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Research article

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20

21 **Abbreviations:** ANOVA, analysis of variance; AST, aspartate aminotransferase; CKD,
22 chronic kidney disease; COPD, chronic obstructive pulmonary disease; DHA, dehydroascorbic
23 acid; EDTA, ethylenediaminetetraacetic acid; HbA1c, hemoglobin A1c; LDL, low-density
24 lipoprotein; TIBC, iron-binding capacity; SEM, standard error of the mean; RDA,
25 recommended dietary allowance

26

Abstract

Background: Low plasma ascorbate levels in hemodialysis patients have been reported worldwide; hence, many end-stage kidney disease patients are forced to restrict their diets, especially potassium-rich fruits and vegetables, to prevent hyperkalemia. In this study, we aimed to clarify whether plasma ascorbate levels are low in Japanese dialysis patients and whether plasma ascorbate levels fluctuate before and after dialysis. In addition, we aimed to clarify whether there are clinical test items that have a causal relationship with plasma ascorbate levels.

Methods: Plasma ascorbate levels in 27 chronic kidney disease (CKD) stage G3–G5 patients (mean age 84 years) and pre- and post-dialysis plasma ascorbate levels in 19 CKD stage G5D hemodialysis patients (mean age 79 years) were determined using high-performance liquid chromatography and electrochemical detection.

Results: Pre-dialysis plasma ascorbate levels in hemodialysis patients (12.0 ± 1.4 μM) were significantly lower (by 56%) than those in CKD stage G3–G5 patients (27.1 ± 2.7 μM). After dialysis, there was a 40% reduction in plasma ascorbate levels. Moreover, pre-dialysis ascorbate levels correlated significantly with plasma potassium levels.

Conclusions: The study results indicate that Japanese hemodialysis patients have lower plasma ascorbate levels than CKD stage G3–G5 patients and that these low plasma ascorbate levels in hemodialysis patients were further reduced by hemodialysis. To avoid the development of scurvy in hemodialysis patients, it is necessary to take sufficient ascorbate from supplements or medicines.

Keywords: ascorbate; chronic kidney disease (CKD); hemodialysis; hyperkalemia; oxalate; potassium; scurvy; vitamin C

Background

Vitamin C (L-ascorbic acid) is a water-soluble micronutrient and antioxidant that scavenges reactive oxygen species [1-3]. Under physiological pH conditions, ascorbic acid most commonly exists in its mono-anion form, ascorbate [4]. In addition to its antioxidant property, ascorbate contributes to numerous well-defined enzymatic reactions involving collagen hydroxylation, carnitine and norepinephrine biosynthesis, tyrosine metabolism, and peptide hormone amidation [5-7]. Many vertebrates have the ability to synthesize ascorbate from glucose de novo in the liver [8]. However, primates, including humans, are unable to synthesize ascorbate since they carry multiple mutations in the *Gulo* gene encoding L-gulono-γ-lactone oxidase, the last enzyme in the ascorbate biosynthesis pathway [9]. Therefore, humans must consume ascorbate from dietary sources such as fresh fruits and vegetables to prevent scurvy. Scurvy is a condition that results from insufficient ascorbate in the body. Most scurvy symptoms such as anemia, weakness, and gingival bleeding are often seen in hemodialysis patients [10].

In the recent years, the increasing number of patients undergoing dialysis has become a social problem worldwide. Currently, there has been an increase in the mean age of incident dialysis

among patients aged ≥45 years, especially among those aged ≥65 years [11]. The proportion of patients aged ≥65 years at the end of 2012 was 65.5% in Japan, indicating the increase in dialysis incidence in the aging population [11].

Low plasma ascorbate levels have been observed in some hemodialysis patients for many years globally [12-18]. However, it is unclear why plasma ascorbate levels are low in hemodialysis patients. Hemodialysis patients are forced to have dietary restrictions, such as protein, salt, and potassium restriction. The consumption of fruits and vegetables that contain high amounts of ascorbate is also restricted due to the high potassium content.

In this study, we examined whether Japanese hemodialysis patients have low plasma ascorbate levels compared to non-hemodialysis-dependent patients with chronic kidney disease (CKD). In addition, we also examined whether dialysis reduces plasma ascorbate levels. Furthermore, we analyzed whether there are any clinical test items that have a causal relationship with plasma ascorbate levels in Japanese hemodialysis patients.

Methods

Ethical consideration

This study was conducted according to the principles expressed in the Declaration of Helsinki. This study was approved by the Clinical Research Ethics Committee of the Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan (permit number: R18-40) and Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (permit number: H30-25). All patients gave their written informed consent and patient anonymity is preserved.

Study patients

Patients with CKD stage G3–G5 (mean eGFR 23 mL/min/1.73 m^2) (n=34) and CKD stage G5D hemodialysis (n=19) who had been regularly visiting an outpatient clinic of Tokyo Metropolitan Geriatric Medical Center from October 2018 through December 2019 were recruited in this study. A diagnosis of CKD was made based on the guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative [19].

Hemodialysis regimen

Of the 19 patients, 10 were under maintenance hemodialysis and 9 patients were undergoing hemodiafiltration. The procedures were performed three times weekly for 9–12 h per week at a blood flow rate of 150–200 mL/min and dialysis flow rate of 500 mL/min using dialyzers with a surface area of 1.1–2.1 m^2. The dialysate sodium concentration was 140 mEq/L and the potassium concentration was 2.0 mEq/L. The mean duration of dialysis therapy was 3.6 years. The mean Kt/V was 1.3.

