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Article

Safety of Oral Administration of 5-Aminolevulinic Acid Phosphate Combined with Ferrous Iron in Healthy Subjects: An Open-Label Trial

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Abstract: The combination of 5-aminolevulinic acid (5-ALA) phosphate and sodium ferrous citrate (SFC) has been approved as an ingredient in dietary supplements in several countries, owing to its broad applicability in healthcare. This study aimed to assess the safety of oral administration of 5-ALA and SFC in healthy adults at doses several times higher than those commercially available. This study included 22 healthy subjects (11 males and 11 females) aged 21–59. Doses of 250 mg 5-ALA phosphate and 143.4 mg SFC (15 mg Fe) per day were administered orally for 28 days. Blood tests, urinalysis, and medical interviews were performed to assess safety. No test compound-related adverse events or abnormal changes were observed, except for elevated serum Fe levels, which were mild-to-moderate and transient. In conclusion, the combined oral administration of 5-ALA phosphate and SFC in healthy adults was safe and well-tolerated at the dose and duration investigated in this study.

Keywords: 5-aminolevulinic acid; sodium ferrous citrate; safety; iron; dietary supplement

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1. Introduction

5-Aminolevulinic acid (5-ALA) is a precursor for the biosynthesis of tetrapyrrole compounds such as heme, chlorophyll, and cytochromes, which are essential for various biological functions, including oxygen transport, electron transfer reactions, and drug metabolism [1]. Orally administered 5-ALA reaches the systemic circulation at approximately 60% [2] and is incorporated into heme by the endogenous biosynthesis pathway in humans [3,4]. Currently, two forms of 5-ALA, 5-ALA hydrochloride and 5-ALA phosphate, are available as a drug and a dietary supplement, respectively. 5-ALA hydrochloride is used as an intraoperative cancer photodiagnostic drug, in which the heme precursor 5-ALA-derived protoporphyrin IX (PpIX) selectively accumulates in tumor cells and emits fluorescence in response to specific wavelengths. 5-ALA phosphate is a less irritating salt than 5-ALA hydrochloride and is widely used as a dietary supplement in combination with iron in Japan, Southeast Asia, and the Middle East. Sodium ferrous citrate (SFC), a divalent iron compound, is often used as an iron source and is co-administered with 5-ALA as a supplement.

Although several studies below have tested 5-ALA phosphate for treating some diseases, there is limited scientific evidence supporting the safety of repetitive oral administration of 5-ALA phosphate with iron as a dietary supplement in the general population.

It is beneficial for patients with type 2 diabetes and individuals with prediabetes, and no severe adverse events have been observed at maximum 5-ALA phosphate doses of 200 mg/day for 12 weeks [5], 15 mg/day for 12 weeks [6], and 50 mg/day for 12 weeks [7]. A high dose of 5-ALA phosphate/SFC was administered to COVID-19 patients for 14 days. Doses of 250 mg 5-ALA phosphate/145 mg SFC three times per day for the first seven days and 150 mg 5-ALA phosphate/87 mg SFC three times per day for the next seven days resulted in a limited effect on COVID-19 symptoms and 22.7% of adverse events, mainly gastrointestinal symptoms, such as decreased appetite, constipation, and vomiting [8]. Moreover, 5-ALA phosphate/SFC was orally administered to 100 patients with sarcopenia at various doses of up to 150 mg 5-ALA-phosphate for 12 weeks. There was no change in the skeletal muscle mass index, but grip strength improved. The frequency of adverse events did not differ between the groups [9]. Furthermore, when patients with late-onset hypogonadism were treated orally with 30 mg 5-ALA-phosphate/3.6 mg Fe per day for eight weeks, a therapeutic effect was observed, and there were no treatment-emergent adverse events [10]. Thus, none of these studies simultaneously included the following conditions: sufficiently high doses, adequate administration periods, and healthy individuals as subjects.

The accumulation of 5-ALA in the body is known to be associated with acute intermittent porphyria. 5-ALA may cause neurotoxicity by competing with GABA for binding synaptic receptors in the central nervous system because of its structural similarity to GABA. 5-ALA treatment resulted in decreased GABAA receptor density in the rat brain and neuronal cells [11]. Moreover, 5-ALA induces oxidative stress by altering iron metabolism in rat brain tissue and damages myelinating Schwann cells [12,13]. Animal studies suggest that 5-ALA may elevate AST, ALT, and total bilirubin levels [14]. Therefore, it is crucial to ensure the safety of 5-ALA for users.

Hence, we investigated whether a four-week consecutive administration of a combination of 5-ALA phosphate (250 mg) and SFC (143.4 mg) at relatively high doses when considered as marketed 5-ALA supplements has deleterious effects in healthy adults for safe use as a dietary supplement.

2. Results

Of the 117 individuals who responded to the advertisement, 28 participated in the study orientation sessions. All 28 participants signed an informed consent form and met the inclusion and exclusion criteria. According to the study protocol, 22 of 28 patients (11 males and 11 females) were enrolled in the study in the order of application. The background of the study participants is summarized in Table 1. All participants completed the study without dropouts (Figure 1). According to the subject diaries, the compliance with capsule intake was 97.6 $\pm$ 5.8%, of which 15 displayed 100% compliance; 4 displayed 96.4% compliance; 1 displayed 89.3% compliance; and 1 displayed 75.0% compliance, not taking the test capsules for six consecutive days (days 21–26) due to diarrhea and abdominal pain. The last male participant did not submit his diary; therefore, the capsule consumption rate was unknown. However, his serum Fe and ferritin levels were elevated during the capsule intake period and then decreased at week 6 in the post-intervention observation period. According to the changes in serum Fe and ferritin, he should have consumed the capsules at a rate that is not low.

Table 1. Background of the study subjects.

