# Erythrocyte concentrations of B1, B2, B6 but not plasma C and E are reliable indicators of nutrition status in the presence of systemic inflammation.

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Short running head: Vitamin B1, B2, B6, C and E concentrations and systemic inflammation.

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**Abstract**

**Background & aim:** There is increasing evidence that the plasma concentration of vitamin D, carotenoids, zinc and selenium are associated with the magnitude of the systemic inflammatory response. In order to examine whether other vitamins may be affected and whether red cell concentrations are less affected by systemic inflammation the aim of the present study was to examine the effect of the systemic inflammatory response on red cell measurements of vitamins B1, B2 and B6, and plasma concentration of vitamin C and E in a large cohort of patients referred for a nutritional screen.

**Methods:** Patients referred for nutritional assessment of B1 (n=551), B2 (n=251), B6 (n=313), ascorbic acid (n=494) and α-tocopherol (n=395) concentrations. These vitamins were measured using routine laboratory methods.

**Results:** The median concentrations of vitamin B1 grouped according to C-reactive protein concentrations ≤10, 11-80 and >80 mg/L were 543, 664 and 766 ng/g Hb respectively ($p$<0.001, 41% higher). The median concentration of vitamin B1 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 547, 664 and 701 ng/g Hb respectively ($p$<0.001, 28% higher). The median concentrations of red cell vitamin B2 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 2.2, 2.3 and 2.4 nmol/g Hb respectively ($p$<0.001, 9% higher). The median red cell concentrations of vitamin B2 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 2.1, 2.4 and 2.3 nmol/g Hb respectively ($p$<0.001, 14% higher). The median concentrations of red cell vitamin B6 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 534, 548 and 767 pmol/g Hb respectively ($p$<0.001, 44% higher). The median red cell concentrations of vitamin B6 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 462, 644 and 840 pmol/g Hb respectively ($p$<0.001, 82% higher).

In contrast, the median plasma concentrations of ascorbic acid grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 25.0, 15.0 and 6.0 µmol/l respectively (78% lower, p<0.001). The median plasma concentrations of ascorbic acid grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 32.0, 13.0 and 5.0 µmol/l respectively (84% lower, p<0.001). The median α-tocopherol/ cholesterol grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 5.9, 4.6 and 2.1 umol/l respectively (64% lower, p<0.001). The median α-tocopherol/cholesterol grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 6.0, 5.5 and 2.1 umol/l respectively (65% lower, p<0.001).

**Conclusion:** Red cell concentrations of vitamins B1, B2 and B6 were not lower with an increasing systemic inflammatory response. In contrast, plasma concentrations of vitamin C and E were lower. Therefore, compared with plasma concentration, red cell concentrations of B1, B2 and B6 are likely to be more reliable measures of status in the presence of a systemic inflammatory response.

**Key words**: Vitamin B1, B2; B6, ascorbic acid; α-tocopherol; C-reactive protein; albumin.

## 1.1. Introduction

There is increasing evidence that the plasma concentration of a variety of trace elements and vitamins are associated with the magnitude of the systemic inflammatory response [1-4]. There was significant lowering of plasma concentrations of vitamins A, B6, C, E, carotenoids and trace elements zinc and selenium associated with an elevated systemic inflammatory response, when adjusted for CRP concentrations alone [3]. Indeed, it was shown that as the severity of systemic inflammatory response increased, the proportion of results below the lower reference limit increased. However, the variability of the association was considerable such that plasma α-tocopherol and ascorbic acid concentrations were not readily adjustable by CRP concentrations. Ghashut and co-workers (2013; 2014; 2016) have recently reported that plasma concentrations of fat soluble micronutrients such as 25 (OH) D, carotenoids, zinc and selenium may be confounded in the presence of a systemic inflammatory response with both CRP and albumin having an independent effect on plasma concentrations [5-7].

Therefore, it is of interest that red cell concentrations of some micronutrients appear to be less affected by the magnitude of the systemic inflammatory response [8;9]. Therefore, the aim of the present study was to examine the effect of the systemic inflammatory response, as evidenced by both CRP and albumin, on red cell measurements of vitamins B1, B2 and B6, and plasma measurements of vitamins C and E in a cohort of patients referred for nutritional screen.