Collection of blood and urine samples

Blood samples for the measurement of clinical test items and ascorbate were obtained at the same time. For the determination of ascorbate levels, blood samples were drawn into a VENOJECT® collection tube (Terumo Corporation, Tokyo, Japan) containing ethylenediaminetetraacetic acid (EDTA)-2Na as an anticoagulant. All of the following procedures were performed within 2 h after sampling since we confirmed in advance that the values of ascorbate are unstable if it is beyond 2 h [20]. Plasma was obtained by centrifugation at 1,700 *g* for 10 min. After the plasma was collected, 0.5 mL of supernatant was immediately mixed with 0.5 mL of cold 10% metaphosphoric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 1 mmol/L of EDTA (Dojindo Laboratories, Kumamoto, Japan) and

118 centrifuged at 21,000 g for 15 min at 4 °C for the analysis of ascorbate level. After collecting
119 the urine samples, 0.5 mL of urine was immediately mixed with 0.5 mL of cold 10%
120 metaphosphoric acid containing 1 mmol/L of EDTA and centrifuged at 21,000 g for 15 min at
121 4 °C for the analysis of ascorbate level. All samples were stored at -80 °C until use.
122
123 **Collection of blood from dialysis**
124 To establish the basal plasma ascorbate levels in hemodialysis patients and the effect of
125 hemodialysis, blood samples were drawn (from the arteriovenous fistula or catheter) prior to
126 the start of dialysis (pre-dialysis sample) and immediately after ending the dialyzing period
127 (post-dialysis sample).
128
129 **Determination of ascorbate and dehydroascorbic acid (DHA)**
130 Ascorbate and DHA, which is an oxidized form of L-ascorbic acid, levels were measured using
131 high-performance liquid chromatography and electrochemical detection according to the
132 methods described previously [21]. After thawing, the plasma and urine were centrifuged at
133 21,000 *g* for 10 min at 4°C. For determination of total ascorbate including DHA, the centrifugal
134 supernatants were reduced with tris(2-carboxyethyl)phosphine hydrochloride for 2 h on ice.

After reduction, the reaction mixture was diluted with 5% metaphosphoric acid containing 0.5 mmol/L EDTA and analyzed for total ascorbate by high-performance liquid chromatography coupled with electrochemical detection. Separation was achieved on an Atlantis dC18 5-μm column (4.6 × 150 mm) combined with an Atlantis dC18 5-μm guard column (4.6 × 20 mm) from Nihon Waters (Tokyo, Japan). The mobile phase consisted of 50 mM phosphate buffer (pH 2.8), 540 μM EDTA, and 2% methanol at a flow rate of 1.3 mL/min, and electrical signals were recorded using an electrochemical detector with a glassy carbon electrode at +0.6 V. All electrical signal data from the electrochemical detector were collected using Waters Empower 2 software (Nihon Waters). The value of DHA was determined by subtracting ascorbate from total ascorbate. The ascorbate level of urine is immediately affected after a meal. Therefore, we evaluated the plasma ascorbate level in the samples whose urinary ascorbate was <0.5 mM.

Study items

The following data were collected from the medical records: age, gender, and clinical investigations, i.e., white blood cell, hemoglobin, hematocrit, platelet, total protein, albumin, C-reactive protein, aspartate aminotransferase (AST), blood urea nitrogen, creatinine, uric acid, sodium, potassium, calcium, phosphorus, triglyceride, total cholesterol, low-density lipoprotein

(LDL) cholesterol, iron, transferrin and iron-binding capacity (TIBC), ferritin, β2-microglobulin, prealbumin, hemoglobin A1c (HbA1c), and parathyroid hormone.

Statistical analysis

The results and clinical characteristic data are expressed as means ± standard error of the mean (SEM). The probability of statistical differences between experimental groups was determined by Welch's t-test, paired t-test, and one-way analysis of variance (ANOVA) followed by Tukey-Kramer test. We verified that Pearson correlation coefficient between ascorbate concentration and clinical characteristics data is different from zero. Statistical differences were considered significant at $p < 0.05$.

Results

Clinical characteristics of CKD stage G3–G5 and hemodialysis patients

A total of 34 CKD stage G3–G5 and 19 CKD stage G5D hemodialysis patients were enrolled. Since seven CKD stage G3–G5 patients showed that urine ascorbate level was higher than 0.5 mM, suggesting supplemental intake of ascorbate just before collecting blood and urine, they were excluded from analysis. Therefore, the results of 27 CKD stage G3–G5 patients were used for analysis. Clinical characteristics of CKD stage G3–G5 and hemodialysis patients are shown in Table 1. The levels of the following were beyond the normal range and differed between CKD stage G3–G5 and hemodialysis patients: albumin, blood urea nitrogen, creatinine, phosphorus, and β2-microglobulin. Albumin values of both CKD stage G3–G5 and hemodialysis patients were below the normal range and blood urea nitrogen, creatinine, and β2-microglobulin values of both CKD stage G3–G5 and hemodialysis patients were higher than the normal range. Phosphorus values of hemodialysis-only patients were higher than the normal range.

Plasma ascorbate levels in CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in hemodialysis patients

The plasma ascorbate levels in 27 CKD stage G3–G5 and pre- and post-dialysis plasma ascorbate levels in 19 hemodialysis patients were measured (see Fig. 1). Pre-dialysis plasma ascorbate levels in hemodialysis patients (12.0 ± 1.4 μM) were significantly lower (by 56%) than that in CKD stage G3–G5 patients (27.1 ± 2.7 μM) (Fig. 1a). Moreover, after dialysis, there was a 40% reduction in the plasma ascorbate levels (7.2 ± 0.9 μM) (Fig. 1b). In addition, the individual pre- and post-dialysis plasma ascorbate levels and distribution of ascorbate levels in CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in hemodialysis patients are shown in Figure 1b and c.

Relationships between clinical characteristics and plasma ascorbate levels

We then analyzed the relationships between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients (Fig. 2). In Pearson correlation coefficient, pre-dialysis ascorbate levels correlated significantly with those of plasma potassium levels (positive correlation; Pearson correlation coefficients (r) = 0.6; p = 0.006) (Table 2). However, no

association was found between plasma ascorbate levels and other clinical characteristics except for plasma potassium levels.

198 ## Discussion

199

200 In this study, we revealed that Japanese hemodialysis patients have low plasma ascorbate levels
201 compared to non-hemodialysis CKD patients and these low plasma ascorbate levels in
202 hemodialysis patients were further reduced by a single hemodialysis treatment session.
203 Moreover, we found that ascorbate levels in hemodialysis patients correlated with those of
204 plasma potassium levels. In general, hemodialysis patients are forced to restrict their diets,
205 especially potassium-rich fruits and vegetables, to prevent hyperkalemia, which is a risk factor
206 for dialysis morbidity and mortality [22]. Most of these fruits and vegetables also contain high
207 amounts of ascorbate. Thereby, plasma ascorbate levels in Japanese hemodialysis patients
208 might be correlated with plasma potassium levels.
209 The recommended dietary allowance (RDA) of vitamin C for a healthy adult is 100 mg per day
210 in Japan and 90 mg and 75 mg per day for men and women, respectively, in the United States
211 to prevent scurvy [23]. The average concentration of ascorbate in the plasma of healthy humans
212 is 40–60 μM [24, 25]. When the plasma ascorbate concentration drops to below 11 μM, there
213 is a risk of developing scurvy, which is thus conventionally considered deficient [24, 25]. In
214 our previous report regarding chronic obstructive pulmonary disease (COPD) and plasma

