

Safety Assessment of Oral 5-aminolevulinic Acid and Ferrous iron in Healthy Human Subjects: A Non-randomized, Open-label, Non-placebo-controlled 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 of food supplement in several countries because of its wide applicability to healthcare areas.

We aimed to assess the safety of 5-ALA phosphate and SFC in healthy adult subjects at the dose several times higher than that available on market.

This was an open, non-randomized, non-placebo-controlled trial that included 11 men and 11 women. Doses of 250 mg 5-ALA phosphate and 143.4 mg SFC (15 mg as Fe) per day were orally administered for 28 days. Blood and urine analyses and interviews were conducted to assess the safety.

No test compound-related adverse events or abnormal values were observed, except for elevated serum iron levels, which were mild to moderate and transient.

Combined administration of 5-ALA phosphate and SFC to healthy adults is well-tolerated and safe at the dose and duration investigated in this study.

Introduction

5-aminolevulinic acid (5-ALA) is a universal precursor of tetrapyrroles, such as heme, chlorophyll and cobalamin (vitamin B₁₂), that is conserved across all organismal domains. In humans, 5-ALA serves as a precursor of heme¹. Orally administered 5-ALA is absorbed via the upper gastrointestinal tract and is used in endogenous biosynthesis of heme in the body²⁻⁴.

It is known the heme precursor 5-ALA-derived protoporphyrin IX (PpIX) selectively accumulates in cancer cells and emits fluorescence in response to specific wavelengths. Repetitive use of 5-ALA was suspected to cause damage to Schwann cells, which comprise the neuronal myelin sheath, by inactivating gamma aminobutyric acid (GABA) receptors owing to its structural similarity to GABA⁵⁻⁷. However, most investigations demonstrating the neuropathic effects of 5-ALA examined slices of rodent brains or cultured cells, or consisted of *in vivo* studies where 5-ALA was injected^{8,9}. In human use, 5-ALA is orally administered or applied topically. Although safety studies of repetitive oral administration to animals and humans are relatively limited¹⁰⁻¹⁴, 5-ALA phosphate is widely used as a dietary supplement in combination with iron in Japan, Southeast Asia, and the Middle East.

Herein, we investigated whether a four-week repetitive administration of a combination of 5-ALA phosphate (250 mg) and sodium ferrous citrate (SFC; 143.4 mg), a divalent iron compound also used as supplement and drug, had deleterious effects in healthy adults.

Results

Of the 117 individuals who replied to the advertisement, 28 participated in the study orientation sessions (Fig. 1). Consent was obtained from all participants, who all satisfied the inclusion criteria. Of the 28 individuals, 22 (11 men and 11 women) were allocated to the study in order of application. There were no dropouts during the study and all subjects completed the study (Table 1). According to subject diaries, 15 followed a 100% administration rate, 27 followed a 96.4% rate, 4 followed an 89.3% rate, 1 followed a 75.0% rate (where drugs were administered 21 out of 28 days and discontinued for 6 days), and 1 was unknown due to non-submission of the diary.

Table 1
Background of subjects

	No. of Subjects	Age \pm SD (years)	Height \pm SD (cm)
Total	22	43.5 \pm 12.1 (21 ~ 59)	164.1 \pm 7.2
Male	11	40.8 \pm 15.4 (21 ~ 59)	168.9 \pm 4.6
Female	11	46.2 \pm 7.5 (29 ~ 58)	159.2 \pm 6.0

No clinically problematic changes were observed for the physiological parameters examined (Fig. 2A to G), haematological test items (Fig. 2H to O), and blood biochemical test items (Figs. 3 and 4).

Comparison of the mean value of physiological, haematological, and blood biochemical examination data from baseline and end of administration (week 4) revealed statistically significant fluctuations in body temperature, body fat percentage, red blood cell count, haemoglobin, haematocrit, MCV, MCHC, platelets, γ -GTP, amylase, Cl⁻, total bilirubin, albumin, uric acid, urea nitrogen, creatinine, eGFR, total cholesterol, LDL cholesterol, LDL/HDL ratio, triglyceride, fasting blood glucose level, glycoalbumin, and ferritin in the male group, female group, or integrated data of both (Fig. 2, 3, 4, **Table S1, S2, and S3**). In urinalysis we observed only one grade 1 occult blood in one patient, which were diagnosed that the causal relationship between the test compound was unlikely.

The number of adverse events that were related or not related with a possibility of causal relation not ruled out to test compounds administration is summarized in Table 2. Only elevated serum iron was judged to have a causal relationship with the test compounds in the study. Individual serum iron data from subjects judged to be related or causal relation not be ruled out, showed that women experienced serum iron elevation more frequently than men; however, the elevated serum iron levels in all subjects were reduced to normal at week 6 (Fig. 5). No subjects reported any changes of concern in physical condition other than individual #20 (mild abdominal pain and diarrhoea) and individual #65 (decreased frequency of drowsiness and shallow sleep) in examination interviews. The abdominal pain and diarrhoea in #20, which occurred during days 16 to 20, were judged to be adverse events in which the

possibility of causality could not be ruled out. This subject, a woman age of 29 who also showed elevated serum iron at week 2, discontinued administration on days 21–26. All subjective symptoms from diaries and value fluctuations of cases where the grade became worse after starting administration is shown in **Table S4 and S5**.

