	0.25
Nervous system disorders					
Dizziness	4 (8%)	1 (2%)	3 (5%)	2 (4%)	0.60
Headache	11 (21%)	10 (19%)	12 (22%)	12 (23%)	0.98
Sleep phase rhythm disturbance	1 (2%)	5 (10%)	6 (11%)	4 (8%)	0.28
Inflammatory disorders					
Stomatitis	0	1 (2%)	4 (7%)	2 (4%)	0.22
Irritation of bladder	3 (6%)	0	1 (2%)	0	0.10
Ear	0	0	1 (2%)	0	1.0
Asthenopia	0	1 (2%)	0	3 (6%)	0.13
Psychiatrtic disorders	1 (2%)	1 (2%)	0	0	0.37

Data are number (%). *P* values were obtained via the Fisher's exact test

high among the participants, considering that the normal lower limit is 1.6. Hence, the effects of ALA-P may be restricted because of this tight margin between the baseline and normal range. They may become detectable when applied to participants with higher insulin resistance, or a longer-term consumption of ALA-P and iron may be necessary to detect a reduction in HOMA-IR.

It has been established that the pro-oxidant properties of iron may play a causal role in the onset of T2DM [17–20]. In our present clinical study, the iron was administered in the form of sodium ferrous citrate together with ALA-P. The intake of non-heme iron was found to induce a beneficial effect rather than a harmful one judging from our finding that the fasting plasma glucose was improved in the 5 mg ALA-P plus 1.8 mg iron group but not in the 5 mg ALA-P plus 0.6 mg iron group. This result may be consistent with the previous observations that the dietary intake of heme iron, but not non-heme iron, is associated with an increased risk of T2DM [20–22], and that the intake of non-heme iron is inversely associated with the incidence of T2DM [23].

Mitochondrial dysfunction has been reported to be linked to insulin resistance and DM [5,6]. The reduction in ALA-dehydratase activity reported in diabetic rats [8–10], and the high prevalence of diabetes or IGT in porphyria cutanea tarda [7], may indicate a connection between the heme biosynthetic pathway and diabetes. Heme deficiency may be a factor in neurodegenerative process [24,25], and it has been suggested that hyperinsulinemia and/or abnormal glucose tolerance are associated with a higher risk for Alzheimer's disease [26,27]. Cellular respiration consists of three stages: glycolysis, the citric acid cycle, and the electron transport chain. Heme is an essential component of the mitochondrial electron transport chain during cellular energy (ATP) production. It has been shown, at least in *Escherichia coli*, that heme biosynthesis is coupled to the electron transport chains for energy generation [28]. If heme biosynthesis is enhanced by the intake of ALA supplemented with iron, the electron transport chain may be activated, and glycolysis may in turn be stimulated, resulting in an increased consumption of serum glucose. It has also been shown that ALA administration in mice gives rise to an increased cytochrome c oxidase (COX) activity in the liver mitochondria [29]. COX, which requires heme for its activity, is one of the enzymes involved in the electron transport chain. The deficiency of heme decreased the activity and protein content of mitochondrial COX [30,31], and the lack of COX activity in mitochondria has been proposed to be associated with aging and diverse diseases including DM, Alzheimer's disease, and cancer [32–35]. The inhibition of COX increases the oxidative and nitrosative stress in vascular endothelial cells [36]. Consequently, the oral administration of ALA-P might be a viable therapy for diabetes by enhancing the COX activity levels, which is followed by reduction of the reactive oxygen species–induced damage of DNA, protein, and/or lipids, and ultimately of the cells themselves.

The results of this study have demonstrated that the combined consumption of ALA-P and iron improves the fasting and postprandial glucose levels in human without any appreciable side effects. The effectiveness of the ALA-P and iron intake is not due to the stimulation of insulin secretion, and it would be advantageous not to damage the β cells. Because ALA-P is speculated to function in different mechanisms than the current antidiabetic agents, the results of a combination therapy of ALA-P and these agents will be of interest in the future.

Conclusion

ALA-P supplemented with iron has potential as a novel preventive strategy for the onset of T2DM in persons who are at risk for this disease.

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