215 ascorbate levels, we reported that plasma ascorbate levels were significantly lower in COPD
216 patients (mean age 72.7 ± 6.9 years) than those in healthy elderly people (mean age 68.8 ± 3.8
217 years) using the same procedure and method as here [20]. Plasma ascorbate levels in COPD
218 patients and healthy elderly people were 31.2 ± 2.2 μM and 42.3 ± 2.9 μM, respectively.
219 Furthermore, the observed plasma levels in non-hemodialysis CKD patients and hemodialysis
220 patients in the present study were lower than those in COPD patients. Plasma ascorbate levels
221 in non-hemodialysis CKD patients and hemodialysis patients were 27.1 ± 2.7 μM and 12.0 ±
222 1.4 μM, respectively. Since there is a risk of developing scurvy when the plasma ascorbate
223 concentration drops to below 11 μM [24, 25], many Japanese hemodialysis patients are likely
224 develop scurvy (see Fig. 3). Worldwide, many hemodialysis patients have developed scurvy
225 [10, 26, 27].
226 Moreover, we tried to compare the percentages of DHA per total ascorbate in plasma from
227 healthy elderly people, COPD patients, non-hemodialysis CKD patients, and hemodialysis
228 patients, and found that the percentages of DHA in non-hemodialysis CKD patients (33.5%)
229 and hemodialysis patients (37.4%) were notably higher percentages than those in COPD
230 patients (12.4%) and healthy elderly people (10.0%) (see Fig. 3) [20]. High percentage of DHA

231 in non-hemodialysis CKD patients and hemodialysis patients may reflect a higher oxidative
232 stress levels in their body.
233 Wang *et al.* [16] reported that plasma ascorbate concentrations were reduced by a median of
234 33% following dialysis. Deicher *et al.* [28] have also reported that hemodialysis causes a 50–
235 75% decrease in plasma ascorbate levels. In the present study, plasma ascorbate levels reduced
236 to 40% by hemodialysis. Thus, hemodialysis certainly reduces plasma ascorbate concentration
237 in hemodialysis patients.
238 For a long time, there has been concern about the accumulation and deposition of oxalate with
239 increased intake of vitamin C because oxalate is a breakdown product of vitamin C and is
240 heavily excreted by the kidneys [29]. Oxalate crystallization occurs at levels above 30 mM [30]
241 and high plasma oxalate levels were seen in hemodialysis patients [31-33]. However, in a recent
242 prospective case series exploring high-dose intravenous vitamin C (15–100 g) administration,
243 increased vitamin C intake was not associated with any cases of symptomatic renal stones and
244 kidney injury [34]. Moreover, significant side effects of vitamin C are not reported in any of
245 the mentioned controlled trials, including the most recent VITAMIN randomized trial [35].
246 CKD patients with higher levels of plasma calcium, phosphate, and parathyroid hormone have
247 a high risk of death because CKD often causes abnormal calcium and phosphate metabolism

248 and hyperparathyroidism [36-40]. Therefore, it is important to control the plasma calcium,
249 phosphate, and parathyroid hormone levels in the non-hemodialysis CKD and hemodialysis
250 patients [41]. Through a systematic review and meta-analysis, Ke *et al.* [42] have reported that
251 vitamin C supplementation in CKD patients has no positive effect that influence the plasma
252 phosphate or parathyroid hormone levels, but it increase plasma calcium levels in the short term.
253 In the present study, we could not detect any correlation between plasma ascorbate and plasma
254 calcium, phosphate, and parathyroid hormone levels in Japanese hemodialysis patients.
255 Meanwhile, we only found the positive correlation between plasma ascorbate and plasma
256 potassium levels in Japanese hemodialysis patients. Perhaps Japanese hemodialysis patients
257 that have dietary potassium restrictions to prevent hyperkalemia may limit their consumption
258 of fresh vegetables and fruits that are rich in ascorbate. Thereby, there is a possibility that
259 Japanese hemodialysis patients have low plasma ascorbate levels.
260 Recently, the increase of frailty in the elderly has become a social problem globally. The
261 Dialysis Morbidity and Mortality Wave 2 cohort study revealed that >60% of end-stage kidney
262 disease patients over the age of 40 met a definition of frailty, which impairs the prognosis [43].
263 Ascorbate is known to be one of the anti-aging factors because of its strong antioxidant

properties [3]. Therefore, ascorbate may be causally associated with life prognosis and aging in hemodialysis patients.

Conclusion

Japanese hemodialysis patients have low plasma ascorbate levels and are likely to develop scurvy. Furthermore, their plasma ascorbate levels are reduced by approximately 40% by a single hemodialysis. The cause of the low plasma ascorbate levels in hemodialysis patients may be due to the decreased intake of ascorbate from fresh fruits and vegetables due to the strict restriction of potassium intake. To avoid the development of scurvy in hemodialysis patients, it is necessary to consume sufficient ascorbate from supplements or medicine because of the body's inability to synthesize ascorbate.

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Availability of data and materials

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

Authors' contributions

MI, TT, WY, NM, and AI designed the research; MI, TT, YT, AS, TY, and AI conducted the experiments; YD, MI, TT, WY, and AI analyzed the data; and YD, MI, TT, YT, AS, TY, WY, NM, and AI wrote the manuscript and had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors declare no conflicts of interest.

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Figure Legends

Fig. 1 Plasma ascorbate levels in non-hemodialysis CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in CKD stage G5D hemodialysis patients. Total ascorbate levels were determined as described in Methods. (a, b) Dots in boxplots are expressed ascorbate levels in CKD stage G3–G5 (n=27) and hemodialysis (n=19) patients. Center lines are expressed median value of each groups. (c) Distributions of ascorbate levels in each group. $P < 0.05$ by (a) Welch's t-test and (b) paired t-test.

Fig. 2 Scatterplots between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients. Dots are expressed data of individual hemodialysis patients (n=19). Blue and red lines are regression lines of data sets between ascorbate levels and clinical data. Pearson correlation coefficients are described in Table 2.