## 1.2 Patients and Methods

### 1.2.1 Nutritional screen cohort red cell B1, B2, B6, C and E

Consecutive heparin-treated whole-blood samples from individual patients that had red cell B1 (n=553), B2 (n=251) and B6 (n=313) measurements were examined. These samples were received from hospitals throughout Scotland between January 2008 and March 2013 for routine laboratory analysis. Consecutive heparin-treated whole-blood samples from individual patients that had plasma vitamin C (n=494) and E (n=359) measurements were examined. As a regional centre blood samples were sent for analysis if the patient was considered at nutritional risk and was often secondary to a number of disease states. Only the patients who had both CRP and albumin measured with the vitamins were included.

This investigation was conducted with the intent of developing local guidelines to aid in the interpretation of vitamin B1, B2, B6, C and E results. This was a large convenience retrospective sample that arose from an audit of patients who had a sample sent to a regional laboratory for a nutritional screen. The patient was anonymised and de-identified prior to analysis (Andrew Duncan and Donald C McMillan respectively). Approval for audit purposes was obtained from the local ethics committee of the North Glasgow NHS Trust.

### 1.2.2. Analytical methods

Vitamins B1, B2 and B6 were assessed by measuring TDP, FAD and PLP respectively in red blood cells. Vitamins C and E were assessed by measuring ascorbic acid and α-tocopherol. These were measured by HPLC using established routine laboratory methods previously described [1;10;11]. The inter assay precision was <10% for all analytes.

C-reactive protein and albumin were measured using an automated analyzer (Architect; Abbot Diagnosis, Maidenhead, UK). The limits of detection for albumin and C-

reactive protein were 10g/L and 0.2 mg/L respectively. The inter-assay CV was <6% for both analytes.

### 1.2.3. Statistical analysis

Data was presented in median and (range) value. Correlations between variables in the convenience sample were carried out using the Spearman rank correlation. The cohorts were divided into three groups according to CRP concentrations ≤10, 11-80 and >80 mg/L as previously described [12]. With each CRP group the concentration of individual vitamins B1, B2, B6, ascorbic acid and α-tocopherol/ cholesterol were grouped according to 3 categories of albumin concentrations ≥ 35, 25-34,< 25 g/L as previously described [13]. A P-value < 0.05 was considered significant and the analysis of the data was carried out using SPSS software (version 19; SPSS Inc, Chicago, Ill).

## 1.3. Results

### 1.3.1 Nutritional screen cohorts

The characteristics of the convenience sample for whole blood vitamin B1 (n=553) are shown in **Table 1**. The majority were older than 50 years (median 54 years), male (50%) and had plasma albumin concentrations below the normal range whereas plasma CRP and whole blood vitamin B1 were in the normal range. Whole blood vitamin B1 was significantly associated with CRP ($r_s$= 0.290, $p$<0.001) and albumin ($r_s$= -0.265, p<0.001). These relationships are shown in Figures 1a, 1b and 1c.

The characteristics of the convenience sample for red cell vitamin B2 (n=251) are shown in **Table 1**. The majority were older than 50 years (median 51 years), female (56%) and had plasma albumin were below the normal range and plasma CRP concentration and red cell vitamin B2 in the normal range. Red cell vitamin B2 was significantly associated with CRP ($r_s$= 0.189, $p$<0.01) and albumin ($r_s$= 0.186, $p$<0.001). These relationships are shown in Figures 2a, 2b and 2c.

The characteristics of the convenience sample for red cell vitamin B6 (n=313) are shown in **Table 1**. The majority were older than 50 years (median 51 years), female (60%) and had plasma albumin below the normal range and plasma CRP and red cell vitamin B6 in the normal range. Red cell vitamin B6 was significantly associated with and albumin ($r_s$=-0.217, $p$<0.001). These relationships are shown in Figures 3a, 3b and 3c.

The characteristics of the convenience sample for ascorbic acid (n= 494) are also shown in **Table 1**. The majority were older than 50 years (median 54 years), female (53%), and had plasma CRP and albumin and plasma ascorbic acid in the normal range. Plasma

ascorbic acid was significantly associated with CRP ($r_s$= -0.333, $p$<0.001) and albumin ($r_s$=0.427, $p$<0.001). These relationships are shown in Figures 4a, 4b and 4c.

The characteristics of the convenience sample for α-tocopherol (n= 359) are shown in **Table 1**. The majority were older than 50 years (median 52 years), male (52%) and had plasma albumin and cholesterol below the normal range, while plasma CRP and plasma α-tocopherol were in the normal range. Plasma α-tocopherol adjusted to cholesterol was significantly associated with CRP ($r_s$=-0.424, p<0.001) and albumin ($r_s$=0.531, p<0.001). These relationships are shown in Figures 5a, 5b and 5c.