Table 2

Summary of adverse events (“related” and “not related with a possibility of causal relation not be ruled out”)

Adverse event	Number of cases	Incidence rate (%)	Incidence rate (per 100 person-weeks)	Severity	Causal relation
Hemoglobin decrease	1	4.5	1.1	Mild	Not related (with possibility of causal relation not be ruled out)
ALT increase	1	4.5	1.1	Mild	Not related (with possibility of causal relation not be ruled out)
γ-GTP increase	2	9.1	2.3	Mild	Not related (with possibility of causal relation not be ruled out)
Total bilirubin increase	2	9.1	2.3	Mild	Not related (with possibility of causal relation not be ruled out)
eGFR decrease	3	13.6	3.4	Mild – moderate	Not related (with possibility of causal relation not be ruled out)
Triglyceride increase	2	9.1	2.3	Mild – moderate	Not related (with possibility of causal relation not be ruled out)
Serum iron increase	6	27.3	6.8	Mild	Related
Abdominal pain / diarrhea	1	4.5	1.1	Mild	Not related (with possibility of causal relation not be ruled out)

Discussion

In this clinical trial, the safety of four-week repetitive administration of 5-ALA phosphate 250 mg and SFC 143.4 mg was evaluated. No test compounds-related adverse events or abnormal values in physical

examination, haematology, blood chemistry or urinalysis were observed except for serum iron levels. The elevated serum iron values were mild to moderate and transient, suggesting that they were not clinically problematic. Because SFC is known to elevate serum iron level^{15,16}, it is reasonable to consider direct causation; it is unlikely that elevated serum iron level is a consequence of concerning abnormalities, such as hepatic toxicity. Moreover, SFC elevates serum iron level up to 12 h after administration^{15,17}. As we did not specify the drug administration timing, it is possible that subjects showing excess blood serum iron level when the test compounds were administered shortly before blood sampling. However, the result that elevated serum iron level was observed more frequently in women than in men (Fig. 5) does not fit this hypothesis. It is possible that menstruation affected serum iron level via fluctuation during the luteal phase^{18,19}. Furthermore, the female subject who discontinued administration owing to abdominal pain and diarrhoea exceeded normal serum iron level at week 2, close to the occurrence date of the adverse events. Chronic administration of high-dose iron is reported to have some unfavourable effects, including diarrhoea^{20,21}, and 14-day administration of 941.8 mg or 1884 mg as SFC (100 or 200 mg of iron) to healthy adult men can transiently cause similar adverse events¹⁷. The Institute of Medicine in the United States set the adult Lowest Observed Adverse Effect Level (LOAEL) of supplemental iron as 60 mg/day and the upper level of total daily intake as 45 mg/day based on a study in which ferrous fumarate and heme iron was used^{21,22}. The amount of SFC we used in this study was 143.4 mg, which contains approximately 15 mg of iron, much less than the upper level¹⁷. However, we cannot rule out the possibility that the adverse events were caused by iron, considering that abdominal pain and diarrhoea are common adverse drug reactions of iron. In addition, safety data for SFC are poor, especially for non-anaemia patients despite its use in treating iron-deficiency anaemia for over 30 years in Japan. Furthermore, we should consider the involvement of 5-ALA in iron uptake because 5-ALA is suggested to regulate its intestinal absorption^{23,24}. Further studies are needed to assure the safety of 5-ALA phosphate and SFC by including sole-SFC placebo control and measurement of hepcidin, the master regulator of Fe homeostasis.

Previous studies based on *in vitro* or animal injection have suggested that 5-ALA may cause neurotoxicity⁵⁻⁹, but we observed no such symptoms. This may have been due to the route of administration. Jagielska *et al.* (2013) reported that acidic extracellular conditions induce cell death of oligodendrocytes, which is essential in maintaining myelin sheaths in the central nervous system²⁵. As the pH of 3% 5-ALA hydrochloride is 2.2–2.8²⁶, it is possible that intravenous injection or addition to the culture medium exposes oligodendrocytes to severe acidic conditions, causing death. However, the plasma maximum concentration of 5-ALA after oral administration is much lower than that after intravenous injection²⁷, accounting for the lack of neurotoxicity observed here.