	Total (n = 22)	Males (n = 11)	Females (n = 11)
Age (y)	43.5 ± 12.1 (21–59)	40.8 ± 15.4 (21–59)	46.2 ± 7.5 (29–58)
Height (cm)	164.1 ± 7.2	168.9 ± 4.6	159.2 ± 6.0
Body weight (kg)	60.1 ± 9.2	64.7 ± 9.8	55.6 ± 6.1
BMI (kg/m^2)	22.3 ± 2.7	22.6 ± 2.8	22.0 ± 2.6
Body fat percentage (%)	23.3 ± 8.5	17.1 ± 6.2	29.4 ± 5.3
Systolic blood pressure (mmHg)	113 ± 13	119 ± 8	106 ± 14
Diastolic blood pressure (mmHg)	70.1 ± 10.4	75.7 ± 9.0	64.5 ± 8.8
Pulse rate (beats per min)	67.8 ± 10.9	65.2 ± 11.7	70.5 ± 9.8
Body temperature (°C)	36.5 ± 0.3	36.5 ± 0.3	36.6 ± 0.2
White blood cell count ($\times 10^3/\mu$L)	5.10 ± 1.46	5.18 ± 1.69	5.00 ± 1.26
Red blood cell count ($\times 10^6/\mu$L)	4.73 ± 0.45	5.07 ± 0.32	4.39 ± 0.25
Hemoglobin (g/dL)	14.3 ± 1.5	15.4 ± 1.0	13.2 ± 0.9
Hematocrit (%)	44.5 ± 4.2	47.6 ± 3.0	41.4 ± 2.8
MCV (fL)	94.2 ± 3.5	94.0 ± 3.3	94.5 ± 3.9
MCH (pg)	30.3 ± 1.2	30.5 ± 1.0	30.1 ± 1.4
MCHC (g/dL)	32.2 ± 0.9	32.4 ± 0.9	31.9 ± 0.8
Platelet count ($\times 10^4/\mu$L)	24.6 ± 6.4	23.0 ± 4.8	26.3 ± 7.5
AST (IU/L)	21.4 ± 5.6	25.1 ± 5.0	17.6 ± 3.1
ALT (IU/L)	19.0 ± 9.9	23.8 ± 11.6	14.1 ± 4.5
γ-GTP (IU/L)	27.4 ± 20.1	38.6 ± 22.7	16.1 ± 7.6
LDH(IU/L)	173 ±28	180 ± 32	167 ± 22
Cholinesterase (IU/L)	316 ± 57	332 ± 59	300 ± 53
Alkaline phosphatase (IU/L)	167 ± 36	180 ± 37	155 ± 31
Amylase (IU/L)	78.1 ± 19.7	78.6 ± 25.2	77.7 ± 13.5
Na (mEq/L)	141 ± 2	141 ± 2	141 ± 2
K (mEq/L)	4.2 ± 0.4	4.3 ± 0.4	4.2 ± 0.4
Cl (mEq/L)	106 ± 2	105 ± 2	106 ± 2
Total protein (g/dL)	7.4 ± 0.3	7.4 ± 0.4	7.4 ± 0.3
Total bilirubin (mg/dL)	0.84 ± 0.36	0.80 ± 0.30	0.89 ± 0.42
Albumin (g/dL)	4.6 ± 0.3	4.7 ± 0.3	4.5 ± 0.2
Uric acid (mg/dL)	5.2 ± 1.5	6.2 ± 1.4	4.2 ± 0.7
Urea nitrogen (mg/dL)	12.0± 2.8	11.7 ± 2.9	12.2 ± 2.8
Creatinine (mg/dL)	0.69 ± 0.12	0.78 ± 0.10	0.61 ± 0.06
eGFR (ml/min/1.73m^2)	103.4 ± 17.3	92.5 ± 15.0	114.3 ± 12.1
Total cholesterol (mg/dL)	211 ± 36	215 ± 42	208 ± 30
LDL cholesterol (mg/dL)	129 ± 30	131 ± 35	127 ± 25
HDL cholesterol (mg/dL)	67 ± 13	64 ± 16	71 ± 9
LDL/HDL ratio	2.02 ± 0.71	2.22 ± 0.89	1.83 ± 0.42
Triglycerides (mg/dL)	95 ± 65	115 ± 84	75 ± 29
Fasting blood glucose (mg/dL)	95 ± 7	96 ± 8	94 ± 7
HbA1c (%)	5.3 ± 0.3	5.4 ± 0.3	5.3 ± 0.3
Glycoalbumin (%)	13.5 ± 1.0	13.2 ± 0.7	13.8 ± 1.1
Fe (μg/dL)	113 ± 37	119 ± 36	106 ± 39
Ferritin (ng/mL)	52.0 ± 52.5	77.0 ± 63.3	27.1 ± 20.4

Data are expressed as the mean ± SD. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate.

No clinically problematic changes were observed in physical parameters (Figure 2), hematological parameters (Figure 3), blood lipid- and glucose-related parameters (Figure 4), enzymes (Figure 5), and other biochemical parameters (Figure 6). Among the parameters shown in Figures 2–6, statistically significant fluctuations were observed in body fat percentage, body temperature (Figure 2), red blood cell count, hemoglobin, hematocrit, MCV, MCHC, platelets (Figure 3), total cholesterol, LDL cholesterol, LDL/HDL ratio, triglycerides, fasting blood glucose, glycoalbumin (Figure 4), γ-GTP, amylase (Figure 5), ferritin, total bilirubin, uric acid, and urea nitrogen (Figure 6) after four weeks of intake of

5-ALA with iron. However, these changes were within the reference range. No statistically significant fluctuations were observed in the urinalysis.