**Nutritional screen cohort for whole blood vitamin B1**

The median concentrations of whole blood vitamin B1 (**Table 2**) grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 543, 664 and 766 ng/g Hb respectively ($p$<0.001) with an overall elevation of 41%. The median whole blood concentrations of vitamin B1 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 547, 664 and 701 ng/g Hb respectively ($p$<0.001) with an overall elevation of 28%.

When albumin concentrations were ≥ 35 g/L, the median concentrations of whole blood vitamin B1 grouped according to CRP concentrations ≤10, 11-80 and >80 were 535, 619 and 812 ng/g Hb respectively ($p$=0.003) with an overall elevation of 52%. When albumin concentrations were 25-34 g/L, the median concentrations of whole blood vitamin B1 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 611, 693 and 776 ng/g Hb respectively ($p$=0.011) with an overall elevation of 27%. When albumin concentrations were < 25 g/L, the median concentrations of whole blood vitamin B1 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 574, 671 and 745 ng/g Hb respectively ($p$=0.118) with an overall elevation of 30%. Median concentrations of vitamin

B1/ albumin x100 were significantly increased from 1450 to 2250 to 4030 respectively ($p<0.001$).

### 1.3.2. Nutritional screen cohort for red cell vitamin B2

The median concentrations of red cell vitamin B2 (**Table 3**) grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 2.2, 2.3 and 2.4 nmol/g Hb respectively ($p<0.001$) with an overall elevation of 9%. The median concentrations of red cell vitamin B2 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 2.1, 2.4 and 2.3 nmol/g Hb respectively ($p<0.001$).

When albumin concentrations were ≥ 35 g/L, the median red cell concentrations of vitamin B2 grouped according to CRP concentrations ≤10 and 11-80 were 2.1 and 2.2 nmol/g Hb respectively ($p=0.147$) with an overall elevation of 4%. When albumin concentrations were 25-34 g/L, the median red cell concentrations of vitamin B2 grouped according to CRP concentrations ≤10 and 11-80 mg/L were 2.4 and 2.4 nmol/g Hb respectively ($p=0.265$). When albumin concentrations were < 25 g/L, the median red cell concentrations of vitamin B2 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 2.5, 2.3 and 2.5 nmol/g Hb respectively ($p=0.334$). Median red cell concentrations of vitamin B2/ albumin x100 were significantly increased from 6 to 8 to 14 respectively ($p<0.001$).

### 1.3.3. Nutritional screen cohort for red cell vitamin B6

The median concentrations of red cell vitamin B6 (**Table 4**) grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 534, 548 and 767 pmol/g Hb respectively ($p<0.001$) with an overall elevation of 9%. The median red cell concentrations of vitamin B2 grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 462, 644 and 840 pmol/g Hb respectively ($p<0.001$).

When albumin concentrations were ≥ 35 g/L, the median red cell concentrations of vitamin B6 grouped according to CRP concentrations ≤10 and 11-80 were 478 and 413 pmol/g Hb respectively were not significantly different ($p$=0.286). When albumin concentrations were 25-34 g/L, the median red cell concentrations of vitamin B6 grouped according to CRP concentrations ≤10 and 11-80 mg/L were 818, 547 and 669 pmol/g Hb respectively ($p$=0.056). When albumin concentrations were < 25 g/L, the median red cell concentrations of vitamin B6 grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 1037, 822 and 81 pmol/g Hb respectively ($p$=0.802). Median red cell concentrations of vitamin B6/ albumin x100 were significantly increased from 1380 to 2030 to 3560 respectively (p<0.001).

**1.3.4. Nutritional screen cohort for plasma ascorbic acid.**

The median plasma concentrations of ascorbic acid (**Table 5**) grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 25.0, 15.0 and 6.0 µmol/l respectively (p<0.001) with an overall reduction of 78%. The median plasma concentrations of ascorbic acid grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 32.0, 13.0 and 5.0 µmol/l respectively (p<0.001) with an overall reduction of 84%.