Miyanari *et al.* (2011) showed that 13-week repeated administration of 60 mg/kg/day 5-ALA phosphate without iron to rats caused anaemia, necrosis of hepatocytes, proliferation of bile ducts, and increased total bilirubin level²⁸. This corresponds to approximately 580 mg/day 5-ALA phosphate in human with 60 kg body weight, using the human equivalent dose (HED) estimation²⁹. In a study of dogs, four-week

administration of 10 mg/kg/day 5-ALA hydrochloride without iron caused vomiting; slight increases in AST and ALT levels; and yellow-brown pigmentation in capillary vessels, Kupffer cells, and hepatocytes³⁰. This dose corresponds to approximately 460 mg/day 5-ALA phosphate in humans with 60 kg body weight. In humans, the 250 mg/day dose applied in this study is the highest in four-week (or longer) studies. Although we have previously shown that 600 mg/body 5-ALA hydrochloride (820 mg/body 5-ALA phosphate) yielded no serious adverse effects (unpublished), this was administered only for one week. We cannot rule out the possibility that 5-ALA phosphate administered for longer than the time period in this study or at higher dose could cause toxicity.

In conclusion, the combination of 5-ALA phosphate 250 mg and SFC 143.4 mg was well-tolerated and safe for healthy adults for administration up to four weeks. No severe adverse effects were observed during the study period.

Materials And Methods

Study design

This study was a single-centre, open label, non-placebo, prospective intervention study aimed at assessing the safety of a combination of 5-ALA phosphate and SFC. It was conducted from June 2016 to December 2016. The study was approved by the institutional review board of Hiroshima University Hospital and registered in the University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) database (UMIN000022825) on 22/06/2016 and conducted at Hiroshima University Hospital in accordance with Ethical Guidelines for Medical and Health Research Involving Human Subjects issued by the Japanese Ministry of Health, Labour and Welfare. SBI Pharmaceuticals is a sponsor of this study and was involved in the study design, writing of the report, and has its own right to submit the report for publication.

A combination of 5-ALA phosphate (250 mg) and SFC (143.4 mg; 15 mg as Fe) was administered for 28 days to 22 healthy male and female volunteers aged 20 to 59 years. The compounds were orally administered once per day. Fourteen days after administration was set as the post-observation period. Health was monitored via interview, blood test, urinalysis, and physical examination conducted before administration, 2 and 4 weeks after the initiation of test compound administration, and two weeks after completion of administration. A ± 1 week allowance was set to each time point to diminish protocol aberration.

Study population

Eleven male and 11 female healthy volunteers were recruited from the local community in Hiroshima using advertisements by the Hiroshima University Hospital. The ethnicity of the volunteers was not ascertained. After written informed consent was obtained, eligibility of the subjects was confirmed within seven days of drug administration. Individuals were excluded if they regularly received drugs for a chronic

disease, consumed food supplements that could affect the study, were currently participating in a clinical study or had participated in a study within the past three months, were pregnant or breastfeeding, had a history of serious 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 anaemia characterized by haemoglobin less than 10 g/dL.

Test compound administration and subject management

Both 5-ALA phosphate (neo ALA Co., Ltd., Tokyo, Japan) and SFC (Mitsubishi-Chemical Foods Corporation, Tokyo, Japan; provided as Sanferol®) were administered orally. Each compound was prepared in capsules of 50 mg of 5-ALA phosphate and approximately 28.68 mg of SFC, and five capsules were administered once daily to yield a total dose of 250 mg 5-ALA phosphate and 143.4 mg SFC (15 mg as Fe) daily. The capsules also contained starch and microcrystalline cellulose as excipient, calcium stearate and fine silicon dioxide as lubricant, and titanium dioxide as colorant. The capsules were made of hydroxypropyl methylcellulose. Administration time was not specified. During the study period, subjects were advised to avoid administering antacids or tannic acid albumin concomitantly with the test compounds, and to avoid additional supplements containing iron and excessive dietary iron. Subjects were asked to keep a daily food diary and to abstain from excessive eating and drinking, food supplements, intense exercise, and donating blood.

Background check and physical examination

Name, gender, date of birth, age, height, body temperature, body weight, body fat percentage, blood pressure, pulse rate, drugs being taken, use of health food, medical history, family history of porphyria, current medical history, allergies, and life history (smoking/drinking) were recorded at the beginning of the trial.

At weeks 2, 4, and 6, the subjects were required to fast for 9 h prior to examination. Body temperature, body weight, body mass index (BMI), body fat percentage, systolic blood pressure, diastolic blood pressure, and pulse rate were recorded. Body fat percentage was measured using a BC-118E (TANITA Corporation, Tokyo, Japan) and blood pressure was measured using an HBP-9020 (Omron Healthcare Co., Ltd., Kyoto, Japan). Additionally, blood and urine samples were collected at these weeks 2, 4 and 6 time points. White blood cell count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (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, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, LDL/HDL ratio, triglyceride, fasting blood glucose, glycosylated

haemoglobin (HbA1c), glycoalbumin, serum iron, and ferritin were recorded. Urine-specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, glucose, and occult blood were also examined.