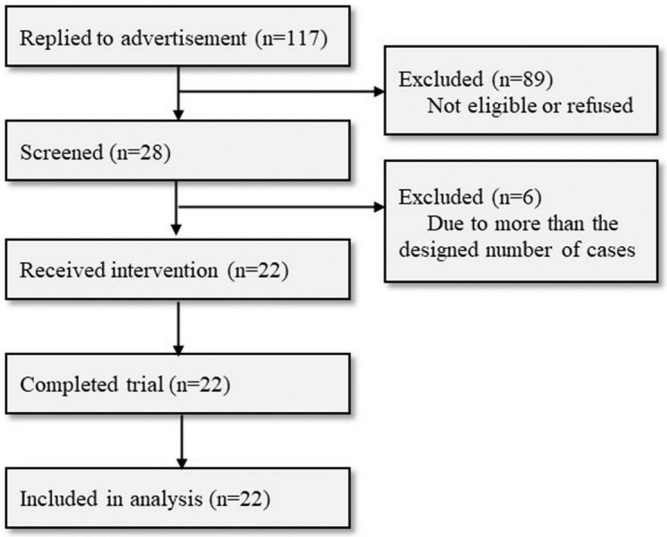

Figure 1. Flow diagram of the study subjects.

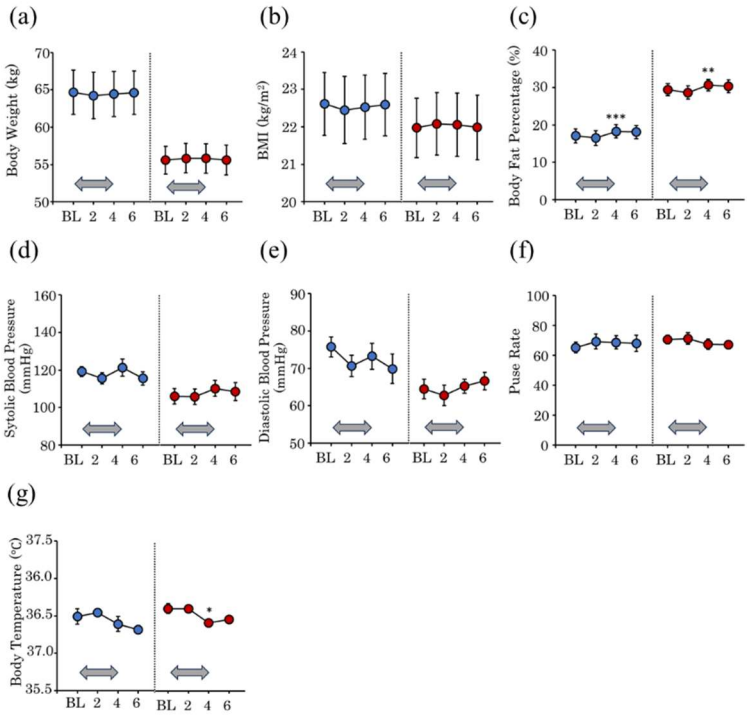

Figure 2. Changes in physical examination parameters of males (**left**) and females (**right**). (**a**) Body weight; (**b**) body mass index (BMI); (**c**) body fat percentage; (**d**) systolic blood pressure; (**e**) diastolic blood pressure; (**f**) pulse rate; (**g**) body temperature. All data are expressed as the mean ± SE; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ (paired *t*-test between baseline and week 4). The intake period was between BL and week 4 (double-headed arrows).

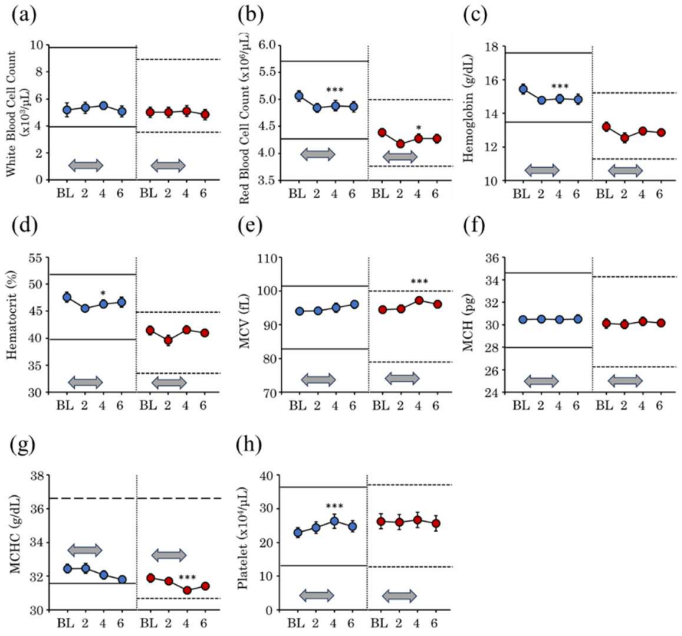

Figure 3. Changes in hematological parameters of males (**left**) and females (**right**). The solid (male), dotted (female), and dashed (both male and female) lines indicate the upper and lower limits of the reference range, respectively. (**a**) White blood cell count; (**b**) red blood cell count; (**c**) hemoglobin; (**d**) hematocrit; (**e**) mean corpuscular volume (MCV); (**f**) mean corpuscular hemoglobin (MCH); (**g**) mean corpuscular hemoglobin concentration (MCHC); (**h**) platelet count. All data are expressed as the mean ± SE; * $p < 0.05$, *** $p < 0.005$ (paired *t*-test between baseline and week 4). The intake period was between BL and week 4 (double-headed arrows).