Fig. 3 Plasma ascorbate levels in healthy controls (n=28) [20] and chronic obstructive pulmonary disease (COPD) (n=39) [20], non-hemodialysis chronic kidney disease (CKD) stage G3–G5 (n=27), and hemodialysis (n=19) patients. Ascorbate (blue column) and DHA (yellow

441 column) levels were determined as described in Methods. The average concentration of
442 ascorbate in the plasma of healthy humans is 40–60 μM (blue zone). There is a risk of
443 developing scurvy when the plasma ascorbate concentration drops to below 11 μM (red zone).
444 Values are expressed as a mean ± SEM. $P < 0.05$ by one-way ANOVA followed Tukey-Kramer
445 test.
446

Table 1 Clinical characteristics of CKD stage G3–G5 and hemodialysis patients

Characteristic	Normal range	CKD stage G3–G5 *(n=27)*	Hemodialysis *(n=19)*
Age (years)		83.9 ± 1.3	78.9 ± 2.5
Sex (male/female)		10/17	9/10
White blood cell (x10³/μl)	M: 3.9–9.8	M: 6.7 ± 0.6	M: 6.5 ± 0.4
	F: 3.5–9.1	F: 7.0 ± 0.4	F: 7.1 ± 1.1
Hemoglobin (g/dL)	M: 13.5–17.6	M: 12.1 ± 0.4	M: 11.3 ± 0.2
	F: 11.3–15.2	F: 11.8 ± 0.3	F: 11.2 ± 0.2
Hematocrit (%)	M: 39.8–51.8	M: 36.5 ± 1.2	M: 34.3 ± 0.8
	F: 33.4–44.9	F: 36.2 ± 0.7	F: 35.1 ± 0.8
Platelet (x10^4/μl)	M: 13.1–36.2 *	M: 20.5 ± 2.0	M: 23.8 ± 2.3
	F: 13.0–36.9	F: 22.1 ± 1.4	F: 16.0 ± 1.9
Total protein (g/dL)	6.7–8.3 *	6.9 ± 0.1	6.3 ± 0.1
Albumin (g/dL)	3.8–5.2 *	3.6 ± 0.1	3.1 ± 0.1
C-Reactive protein (mg/dL)	< 0.3	0.4 ± 0.1	0.8 ± 0.6
AST (IU/L)	37.0–125.0	21.2 ± 1.0	24.7 ± 8.4
Blood urea nitrogen (mg/dL)	8.0–22.0 *	32.9 ± 2.8	62.7 ± 3.1
Creatinine (mg/dL)	M: 0.6–1.0 *	M: 2.0 ± 0.3	M: 10.4 ± 0.6
	F: 0.5–0.8 *	F: 2.1 ± 0.3	F: 7.4 ± 0.4
Uric acid (mg/dL)	M: 3.7–7.0	M: 6.0 ± 0.3	M: 5.9 ± 0.5
	F: 2.5–7.0	F: 6.3 ± 0.4	F: 5.9 ± 0.5
Sodium (mEq/L)	136.0–147.0 *	140.4 ± 0.7	137.7 ± 0.8
Potassium (mEq/L)	3.6–5.0 *	4.4 ± 0.1	4.9 ± 0.2
Calcium (mg/dL)	8.5–10.2 *	9.1 ± 0.1	8.5 ± 0.1
Phosphorus (mg/dL)	2.4–4.3 *	3.8 ± 0.1	5.1 ± 0.3
Triglyceride (mg/dL)	50.0–149.0 *	150.9 ± 14.1	108.7 ± 7.5
Total cholesterol (mg/dL)	150.0–219.0 *	200.9 ± 11.3	152.8 ± 8.7
LDL cholesterol (mg/dL)	70.0–139.0 *	109.5 ± 6.8	81.2 ± 6.1
Iron (μg/dL)	M: 54.0–200.0 *	M: 66.2 ± 4.4	M: 62.8 ± 14.9
	F: 48.0–154.0	F: 76.6 ± 6.8	F: 47.5 ± 15.5
TIBC (μg/dL)	M: 253.0–365.0	M: 234.6 ± 5.2	M: 251.7 ± 12.8
	F: 246.0–410.0	F: 253.6 ± 8.6	F: 230.9 ± 15.5
Ferritin (ng/dL)	M: 3940–34,000	M: 159.6 ± 27.3	M:105.9 ± 27.9

	F: 360–11,400	F: 177.2 ± 60.7	F: 117.3 ± 25.1
β2-Microglobulin (mg/L)	1.0–1.9 *	5.1 ± 0.6	27.0 ± 1.7
Prealbumin (mg/dL)	22.0–40.0	23.2 ± 1.1	24.1 ± 1.3
HbA1c (%)	4.6–6.2	6.1 ± 0.1	5.8 ± 0.2

Values are presented as the mean ± SEM. * significant difference at $p < 0.05$

M, male; F, female; CKD, chronic kidney disease; AST, aspartate aminotransferase; LDL, low-density lipoprotein; TIBC, total iron-binding capacity; HbA1, hemoglobin A1c

447

Table 2 Pearson correlation coefficients (r) between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients

Characteristic	r	*p*-value
Age	0.44	0.056
Dry weight	0.02	0.934
White blood cell	0.12	0.617
Hemoglobin	0.18	0.461
Hematocrit	0.22	0.362
Platelet	-0.38	0.104
Total protein	0.02	0.944
Albumin	-0.12	0.632
C-Reactive protein	0.40	0.087
AST	0.30	0.206
Blood urea nitrogen	0.05	0.835
Creatinine	-0.12	0.633
Uric acid	-0.05	0.828
Sodium	0.15	0.537
Potassium *	0.60	0.006
Calcium	0.05	0.829
Phosphorus	-0.14	0.578
Triglyceride	0.30	0.206
Total cholesterol	0.32	0.179
LDL cholesterol	0.18	0.454
Iron	0.01	0.962
TIBC	-0.33	0.161
Ferritin	0.44	0.060
β2-Microglobulin	0.18	0.465
Prealbumin	0.00	0.995
HbA1c	-0.19	0.430
Parathyroid hormone	-0.06	0.816

* p-value < 0.05

AST, aspartate aminotransferase; LDL, low-density lipoprotein; TIBC, total iron-binding capacity; HbA1, hemoglobin A1c