Adverse events

Severity of adverse events and their causal relationship to the test compounds were determined according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 criteria. We assessed the presence or absence of adverse events and their grade based on inquiries, consultations during health interviews, subject diaries, and clinical / laboratory values. The grade of clinical laboratory values was judged, and the presence of adverse events was assessed based on the reference range of the clinical laboratory and CTCAE v4.0. All undesirable events that occurred to a subject during the test compounds administration period were recorded as adverse effects, regardless of relation to the test compounds intake. Adverse events were judged as not related, not related with slight possibility of causal relationship, not related where the possibility of causal relationship could not be ruled out, or related. Changes in subjective symptoms were individually evaluated for severity and causality from the diary in which daily health status was recorded. The incidence proportion of adverse events occurring and the incidence rate of adverse events during the administration period (person-years method) were calculated. For fluctuations in each laboratory test value, we compared the change from the baseline at each measurement point and evaluated the presence or absence of change.

Sample size and statistical analysis

As this was an exploratory trial, sample size was determined based on practical considerations. Means and standard deviation (*SD*) were used to describe variables of interest. Changes from baseline to week 4 or from week 4 to week 6 were assessed using a two-tailed paired Student's *t*-test, with differences considered significant at $P < 0.05$. The statistically analysed set for safety covers all subjects who had ingested the test compounds at least once, and whose safety was evaluated at least once.

Declarations

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author's Contribution

H.I and T.T designed research; supported the protocol writing conducted by Hiroshima University; and H.I. wrote the paper.

Additional Information

This work was supported by SBI Pharmaceuticals Co., Ltd. This work was commissioned from SBI Pharmaceuticals Co., Ltd. to the Graduate School of Biomedical Sciences, Hiroshima University. H.I. and T.T. were employees of SBI Pharmaceuticals at the time this work was completed.

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Figures

Figure 1

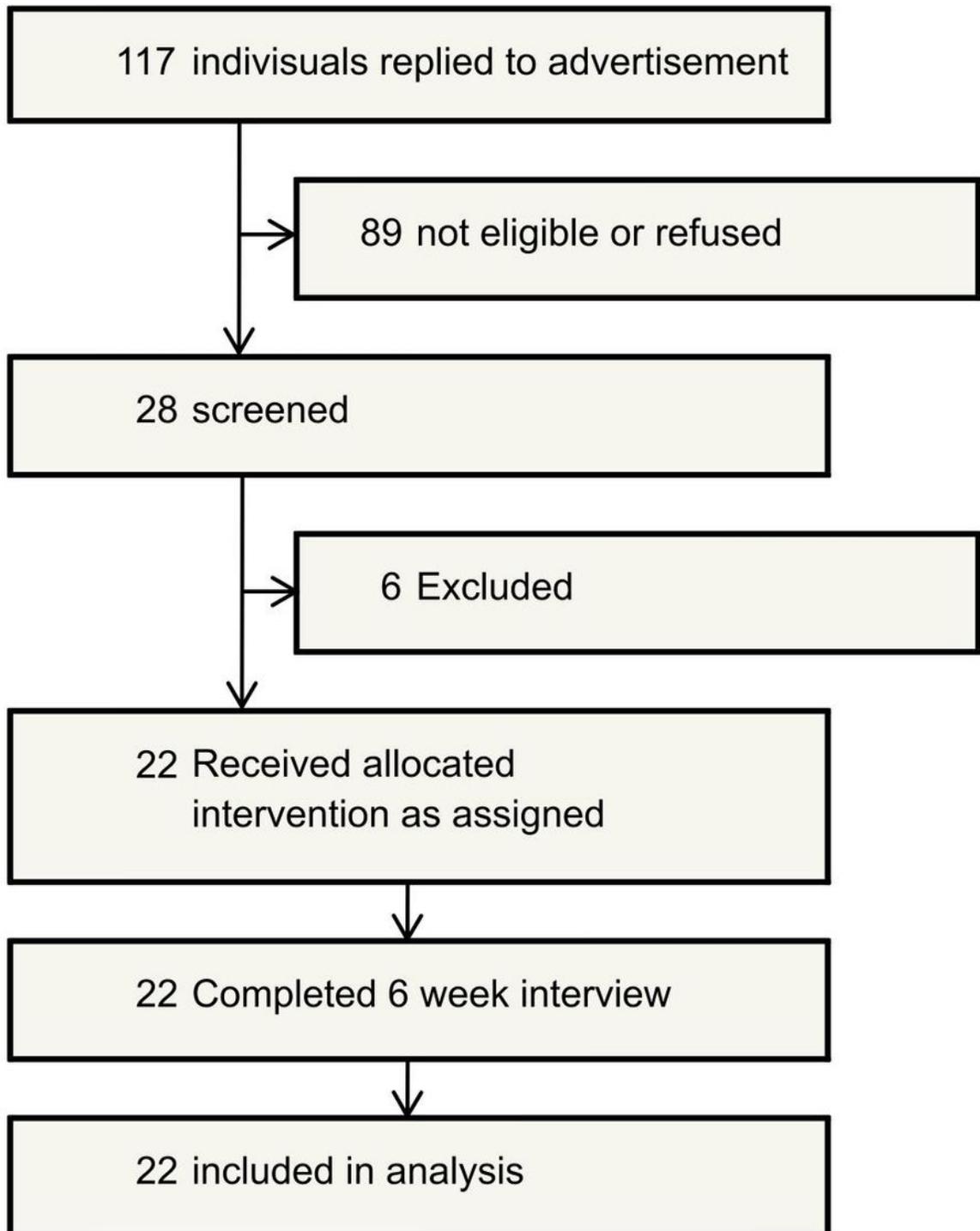


Figure 1

Flow diagram of the study Flow diagram showing participant flow through each stage of the trial (enrolment, intervention allocation, follow-up, and data analysis).