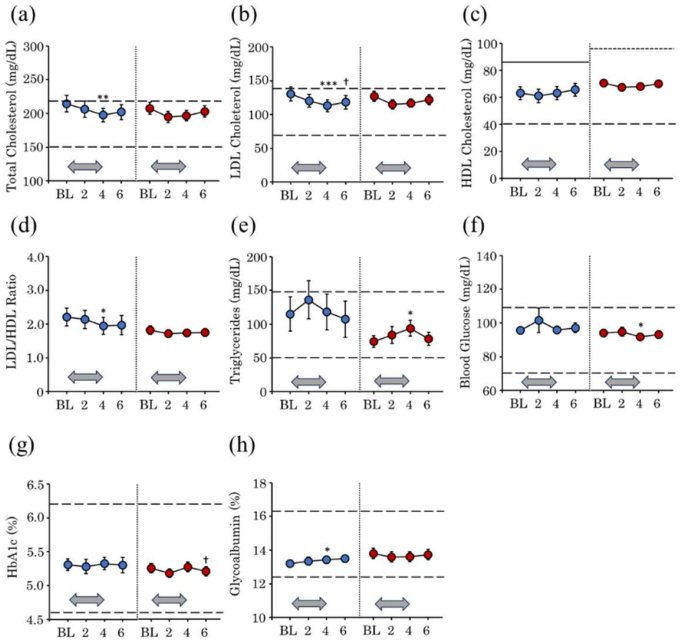

Figure 4. Changes in serum lipid profiles and glucose-related parameters of males (**left**) and females (**right**). The solid (male), dotted (female), and dashed (both male and female) lines indicate the upper and lower limits of the reference range, respectively. Parameters without dotted lines have no reference ranges. (**a**) Total cholesterol; (**b**) LDL cholesterol; (**c**) HDL cholesterol; (**d**) LDL/HDL ratio; (**e**) triglycerides; (**f**) fasting blood glucose; (**g**) HbA1c; (**h**) glycoalbumin levels. All data are expressed as mean ± SE; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ (paired *t*-test between baseline and week 4). † $p < 0.05$ (paired *t*-test between week-4 and week-6). The intake period was between BL and week 4 (double-headed arrows).

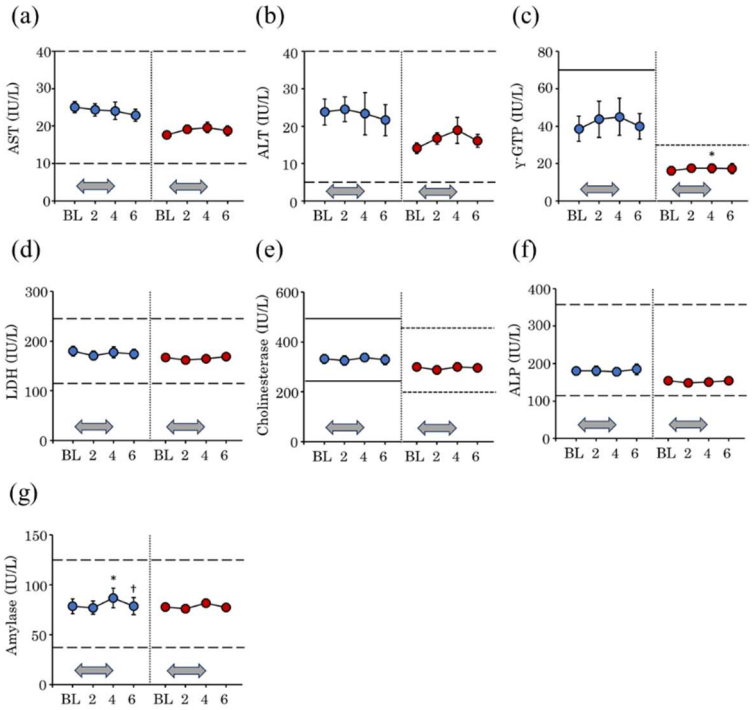

Figure 5. Changes in serum enzyme levels in males (**left**) and females (**right**). The solid (male), dotted (female), and dashed (both male and female) lines indicate the upper and lower limits of the reference range, respectively. (**a**) Aspartate transaminase (AST); (**b**) alanine transaminase (ALT); (**c**) gamma-glutamyl transpeptidase (γ-GTP); (**d**) lactate dehydrogenase (LDH); (**e**) cholinesterase; (**f**) alkaline phosphatase (ALP); (**g**) amylase. All data are expressed as mean ± SE * $p < 0.05$ (paired *t*-test between baseline and week 4). † $p < 0.05$ (paired *t*-test between week-4 and week-6). The intake period was between BL and week 4 (double-headed arrows).

The case number of adverse events that were "related" or "not related, but the possibility of a causal relationship could not be denied" is summarized in Table 2. Only elevated serum Fe levels were determined to have a causal relationship with the test compounds in this study. Individual clinical data that showed a worsening CTCAE grade are summarized in Supplementary Table S1. When judged individually, all fluctuations in clinical parameters were mild to moderate. Two males and five females exceeded the reference range of serum Fe levels during the intervention period (Figure 7). However, the elevated serum Fe levels were reduced within the normal range at week 6, 2 weeks after stopping the intake, in all cases (Figure 7). The self-reported subjective symptoms are summarized in Supplementary Table S2. A female aged 29 (ID 6) complained of abdominal pain and diarrhea on days 16–20, then discontinued capsule intake during days 21–26. These symptoms did not appear either after stopping or restarting the capsule intake.

Table 2. Summary of adverse events ("related" and "not related, but the possibility of causal relationship could not be denied").

Adverse Event	Number of Cases	Incidence Rates (%)	Incidence Rates (per 100 Person-Weeks)	Severity	Causal Relationship
Decrease in hemoglobin	1	4.5	1.1	Mild	Not related (but the possibility of a causal relationship could not be denied)
Increase in ALT	1	4.5	1.1	Mild	Not related (but the possibility of a causal relationship could not be denied)

Table 2. *Cont.*

Adverse Event	Number of Cases	Incidence Rates (%)	Incidence Rates (per 100 Person-Weeks)	Severity	Causal Relationship
Increase in γ-GTP	2	9.1	2.3	Mild	Not related (but the possibility of a causal relationship could not be denied)
Increase in total bilirubin	2	9.1	2.3	Mild	Not related (but the possibility of a causal relationship could not be denied)
Decrease in eGFR	3	13.6	3.4	Mild–Moderate	Not related (but the possibility of a causal relationship could not be denied)
Increase in triglycerides	2	9.1	2.3	Mild–Moderate	Not related (but the possibility of a causal relationship could not be denied)
Increase in serum Fe	6	27.3	6.8	Mild	Related
Abdominal pain/diarrhea	1	4.5	1.1	Mild	Not related (but the possibility of a causal relationship could not be denied)