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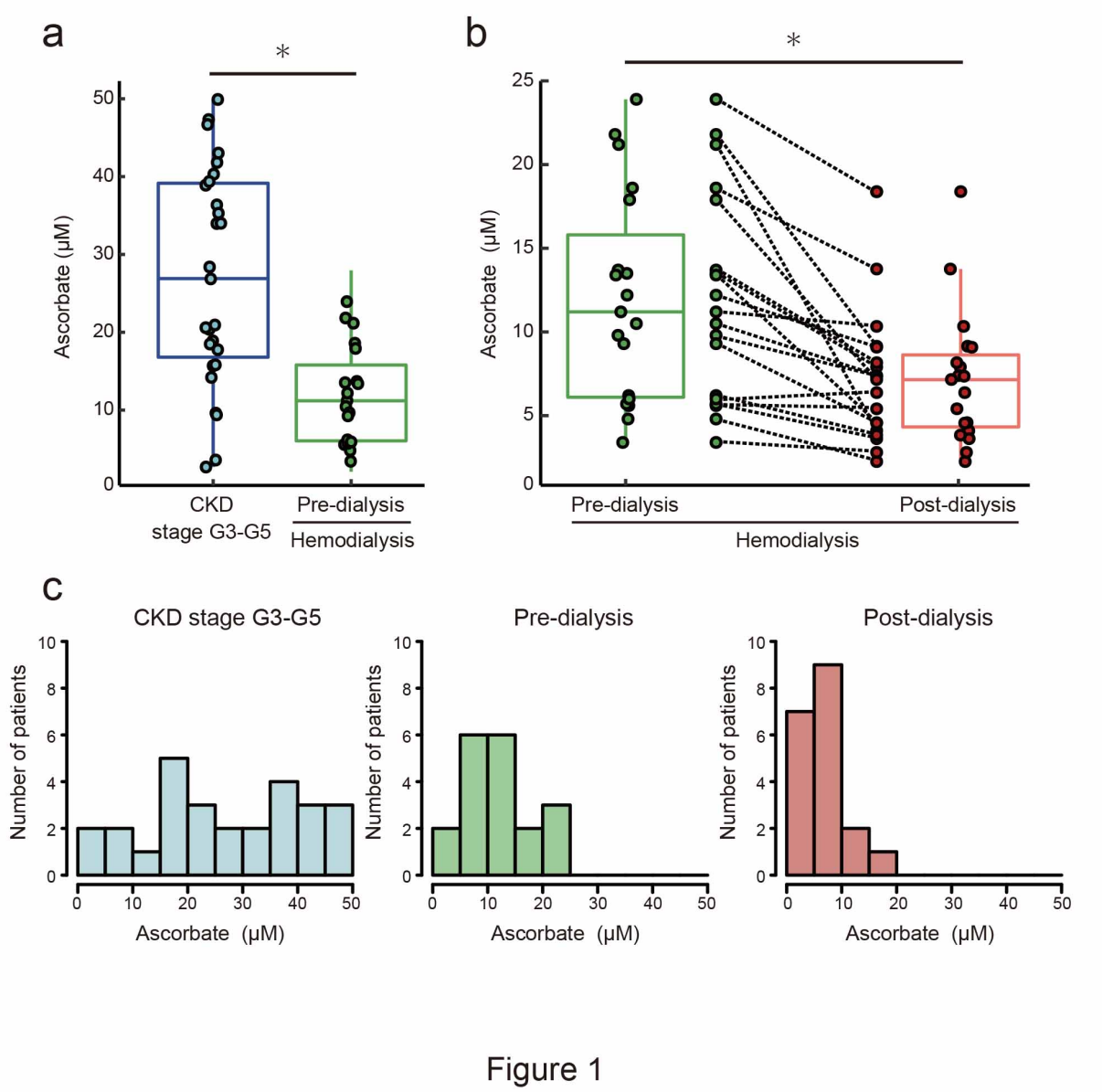
a
*
Ascorbate (μM)
50
40
30
20
10
0
CKD stage G3-G5
Pre-dialysis
Hemodialysis
b
*
Ascorbate (μM)
25
20
15
10
5
0
Pre-dialysis
Post-dialysis
Hemodialysis
c
CKD stage G3-G5
Pre-dialysis
Post-dialysis
Number of patients
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8
6
4
2
0
0
10
20
30
40
50
Ascorbate (μM)

Figure 1

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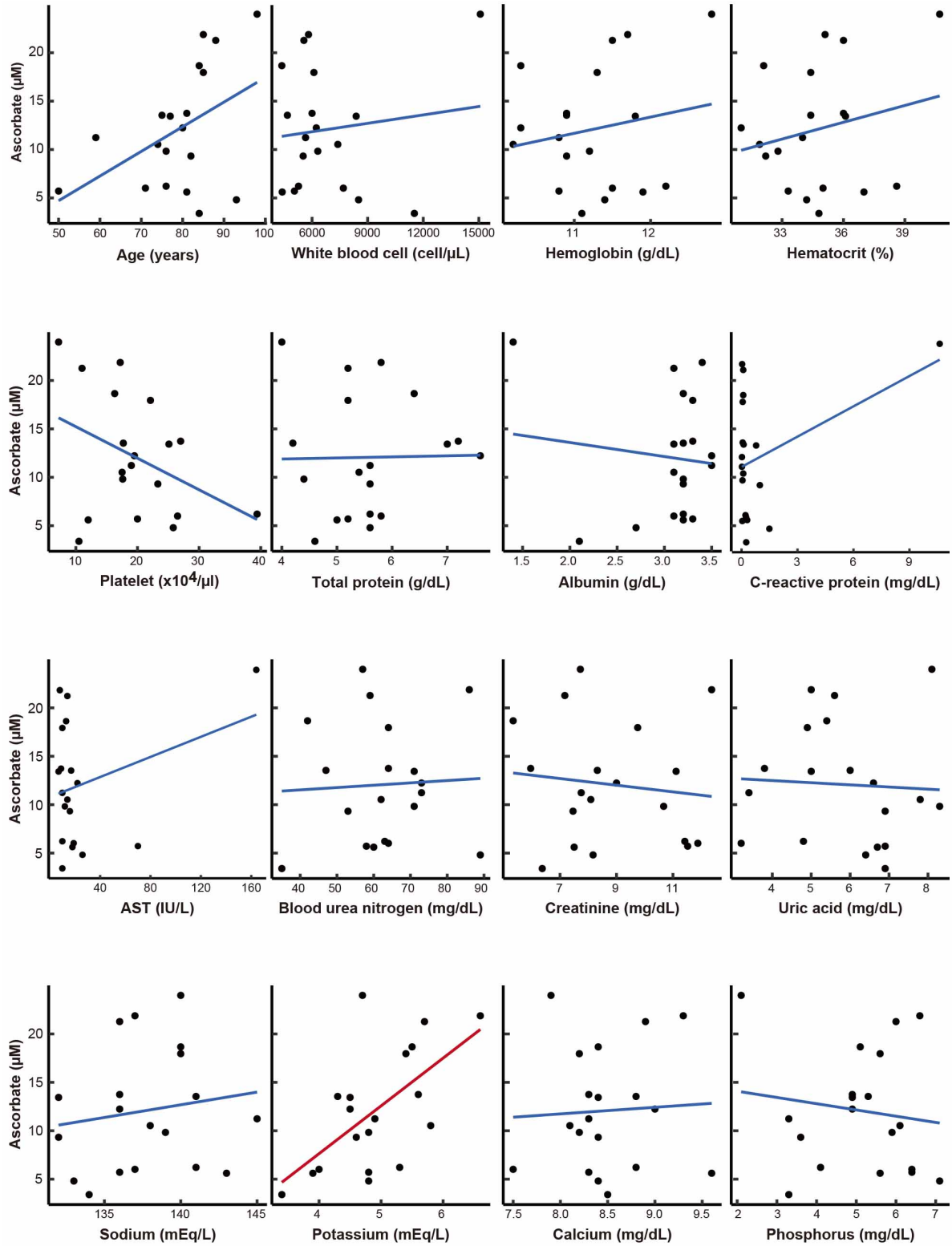