Figure 2

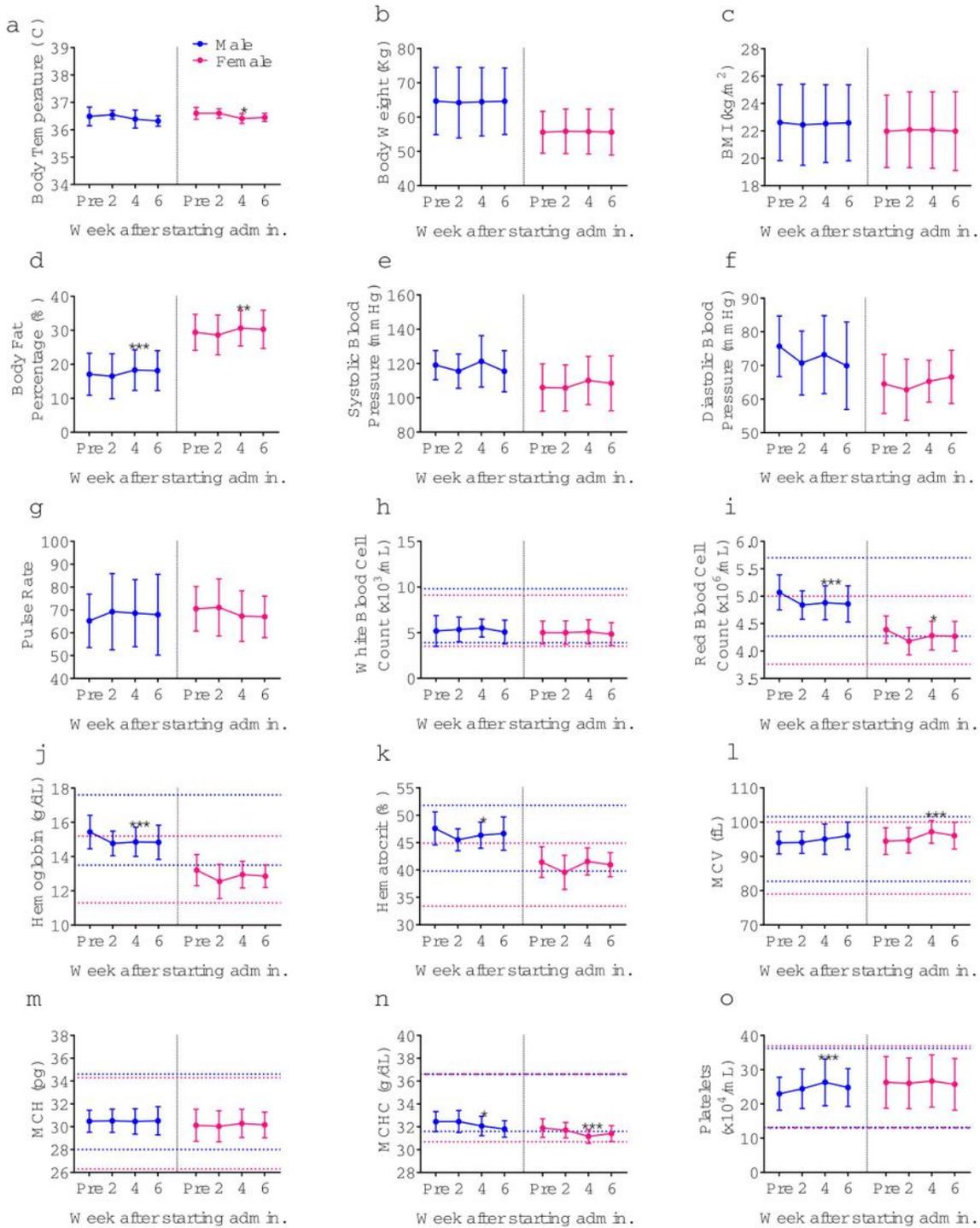


Figure 2

Physiological and haematological test items Fluctuation of physiological and haematological test items of male (blue) and female (pink). Dotted blue (male) and pink (female) lines show the upper and lower limit of reference range. The items without dotted line have no reference range. (A) Body temperature, (B) body weight, (C) body mass index (BMI), (D) body fat percentage, (E) systolic blood pressure, (F) diastolic blood pressure, (G) pulse rate, (H) white blood cell count, (I) red blood cell count, (J) haemoglobin, (K)

haematocrit, (L) mean corpuscular volume (MCV), (M) mean corpuscular haemoglobin (MCH), (N) mean corpuscular haemoglobin concentration (MCHC), and (O) platelet count are shown. All data are expressed as mean \pm SD *p < 0.05, **p < 0.01, ***p < 0.005 (paired t-test comparison of baseline to week 4). †p<0.05, ††p < 0.01, †††p< 0.005 (paired t-test comparison of week 4 to week 6).