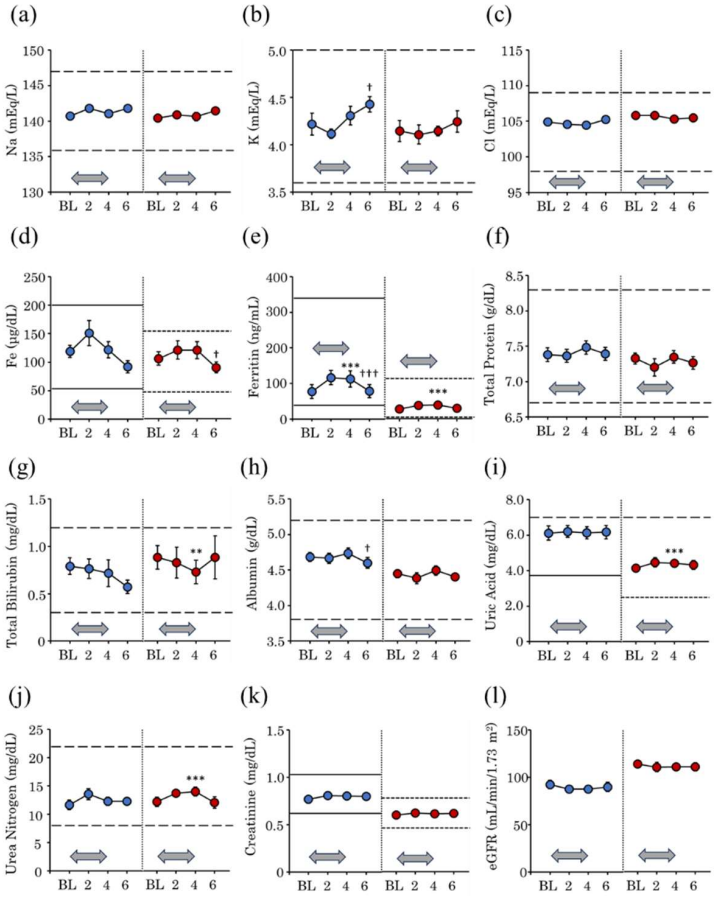

Figure 6. Changes in blood biochemical parameters of males (**left**) and females (**right**). The solid (male), dotted (female), and dashed (both male and female) lines indicate the upper and lower limits of the reference range, respectively. Parameters without dotted lines have no reference ranges. (**a**) Na; (**b**) K; (**c**) Cl; (**d**) Fe; (**e**) ferritin; (**f**) total protein; (**g**) total bilirubin; (**h**) albumin; (**i**) uric acid; (**j**) urea nitrogen; (**k**) creatinine; (**l**) estimated glomerular filtration rate (eGFR). All data are expressed as mean ± SE ** $p < 0.01$, *** $p < 0.005$ (paired *t*-test between baseline and week 4). † $p < 0.05$, ††† $p < 0.005$ (paired *t*-test between week-4 and week-6). The intake period was between BL and week 4 (double-headed arrows).

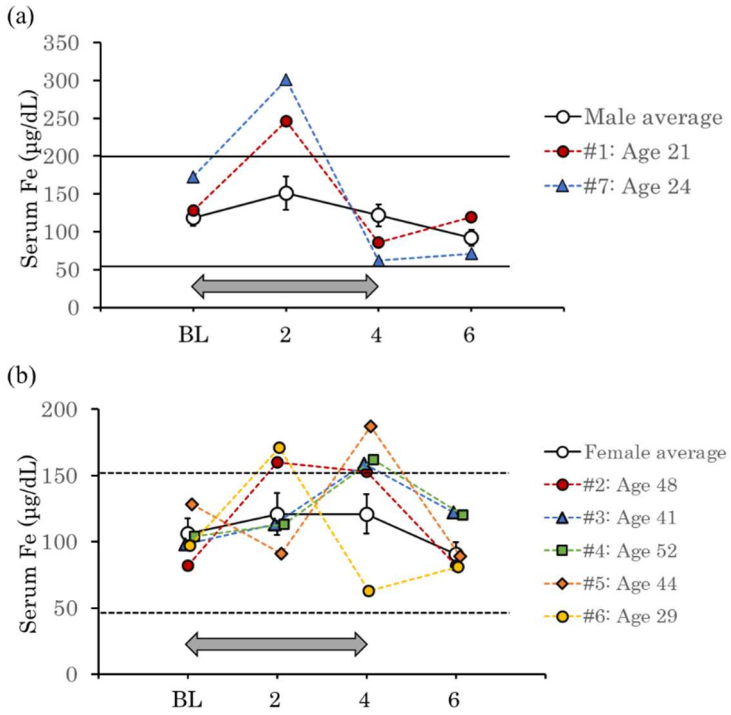

Figure 7. Changes in serum Fe levels in subjects with exceeded reference ranges at any point during the study period. The ID numbers for each data correspond to the IDs listed in Tables S1 and S2. (**a**) Male; (**b**) female. Solid (male) and dotted (female) lines represent the upper and lower limits of the reference range, respectively. The intake period was between BL and week 4 (double-headed arrows).

3. Discussion

This clinical trial evaluated the safety of the combined administration of 5-ALA phosphate 250 mg and SFC 143.4 mg for four weeks. 5-ALA phosphate is administered with iron because both are essential precursors and components of heme synthesis. No test compound-related adverse events or abnormal values on physical examination, hematology, blood chemistry, or urinalysis were observed, except for serum Fe levels. The elevations in serum Fe were mild to moderate and transient and were judged not to be clinically problematic. The frequency of cases in which serum Fe levels exceeded the upper limit at least once during the 5-ALA phosphate/iron administration period was 31.8% (7/22).