Figure 2

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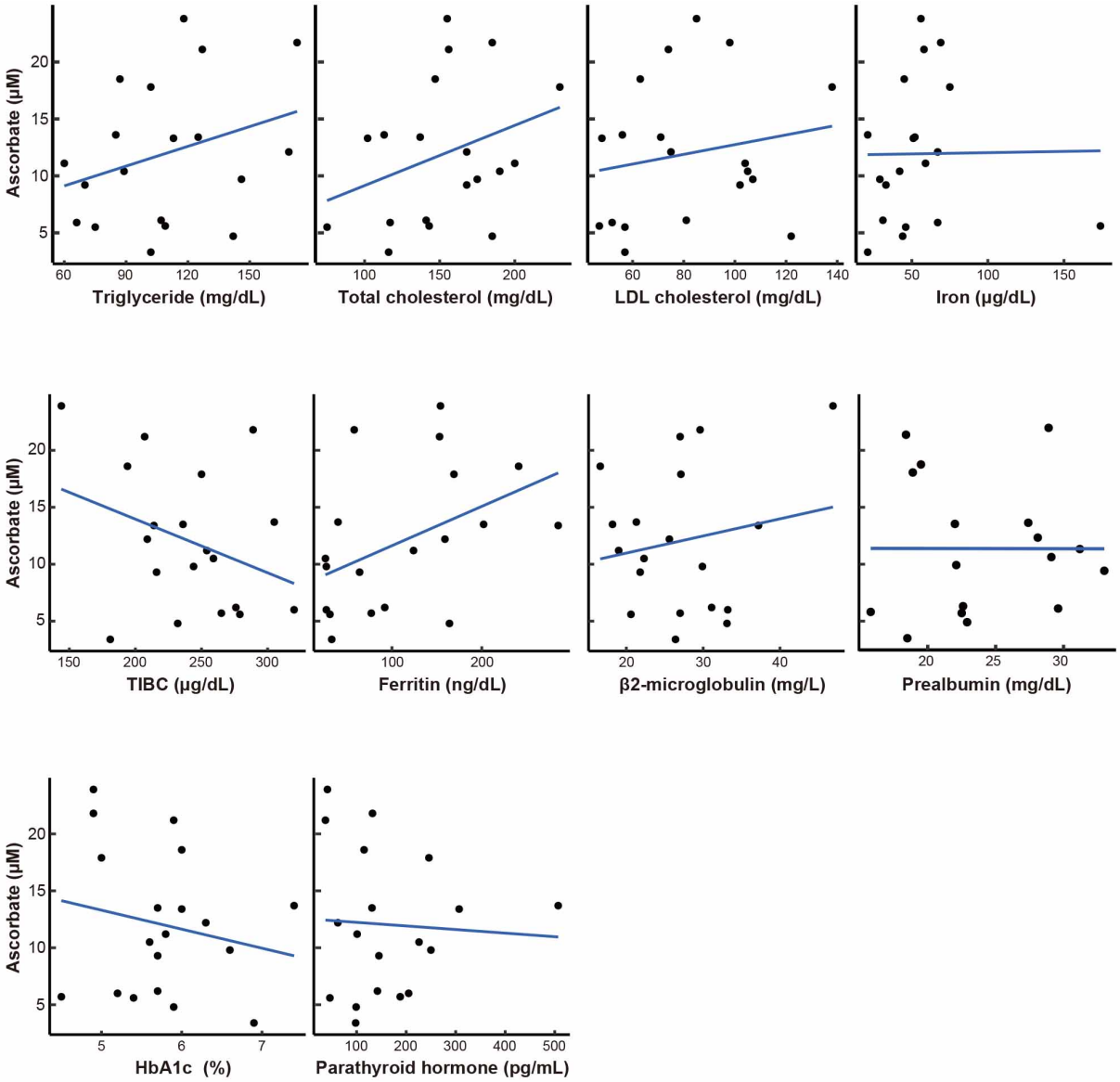