When albumin concentrations were ≥ 35 g/L, the median plasma concentrations of ascorbic acid grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 33.0, 30 and 31µmol/l ($p$=0.765). When albumin concentrations were 25-34 g/L, the median plasma concentrations of ascorbic acid grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 12.0, 15 and 10.0 µmol/l ($p$=0.409) with an overall reduction of 17%. When albumin concentrations were < 25 g/L, the median plasma concentrations of ascorbic acid grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 3, 10 and 5.0 µmol/l ($p$=0.121). The median vitamin C/albumin ration x 100 for CRP concentrations ≤10, 11-80 and >80 mg/L were 71.8, 53. 5 and 37.3 respectively (48%, $p$=0.050). The median

vitamin C /CRP x 100 for albumin concentrations ≥35, 25-34 and <25 g/l were 711.5, 65.8 and 10.3 (99%, $p<0.001$).

### 1.3.5. Nutritional screen cohort for α-tocopherol / cholesterol

The median α-tocopherol/ cholesterol (**Table 6**) grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 5.9, 4.6 and 2.1 umol/l respectively ($p<0.001$) with an overall reduction of 64%. The median α-tocopherol/cholesterol grouped according to albumin concentrations ≥35, 25-34 and <25 g/l were 6.0, 5.5 and 2.1 umol/l respectively ($p<0.001$) with an overall reduction of 65%.

When albumin concentrations were ≥ 35 g/L, the median α-tocopherol/cholesterol grouped according to CRP concentrations ≤10 and 11-80 mg/L were 6.2 and 5.4 ($p=0.134$). When albumin concentrations were 25-34 g/L, the median α-tocopherol/cholesterol grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 5.2, 6.0 and 4.6 ($p=0.518$) with an overall reduction of 12%. When albumin concentrations were < 25 g/L, The median α-tocopherol/cholesterol grouped according to CRP concentrations ≤10, 11-80 and >80 mg/L were 3.8, 2.4 and 1.7 ($p=0.032$) with an overall reduction of 55%.

## 1.4. Discussion

The results of the present study show that red cell concentrations of vitamin B1, B2 and B6 were not lower with an increasing magnitude of the systemic inflammatory response (as evidenced by both CRP and albumin concentrations). Indeed, there was a relatively small but significant increase in red cell B1, B2 and B6 concentrations. In contrast, plasma measurements of vitamin C and E were lower with an increasing magnitude of the systemic inflammatory response with the potential for misclassification of deficiency. Therefore, red cell measurements appear to obviate the potential for misclassification of vitamin B1, B2 and B6 deficiency in the presence of systemic inflammatory response. On this basis red cell measurements are recommended for the routine assessment of B-vitamin status.

The present relationship between the systemic inflammatory response and low plasma vitamin C and E results are consistent with that previously reported for vitamins A, D and carotenoids. Taken together these results would suggest that the systemic inflammatory response is associated with a redistribution of plasma vitamins, perhaps to specific tissues and organs irrespective of vitamin status. Despite this strong and consistent association recognition of this phenomenon remains limited and plasma measurements of vitamins continue to be used to assess status. Therefore, in order to obviate such interpretative problems it is essential that plasma vitamin measurements are accompanied by a measure of the systemic inflammatory response such as CRP and albumin. Alternatively, that new methods of measurements are developed for the assessment of vitamins A, C, D, E and the carotenoids, that better reflect nutritional statuse.

The results of the present study are particularly pertinent to plasma vitamin measurements carried out in patients with critical illness since almost all patients will have evidence of a systemic inflammatory response and may have a true deficiency and a need for supplementation. This may explain the mixed results of vitamin supplementation in the

248 critically ill [14]. In contrast, measurements of thiamine, FAD and PLP concentrations in
249 blood may be considered to more reliably reflect nutritional status of vitamin B1, B2 and B6
250 [1-4] and more reliably benefit from supplementation [14].

251 Vitamin C and E status is usually assessed by plasma measurement of α-tocopherol
252 and ascorbic acid respectively, although their correlation with concentrations in tissues is not
253 clearly established. Moreover, plasma concentrations of vitamin E are strongly associated
254 with their carrier lipids, principally lipoprotein complexes, such as high density lipoprotein
255 (HDL), very low density lipoprotein (VLDL) and chylomicrons. To overcome this limitation
256 it has been proposed that the plasma α-tocopherol concentrations should be expressed in
257 relation to plasma lipid concentrations, usually cholesterol [15-17]. In particular, the results
258 of the present study question the use of the α-tocopherol adjusted for cholesterol to
259 compensate for the effect of the systemic inflammatory response. Since, when expressed per
260 mmol of cholesterol, α-tocopherol, concentrations were lower with decreasing concentrations
261 of albumin.