In Japan, SFC is prescribed to patients with iron-deficiency anemia at 100–200 mg a daily dose as Fe. As we did not specify the timing of 5-ALA phosphate/SFC administration, it is possible that the subjects showed transient excessive blood serum Fe levels if it happened to be around the peak level of serum Fe by absorbed iron due to the timing of taking the test compounds, supported by the evidence that the increment in serum Fe levels correlates with the absorbed iron administered orally [15]. However, according to the ethical drug package insert of sodium ferrous citrate, the ΔCmax was 69.0 ± 12.7 μg/dL, and Tmax was 3.9 ± 0.5 h after oral administration of SFC at 100 mg as Fe. Considering that the iron dose was 15 mg in this study, it is unlikely that the peak of absorbed iron caused the elevation of serum Fe levels because the observed serum Fe increments were between 58 and 128 μg/dL from each baseline in the cases showing exceeded serum Fe levels.

The cases in which the subjects exceeded the reference range of serum Fe were greater in females than in males, possibly due to the lower reference range in females (48–154 μg/dL) than in males (54–200 μg/dL), but there was no significant difference in frequencies between sexes by Fisher's exact test. It is possible that menstruation affected serum Fe levels via fluctuations during the luteal phase [16].

Gastrointestinal issues caused by oral iron administration are well-known in clinical settings [17]. Chronic administration of high-dose (60 mg) non-heme iron has been reported to have some unfavorable effects, including constipation, compared to the administration of low-dose (18 mg) heme and non-heme iron combination [18]. A 14-day administration of 941.8 mg or 1884 mg as SFC (100 or 200 mg as Fe) to healthy adult men caused transiently similar gastrointestinal adverse events [16]. The Institute of Medicine in the United States has set the upper level of total daily intake to 45 mg/day, including food [19]. The amount of SFC used in this study (15 mg of Fe) was much lower than the above iron doses. However, we cannot rule out the possibility that the adverse events observed in the female subject (ID 6) were caused by iron because her serum Fe level on week 2, close to the occurrence date of gastrointestinal symptoms, exceeded the reference range. Furthermore, the involvement of 5-ALA in iron uptake should be considered because 5-ALA is suggested to regulate the intestinal absorption of iron [20,21]. Since the safety data of SFC for healthy people are poor, despite its use in treating iron-deficiency anemia for over 30 years in Japan, the data in this study are valuable. Nevertheless, further studies are needed to ensure the safety of 5-ALA and SFC by including sole-SFC placebo control and measurement of hepcidin, the master regulator of iron homeostasis.

Previous in vitro cell culture or animal studies have suggested that 5-ALA may cause neurotoxicity by reducing GABA(A) receptors and oxidative stress [11–13]. In addition, Jagielska et al. reported that acidic extracellular conditions induce the death of oligodendrocyte precursor cells and reduce their differentiation into oligodendrocytes, which are essential for maintaining myelin sheaths in the central nervous system [22]. As the pH of 3% 5-ALA hydrochloride is 2.2–2.8 [23], the acidic effect of 5-ALA is also a concern, possibly affecting neuronal cells. However, we observed no symptoms suspected of neurotoxicity related to test compound intake. This may have been because the range of 5-ALA concentrations in neuronal cells after oral administration would be much lower than in the studies mentioned above (0.5–10 mM 5-ALA) [11–13]. The peak plasma concentration of 5-ALA ranged between 1.27 and 9.42 μg/mL after 128 mg of 5-ALA hydrochloride (equivalent to approximately 175 mg of 5-ALA phosphate) oral administration in dogs [24]. This range is less than approximately 0.1 mM of 5-ALA, accounting for the lack of neurotoxicity observed in this study.

In an animal study, a four-week administration of 10 mg/kg/day 5-ALA hydrochloride without iron in beagle dogs caused vomiting, slight increases in AST and ALT levels, and yellow-brown pigmentation in capillary vessels, Kupffer cells, and hepatocytes [14]. This dose corresponds to approximately 450 mg/day of 5-ALA phosphate in humans with a body weight of 60 kg using human equivalent dose (HED) estimation [25]. Total bilirubin increased by 48% in male SD rats after oral administration of 300 mg/kg/day 5-ALA hydrochloride, equivalent to approximately 4,000 mg/day of 5-ALA phosphate in 60 kg humans, for 14 days [14]. In our study, AST (n = 1), ALT (n = 1), γ-GTP (n = 2), and total bilirubin (n = 3) levels were slightly increased, but all were judged to be unrelated to the test compounds. Serum ferritin levels were significantly increased during the intake period; however, the extent was sex-dependent and was greater in males. On the other hand, MCV was significantly increased only in females. Both serum ferritin and MCV are biomarkers of iron-deficient anemia; serum ferritin reflects body iron storage, and MCV is the mean red blood cell volume. These sex-dependent fluctuations may be due to the differences in body iron content between males and females. Recommended doses for adequate intake of 5-ALA phosphate are set between 15 and 100 mg daily as dietary supplements on the market in Japan. Therefore, 250 mg of 5-ALA phosphate per day covers the expected dose of marketed dietary supplements containing 5-ALA phosphate for healthy individuals.

This study has three limitations. First, all participants were Japanese; therefore, other ethnicities should be tested in future studies. Second, there was no placebo or arm with iron only. No severe adverse events related to the test compounds were observed; however, it would be more comprehensive if compared with iron alone or a placebo without 5-ALA phosphate and iron for gastrointestinal symptoms and serum Fe elevation. Third, the intake period was four weeks. We cannot dismiss the possibility that 5-ALA, if administered for longer than the period of this study or at higher doses, could potentially cause toxicity. Considering its long use as a dietary supplement, a more extended period should be elucidated.

4. Materials and Methods

4.1. Materials

The test capsule was composed of 50 mg 5-ALA phosphate (neo ALA Co., Ltd., Tokyo, Japan) and 28.68 mg SFC (Mitsubishi Chemical Corporation, Tokyo, Japan; provided as Sanferol®, 3 mg as Fe). The capsules also contained starch and microcrystalline cellulose as excipients, calcium stearate and fine silicon dioxide as lubricants, and titanium dioxide as a colorant. The capsules were composed of hydroxypropyl methylcellulose.