Figure 2

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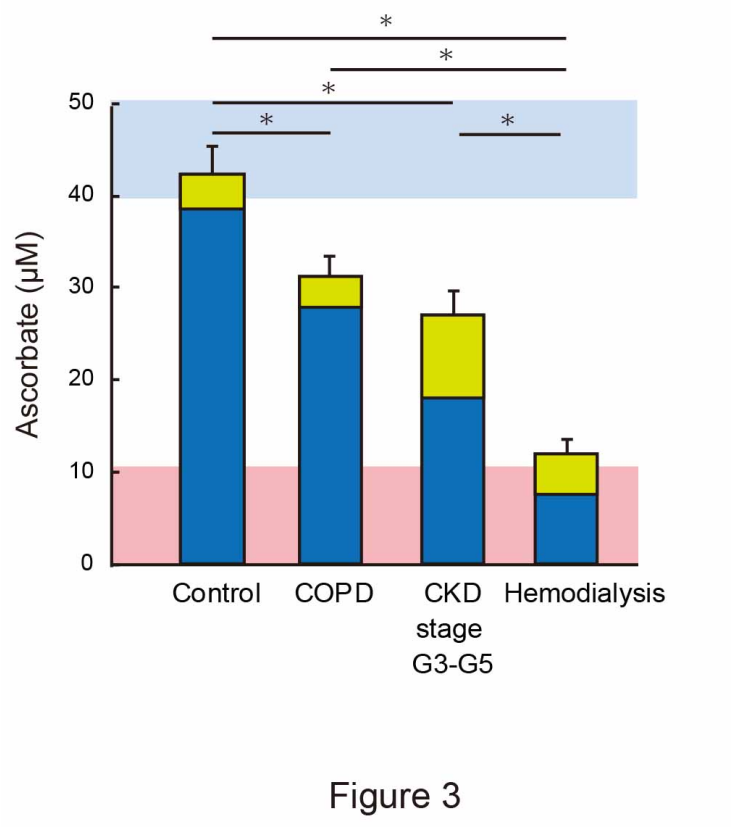

Figure 3

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Figures

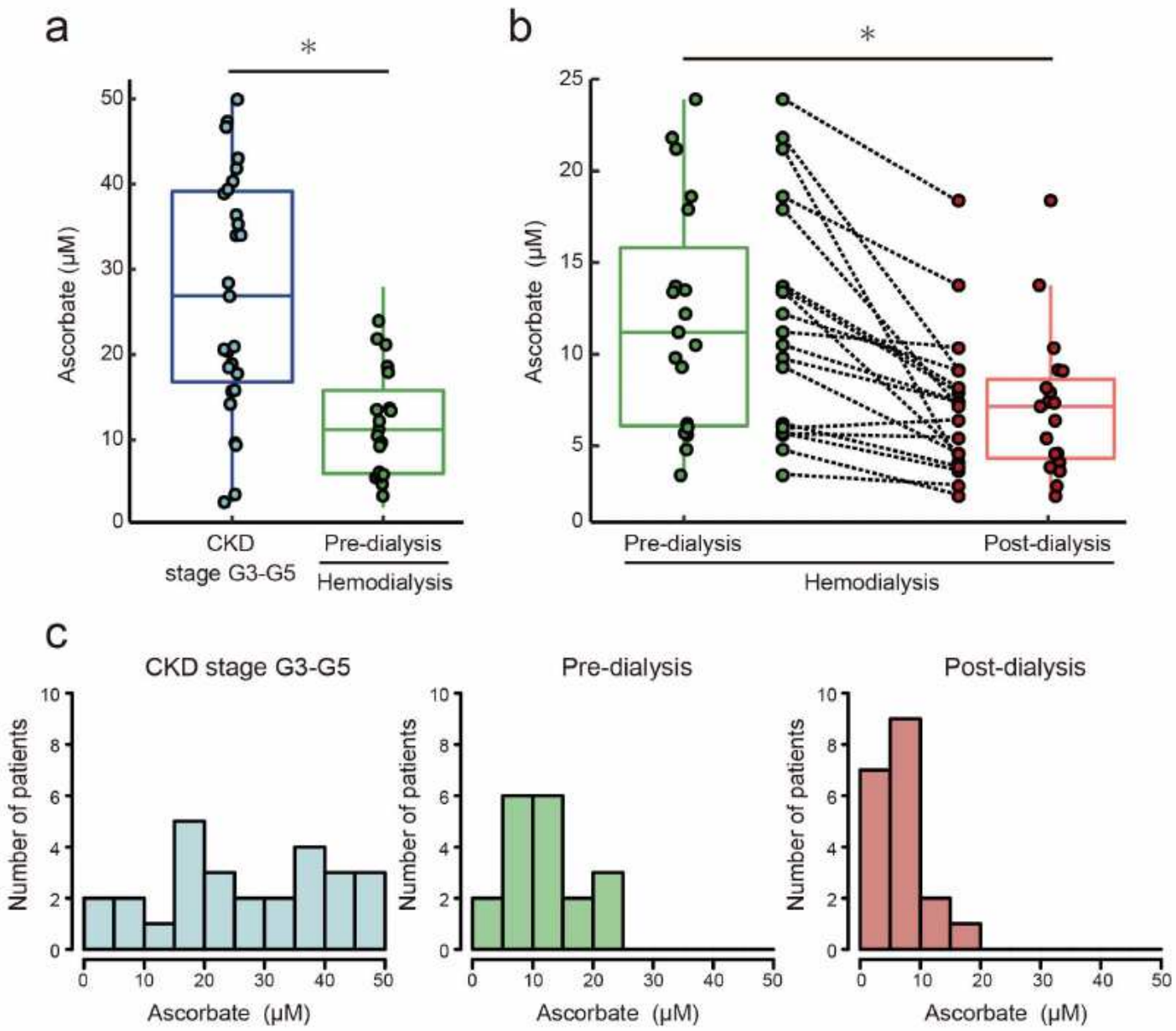

Figure 1

Plasma ascorbate levels in non-hemodialysis CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in CKD stage G5D hemodialysis patients. Total ascorbate levels were determined as described in Methods. (a, b) Dots in boxplots are expressed ascorbate levels in CKD stage G3–G5 (n=27) and hemodialysis (n=19) patients. Center lines are expressed median value of each groups. (c) Distributions of ascorbate levels in each group. $P < 0.05$ by (a) Welch's t-test and (b) paired t-test.