Figure 3

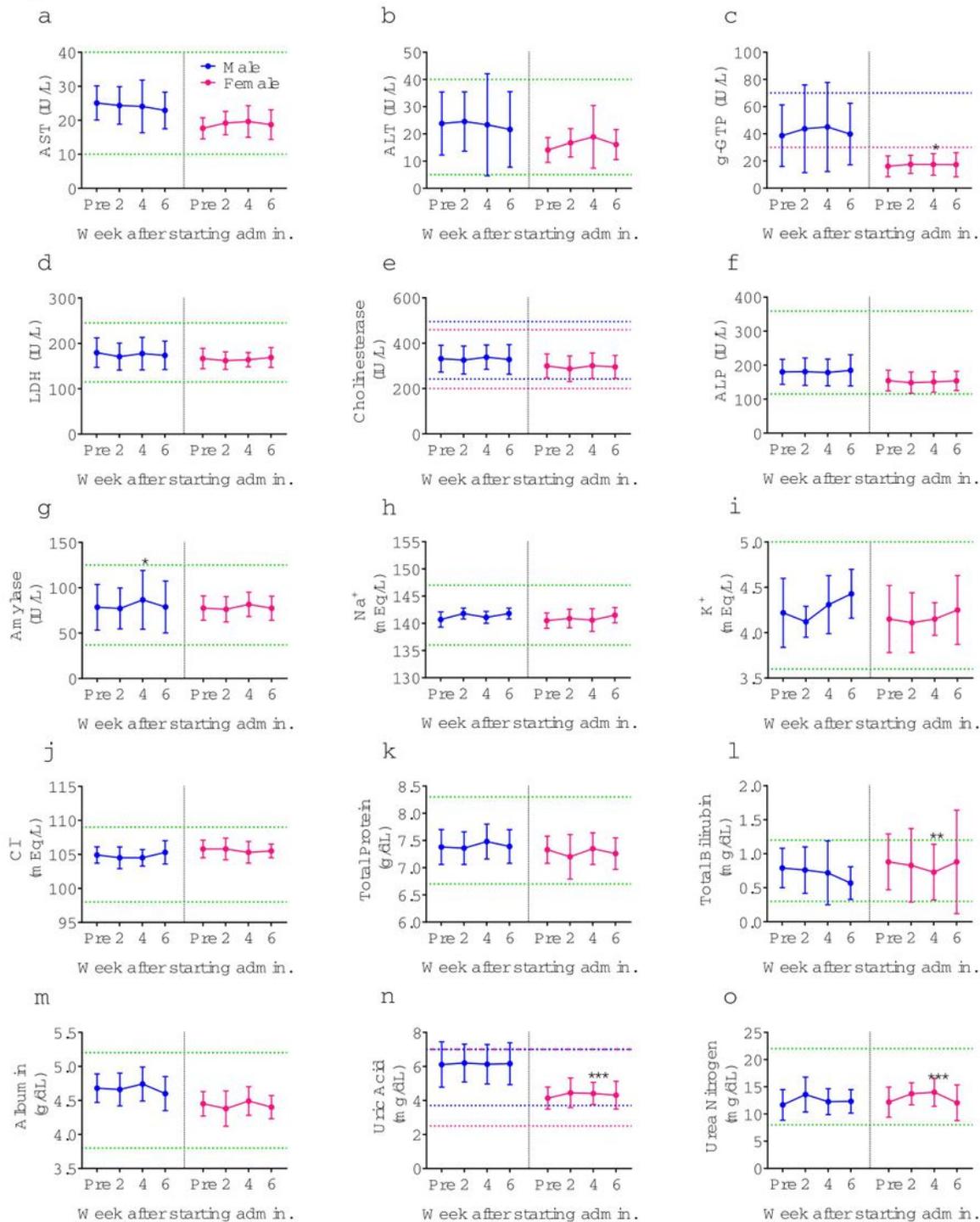


Figure 3

Blood biochemical test items Fluctuation of blood biochemical test items of male (blue) and female (pink). Dotted blue (male), pink (female) and green (items without sexual difference) lines show the upper and lower limit of reference range. The items without dotted line have no reference range. (A) Aspartate transaminase (AST), (B) alanine transaminase (ALT), (C) gamma-glutamyltransferase (γ -GTP), (D) lactate dehydrogenase (LDH), (E) cholinesterase, (F) alkaline phosphatase (ALP), (G) amylase, (H) Na⁺, (I) K⁺, (J) Cl⁻, (K) total protein, (L) total bilirubin, (M) albumin, (N) uric acid, and (O) urea nitrogen are shown. All data are expressed as mean \pm SD * p < 0.05, ** p < 0.01, *** p < 0.005 (paired t-test comparison of baseline to week 4). † p < 0.05, †† p < 0.01, ††† p < 0.005 (paired t-test comparison of week 4 to week 6).