4.2. Study Population

Eleven healthy male and eleven healthy female volunteers, all Japanese, were recruited from the local community in Hiroshima through advertisements. After obtaining written informed consent, the eligibility of the subjects was confirmed. The inclusion criteria were healthy and aged between 20 and 59 years old. Individuals who regularly received drugs for chronic diseases, consumed food supplements that could affect the study results, were currently participating or had participated in a clinical study within the past three months, were pregnant or breastfeeding, had a medical history of severe illness or major surgery, had a history of hypersensitivity to 5-ALA or porphyrin, had been diagnosed with porphyria, had a family member with porphyria, or had severe anemia characterized by hemoglobin less than 10 g/dL were excluded.

4.3. Study Design

This was a single-center, open-label, non-placebo prospective intervention study to assess the safety of oral administration of a combination of 5-ALA and SFC. The study was approved by the Ethical Committee for Clinical Research of Hiroshima University and conducted between Jun and Dec 2016.

Twenty-two healthy male and female participants aged 20–59 years were orally administered five test capsules; the total dose was 250 mg of 5-ALA phosphate and 143.4 mg of SFC (15 mg as Fe) once daily for 28 days. The administration time was not specified, but the subjects were instructed to take five capsules simultaneously. During the study period, subjects were instructed to avoid taking antacids or tannic acid albumin concomitantly with 5-ALA and to avoid additional supplements containing iron and excessive dietary iron. The subjects were asked to maintain a daily food diary, abstain from eating and drinking excess food supplements, exercise intensely, and donate blood. The 14 days after the administration period was set as the post-observation period.

4.4. Clinical Examination

Health conditions were monitored via interviews, blood tests, urinalysis, and physical examinations conducted before administration and at weeks 2, 4, and 6. The subjects were required to fast for 9 h before each clinical visit. Clinical examinations included body weight, body mass index (BMI), body fat percentage, systolic blood pressure, diastolic

blood pressure, pulse rate, body temperature, white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), cholinesterase, alkaline phosphatase (ALP), amylase, Na^+, K^+, Cl^-, total protein, total bilirubin, albumin, uric acid, urea nitrogen, creatinine, estimated glomerular filtration rate (eGFR), total cholesterol, LDL cholesterol, HDL cholesterol, LDL/HDL ratio, triglycerides, fasting blood glucose, HbA1c glycoalbumin, serum Fe, and ferritin. Urine-specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, glucose, and occult blood were also examined.

Body fat percentage was measured using BC-118E (TANITA Corporation, Tokyo, Japan), and blood pressure was measured using HBP-9020 (Omron Healthcare Co. Ltd., Kyoto, Japan). The health status recorded in the participants' diaries was also assessed for safety.

4.5. Safety Assessment

The severity of adverse events was determined according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0, based on medical interviews, subject diaries, and clinical examinations. All undesirable events in the subjects during the study period were recorded as adverse events, regardless of relation to the intake of test compounds. In this study, the medical doctor judged the causal relationship between the adverse events and the test compounds as following four categories: "not related", "not related with a low possibility of a causal relationship", "not related, but the possibility of a causal relationship could not be denied", or "related". A medical doctor individually assessed all self-reported adverse events and abnormal values of clinical examinations for severity and the four categories mentioned above. The incidence rates of adverse events and their incidence rates during the intervention period (per 100 person-weeks) were calculated.

4.6. Sample Size and Statistical Analysis

Because this was an exploratory trial, the sample size was determined to be feasible and sufficient. Changes from baseline to week 4 or from week 4 to week 6 were assessed using a paired *t*-test, with significant differences at $p < 0.05$. The analysis set for safety covers all subjects who had taken the test compounds at least once and whose safety was evaluated at least once after the intervention.

5. Conclusions

The co-administration of 250 mg 5-ALA phosphate and 143.4 mg SFC was well tolerated and safe in healthy adults for up to four weeks with no severe adverse effects based on blood tests, urinalysis, and self-reported symptoms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/scipharm93010005/s1, Table S1: Adverse events in clinical examination. Cases in which the grade worsened after the intervention when judged using CTCAE v4.0.; Table S2: Adverse events in self-reported subjective symptoms.

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Institutional Review Board Statement: The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Hiroshima University (C-94, 20 June 2016).

Informed Consent Statement: Written informed consent was obtained from the participants in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request with the right intent.

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Conflicts of Interest: H.I. is an employee of SBI Pharmaceuticals. T.T. was an employee of SBI Pharmaceuticals when the study was conducted. H.I. and T.T. had no role in the interpretation of the clinical data in terms of safety. F.H. has no conflicts of interest to declare other than study funding.