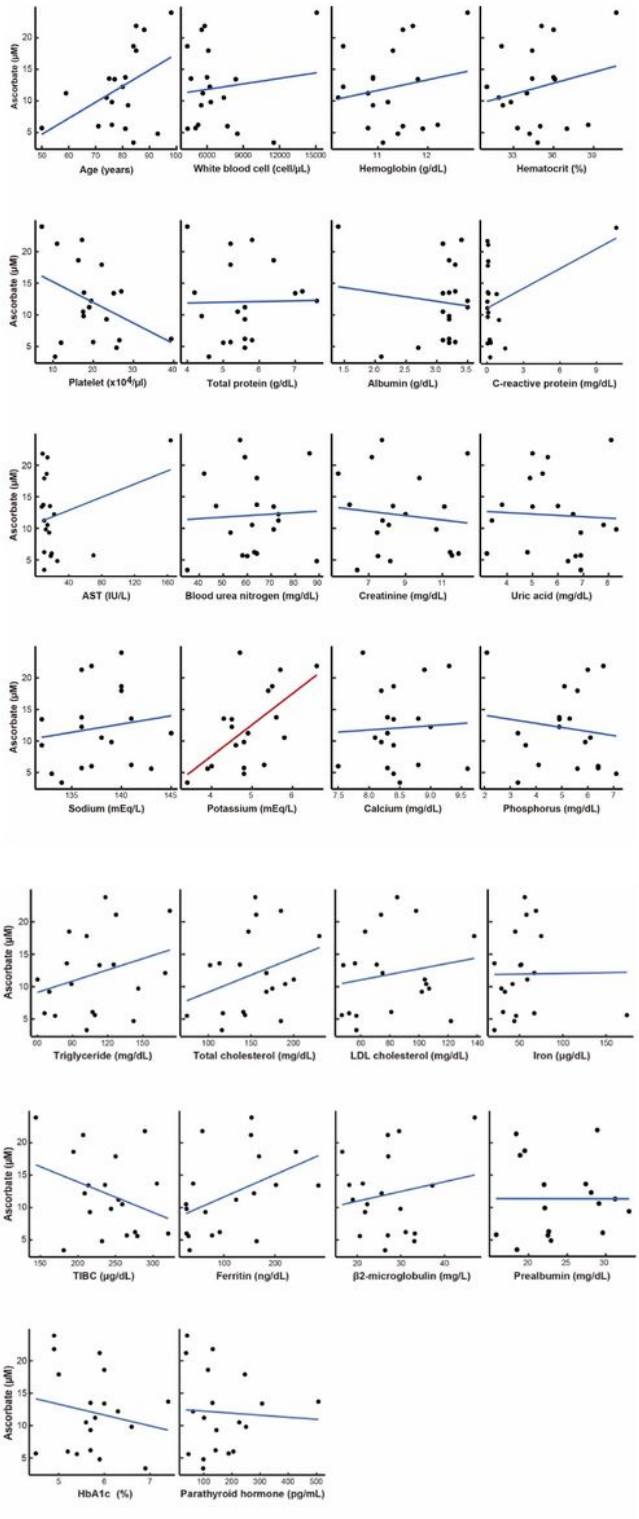

Figure 2

Scatterplots between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients. Dots are expressed data of individual hemodialysis patients (n=19). Blue and red lines are regression lines of data sets between ascorbate levels and clinical data. Pearson correlation coefficients are described in Table 2.

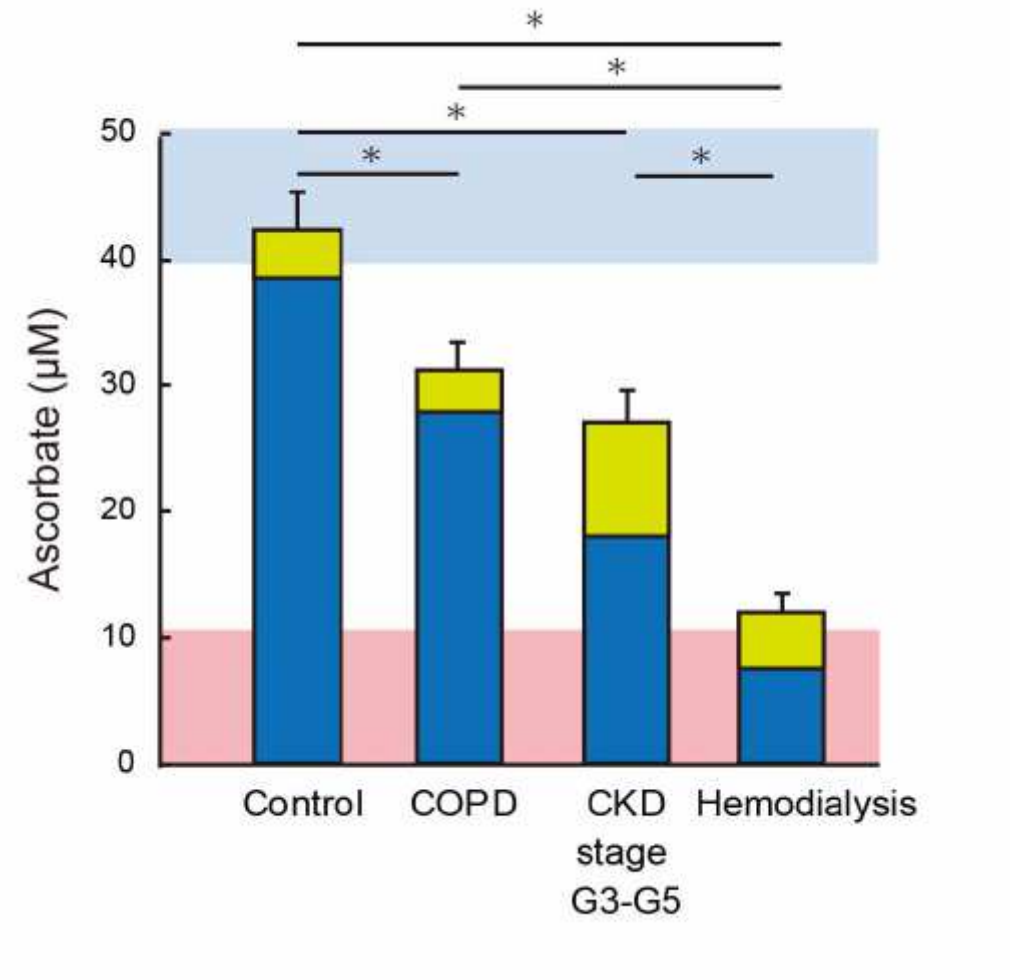

Figure 3

Plasma ascorbate levels in healthy controls (n=28) [20] and chronic obstructive pulmonary disease (COPD) (n=39) [20], non-hemodialysis chronic kidney disease (CKD) stage G3–G5 (n=27), and hemodialysis (n=19) patients. Ascorbate (blue column) and DHA (yellow column) levels were determined as described in Methods. The average concentration of ascorbate in the plasma of healthy humans is 40–60 μM (blue zone). There is a risk of developing scurvy when the plasma ascorbate concentration drops to below 11 μM (red zone). Values are expressed as a mean ± SEM. $P < 0.05$ by one-way ANOVA followed Tukey-Kramer test.