Figure 4

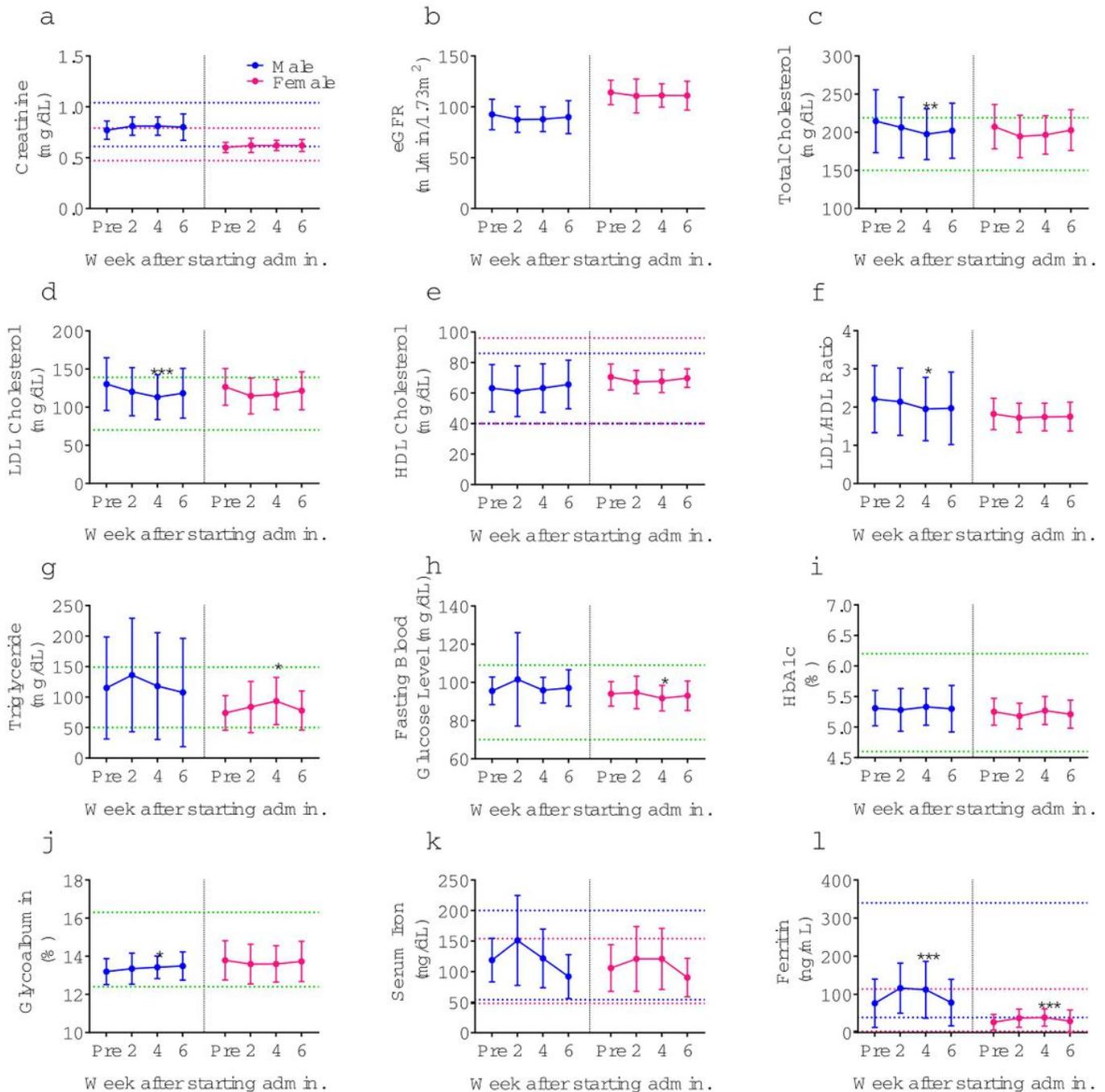


Figure 4

Blood biochemical test items (continued) Fluctuation of blood biochemical test items of male (blue) and female (pink). Dotted blue (male), pink (female) and green (items without sexual difference) lines show the upper and lower limit of reference range. The items without dotted line have no reference range. (A) creatinine, (B) estimated glomerular filtration rate (eGFR), (C) total cholesterol, (D) low-density lipoprotein (LDL) cholesterol, (E) high-density lipoprotein (HDL) cholesterol, (F) LDL/HDL ratio, (G) triglyceride, (H) fasting blood glucose, (I) glycosylated haemoglobin (HbA1c), (J) glycoalbumin, (K) serum iron, and (L) ferritin are shown. All data are expressed as mean \pm SD *p < 0.05, **p < 0.01, ***p < 0.005 (paired t-test comparison of baseline to week 4). †p < 0.05, ††p < 0.01, †††p < 0.005 (paired t-test comparison of week 4 to week 6).