References

1. Heinemann, I.U.; Jahn, M.; Jahn, D. The biochemistry of heme biosynthesis. *Arch. Biochem. Biophys.* **2008**, *474*, 238–251. [CrossRef] [PubMed]
2. Dalton, J.T.; Yates, C.R.; Yin, D.; Straughn, A.; Marcus, S.L.; Golub, A.L.; Meyer, M.C. Clinical pharmacokinetics of 5-aminolevulinic acid in healthy volunteers and patients at high risk for recurrent bladder cancer. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 507–512. [CrossRef] [PubMed]
3. Berlin, N.I.; Neuberger, A.; Scott, J.J. The metabolism of delta-aminolaevulic acid. 1. Normal pathways, studied with the aid of 15N. *Biochem. J.* **1956**, *64*, 80–90. [CrossRef] [PubMed]
4. Berlin, N.I.; Neuberger, A.; Scott, J.J. The metabolism of delta-aminolaevulic acid. 2. Normal pathways, studied with the aid of 14C. *Biochem. J.* **1956**, *64*, 90–100. [CrossRef]
5. Al-Saber, F.; Aldosari, W.; Alselaiti, M.; Khalfan, H.; Kaladari, A.; Khan, G.; Harb, G.; Rehani, R.; Kudo, S.; Koda, A.; et al. The Safety and Tolerability of 5-Aminolevulinic Acid Phosphate with Sodium Ferrous Citrate in Patients with Type 2 Diabetes Mellitus in Bahrain. *J. Diabetes Res.* **2016**, *2016*, 8294805. [CrossRef]
6. Higashikawa, F.; Noda, M.; Awaya, T.; Tanaka, T.; Sugiyama, M. 5-aminolevulinic acid, a precursor of heme, reduces both fasting and postprandial glucose levels in mildly hyperglycemic subjects. *Nutrition* **2013**, *29*, 1030–1036. [CrossRef]
7. Rodriguez, B.L.; Curb, J.D.; Davis, J.; Shintani, T.; Perez, M.H.; Apau-Ludlum, N.; Johnson, C.; Harrigan, R.C. Use of the dietary supplement 5-aminiolevulinic acid (5-ALA) and its relationship with glucose levels and hemoglobin A1C among individuals with prediabetes. *Clin. Transl. Sci.* **2012**, *5*, 314–320. [CrossRef]
8. Tanaka, T.; Tashiro, M.; Ota, K.; Fujita, A.; Sawai, T.; Kadota, J.; Fukuda, Y.; Sumiyoshi, M.; Ide, S.; Tachikawa, N.; et al. Safety and efficacy of 5-aminolevulinic acid phosphate/iron in mild-to-moderate coronavirus disease 2019: A randomized exploratory phase II trial. *Medicine* **2023**, *102*, e34858. [CrossRef]
9. Tamura, Y.; Kaga, H.; Abe, Y.; Yoshii, H.; Seino, H.; Hiyoshi, T.; Kuribayashi, N.; Inoue, I.; Watada, H. Efficacy and Safety of 5-Aminolevulinic Acid Combined with Iron on Skeletal Muscle Mass Index and Physical Performance of Patients with Sarcopenia: A Multicenter, Double-Blinded, Randomized-Controlled Trial (ALADDIN Study). *Nutrients* **2023**, *15*, 2866. [CrossRef]
10. Koyasu, H.; Horie, S.; Matsushita, K.; Ashizawa, T.; Muto, S.; Isotani, S.; Tanaka, T.; Nakajima, M.; Tsujimura, A. Efficacy and Safety of 5-Aminolevulinic Acid for Patients with Symptoms of Late-Onset Hypogonadism: A Preliminary Study. *World J. Men's Health* **2022**, *40*, 456–464. [CrossRef]
11. Adhikari, A.; Penatti, C.A.; Resende, R.R.; Ulrich, H.; Britto, L.R.; Bechara, E.J. 5-Aminolevulinate and 4, 5-dioxovalerate ions decrease GABA(A) receptor density in neuronal cells, synaptosomes and rat brain. *Brain Res.* **2006**, *1093*, 95–104. [CrossRef] [PubMed]
12. Demasi, M.; Penatti, C.A.; DeLucia, R.; Bechara, E.J. The prooxidant effect of 5-aminolevulinic acid in the brain tissue of rats: Implications in neuropsychiatric manifestations in porphyrias. *Free Radic. Biol. Med.* **1996**, *20*, 291–299. [CrossRef] [PubMed]
13. Felitsyn, N.; McLeod, C.; Shroads, A.L.; Stacpoole, P.W.; Notterpek, L. The heme precursor delta-aminolevulinate blocks peripheral myelin formation. *J. Neurochem.* **2008**, *106*, 2068–2079. [CrossRef] [PubMed]
14. APPLICATION NUMBER:208630Orig1s000 NON-CLINICAL REVIEW(S) [Internet]: Food and Drug Administration of United States. 2016. Available online: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/208630Orig1s000PharmR.pdf (accessed on 8 January 2025).
15. Hoppe, M.; Hulth, N.L. Validation of the clinical approach of using the induced serum iron increase after 1h as a measure of iron absorption. *Clin. Nutr.* **2006**, *25*, 163–165. [CrossRef]

16. Kim, I.; Yetley, E.A.; Calvo, M.S. Variations in iron-status measures during the menstrual cycle. *Am. J. Clin. Nutr.* **1993**, *58*, 705–709. [CrossRef]
17. DeLoughery, T.G. Safety of Oral and Intravenous Iron. *Acta Haematol.* **2019**, *142*, 8–12. [CrossRef]
18. Frykman, E.; Bystrom, M.; Jansson, U.; Edberg, A.; Hansen, T. Side effects of iron supplements in blood donors: Superior tolerance of heme iron. *J. Lab. Clin. Med.* **1994**, *123*, 561–564.
19. Micronutrients IoMUPo. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; National Academy Press: Washington, DC, USA, 2001.
20. Laftah, A.H.; Raja, K.; Simpson, R.J.; Peters, T.J. Effect of Tin-mesoporphyrin, an inhibitor of haem catabolism, on intestinal iron absorption. *Br. J. Haematol.* **2003**, *122*, 298–304. [CrossRef]
21. Laftah, A.H.; Raja, K.B.; Latunde-Dada, G.O.; Vergi, T.; McKie, A.T.; Simpson, R.J.; Peters, T.J. Effect of altered iron metabolism on markers of haem biosynthesis and intestinal iron absorption in mice. *Ann. Hematol.* **2005**, *84*, 177–182. [CrossRef]
22. Jagielska, A.; Wilhite, K.D.; Van Vliet, K.J. Extracellular acidic pH inhibits oligodendrocyte precursor viability, migration, and differentiation. *PLoS ONE* **2013**, *8*, e76048. [CrossRef]
23. GLIOLAN® PRODUCT INFORMATION [Internet]: Department of Health, Australian Government. 2014. Available online: https://www.tga.gov.au/sites/default/files/auspar-aminolevulinic-acid-140303-pi.pdf (accessed on 8 January 2025).
24. Dalton, J.T.; Meyer, M.C.; Golub, A.L. Pharmacokinetics of aminolevulinic acid after oral and intravenous administration in dogs. *Drug Metab. Dispos.* **1999**, *27*, 432–435. [CrossRef]
25. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers: Food and Drug Administration of United States. 2005. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers (accessed on 8 January 2025).