262 The role of vitamin C in alleviating the oxidant stress and recycling the oxidized α-
263 tocopherol is well recognised. Plasma α-tocopherol concentrations, in addition to being
264 redistributed as part of the systemic inflammatory response, have been reported to be
265 regenerated by vitamin C. Early work on fat autoxidation performed by Golumbic and
266 Mattill (1941) reported the antioxygenic action of ascorbate in association with tocopherols
267 [18]. More recently, in vitro studies have shown that ascorbic acid reduces the tocopheroxyl
268 radical [19] and thereby restores the radical-scavenging activity of tocopherol [20-24]. It
269 would appear that the tocopheroxyl radical that forms in membranes reacts with ascorbic acid
270 to yield tocopherol and the ascorbyl radical, the result of which is to maintain radical
271 scavenging potential within the membrane by regenerating tocopherol and to transfer the
272 oxidative challenge to the aqueous phase. This scheme is consistent with the observation in

the present study that plasma vitamin C concentrations were lower compared with vitamin E concentrations for the same magnitude of the systemic inflammatory response and even in patients with critical illness in whom vitamin C concentrations were recognised to be profoundly low [25-27].

The importance of the systemic inflammatory response in determining plasma vitamin C concentrations is highlighted by the relative inefficiency, whereby supplementation can prevent low concentrations following an inflammatory insult. For example, in healthy patients with normal pre–operative plasma concentrations of ascorbic acid, a single oral supplementation with 1,000 mg of ascorbic acid was unable to prevent the fall in postoperative plasma concentrations [28]. Therefore, it is reasonable to conclude that ascorbic acid deficiency and the need for supplementation should only be considered on the basis of low plasma ascorbic acid concentrations in the presence of plasma CRP and albumin concentrations in the normal range.

The present study has a number of limitations. In particular there was no information on the presence of chronic disease, co-morbidity or on BMI and dietary intake. Nevertheless, it is likely that the impact of these clinical factors on vitamins B1, B2, B6 C and E will involve the systemic inflammatory response, at least in part.

In summary, the results of the present study show that, unlike plasma concentrations of vitamins C and E, red cell concentrations of vitamin B1, B2 and B6 were not significantly affected by the systemic inflammatory response. Therefore, red cell B1, B2 and B6 concentrations are likely to be a more reliable measure of status in patients with evidence of a systemic inflammatory response

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**Declaration**

The authors confirm that there are no conflicts of interest.

**Authors' statements**

DCM, JK and DT conceived the idea of examining the relationship between red cell vitamin B1, B2 and B6, CRP and albumin concentrations in a large cohort and in patients with critical illness and funded the study. RAG and DCM performed the statistical analysis. All authors contributed to the drafts and final version of the paper and are the guarantors.

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**Tables legends**

**Table 1**. Characteristics of nutritional screen cohorts with measurements of vitamins B1, B2, B6, C and E concentrations.

**Table 2.** Distribution of whole blood vitamin B1 (ng/g Hb) according to C-reactive protein and albumin concentrations (n=553).

**Table 3.** Distribution of red cell vitamin B2 according to C-reactive protein and albumin concentrations (n=251).

**Table 4**. Distribution of red cell vitamin B6 (pmol/g Hb) according to C-reactive protein and albumin concentrations (n=312).

**Table 5**: Distribution of plasma vitamin C (ascorbic acid) (µmol/l) according to CRP and albumin concentrations (n=494).

**Table 6:** Distribution of plasma α-tocopherol adjusted to cholesterol according to CRP and albumin concentrations (n=359).

**Figure legends**

**Figure 1a**.The relationship between CRP (log 10) and whole blood vitamin B1 (ng/g Hb) ($r_s$= 0.290, $p$<0.001) ).

**Figure 1b**.The relationship between albumin and whole blood vitamin B1 (nmol/l) ($r_s$= - 0.265, $p$<0.001).

**Figure 1c**.The relationship between vitamin B1 adjusted for albumin and CRP ($r_s$= 0.583, $p$<0.001)

**Figure 2a**.The relationship between CRP (log 10) and red cells vitamin B2 (nmol/g Hb) ($r_s$= 0.189, $p$=0.003).

**Figure 2b**.The relationship between albumin and red cells vitamin B2 (nmol/g Hb) ($r_s$= 0.186, $p$<0.001).

**Figure 2c**.The relationship between vitamin B2 adjusted for albumin and CRP ($r_s$= 0.543, $p$<0.001).

**Figure 3a**.The relationship between CRP (log 10) and red cells vitamin B6 (pmol/g Hb) ($r_s$= 0.079, $p$=0.161)

**Figure 3b**.The relationship between albumin and red cells vitamin B6 (pmol/g Hb) ($r_s$= - 0.217, $p$<0.001)

**Figure 3c**.The relationship between vitamin B6 adjusted to albumin and CRP ($r_s$= 0.238, $p$<0.001) **4a:** The relationship between CRP (log 10) and vitamin C (µmol/l) ($r_s$= - 0.333, p<0.001)