Figure 5

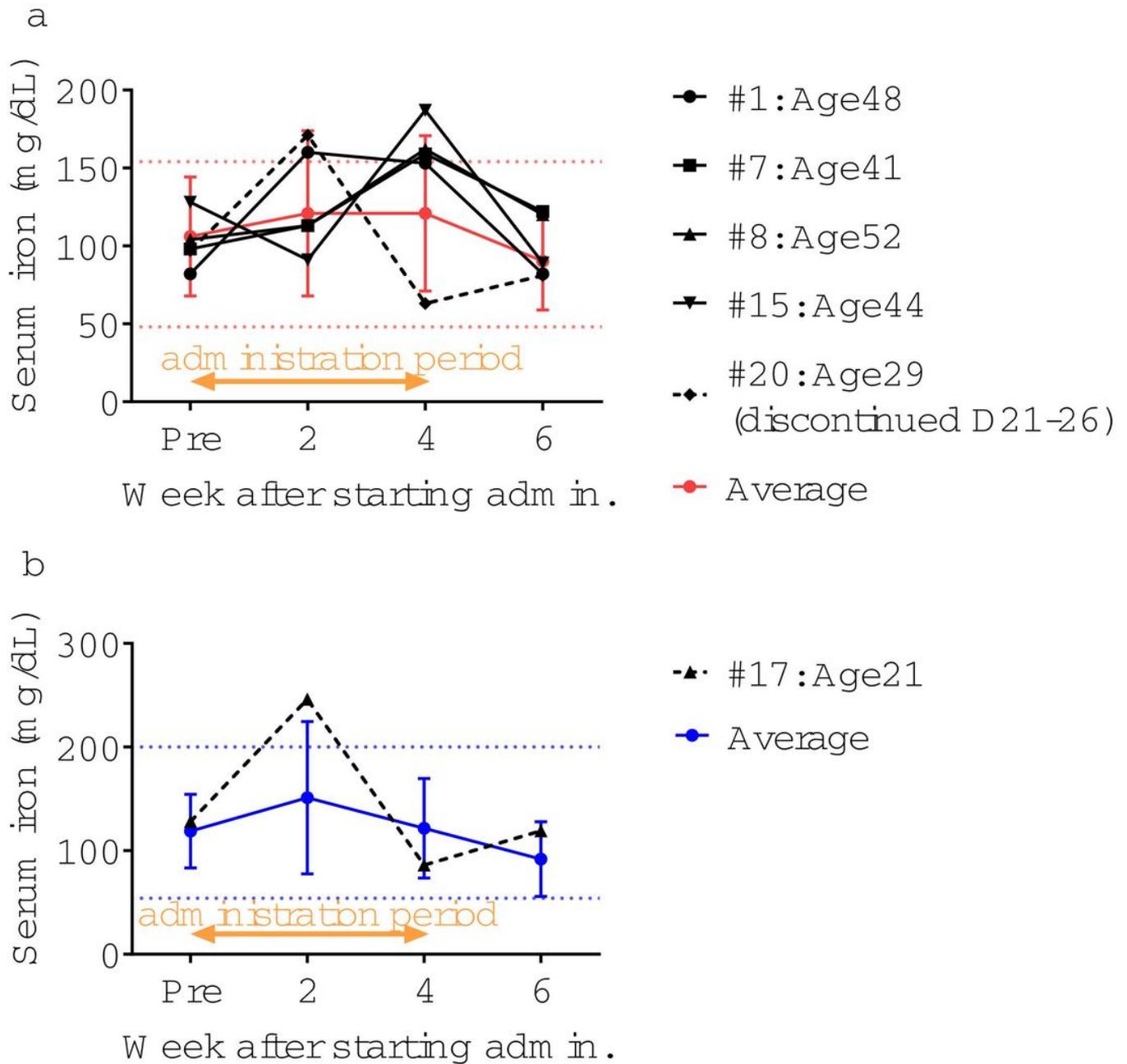


Figure 5

Serum iron level changes in subjects exceeded reference range Elevated serum iron of (A) female (red line: mean \pm SD) and (B) male individuals (blue line: mean \pm SD) diagnosed as related (black solid line) or causal relationship to test compounds not ruled out (black dotted line). The horizontal dotted line shows upper and lower limit of the normal values observed in the Hiroshima University Hospital. Of these subjects, female #20 discontinued administration from day 21 to day 26 owing to abdominal pain and diarrhoea.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation4.pdf](#)