**Figure 4b**: The relationship between albumin and vitamin C (µmol/l) ($r_s$= 0.427, p<0.001).

**Figure 4c:** The relationship between CRP (log 10) and vitamin C adjusted to albumin ($r_s$= - 0.179, p<0.001).

**Figure 5a:** The relationship between α-tocopherol adjusted to cholesterol and CRP (log 10) ($r_s$= - 0.424, p<0.001)

**Figure 5b:** The relationship between α-tocopherol adjusted to cholesterol and albumin ($r_s$= 0.531, p<0.001).

**Figure 5c:** The relationship between CRP (log 10) and vitamin α-tocopherol adjusted to cholesterol and albumin ($r_s$= - 0.094, p= 0.359).

**Table 1. Characteristics of nutritional screen cohorts with measurements of vitamins B1, B2, B6, C and E concentrations.**

| | **Reference interval** | **Nutritional screen cohort Whole blood vitamin B1 (n=553)** | **Nutritional screen cohort Vitamin B2 (n=251)** | **Nutritional screen cohort Vitamin B6 (n=313)** | **Nutritional screen cohort Vitamin C (n=494)** | **Nutritional screen cohort Vitamin E (n=359)** |
|---|---|---|---|---|---|---|
| **Age (years)** | NA | 54 (18-100) | 51 (16-82) | 51 (16-82) | 54 (16-90) | 52 (15-100) |
| **Sex (Male/Female)** | NA | 276 (50%)/ 277 (50%) | 111 (44%)/ 140 (56%) | 125 (40%)/ 188 (60%) | 234 (47%)/ 260 (53%) | 185 (52%)/ 174 (48%) |
| **C-reactive protein (mg/l)** | <10 | 14.0 (<10- 565.0) | 5.0 (<10- 565.0) | 4.3 (<10- 301.0) | 10.0 (0.20- 565.0) | 9.0 (0.20- 565.0) |
| **Albumin (g/l)** | 35-55 | 32 (6-50) | 36 (9-49) | 35 (9-49) | 33 (9-50) | 31 (9-50) |
| **B1 (ng/g Hb)** | 275- 675 | 613 (175-3650) | | | | |
| **B2 (nmol/g Hb)** | 1.0 – 3.4 | | 2.2 (1.2-27.3) | | | |
| **B6 (pmol/g Hb)** | 250 – 680 | | | 540 (79-100755) | | |
| **Plasma ascorbic acid** | 11-114 | | | | 17.0 (0.9-262.0) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **(µmol/l)** | | | | | | |
| **Plasma α-tocopherol (umol/l)** | 12 - 46 | | | | | 21.0 (2.9-58.0) |
| **Cholesterol (mmol/l)** | <5.2 | | | | | 4.5 (1.1-15.7) |
| **α-tocopherol/ cholesterol ratio (µmol/ mmol)** | 5.2 (3.5-8.7) | | | | | 5.2 (0.4-19.5) |

**(Median and range)**

**Table 2. Distribution of whole blood vitamin B1 (ng/g Hb) according to C-reactive protein and albumin concentrations (n=553).**

| **whole blood vitamin B1 (ng/g Hb)** | **CRP ≤10 (mg/l)** | **11-80 (mg/l)** | **>80 (mg/l)** | **whole blood vitamin B1 according to albumin** |
|---|---|---|---|---|
| **Albumin ≥35 (g/l)** | 535 (211-2067) (n=175) | 619 (201-1508) (n=61) | (n=2) | 547 (201-2067) (n=238) |
| **34-25 (g/l)** | 611 (175-1781) (n=56) | 693 (260-2552) (n=77) | 776 (385-3650) (n=18) | 664 (175-3650) (n=151) |
| **<25 (g/l)** | 574 (233-1248) (n=19) | 671 (288-2129) (n=71) | 745 (196-3524) (n=74) | 701 (196-3524) (n=164) |
| **whole blood vitamin B1 according to CRP** | 543 (175-2067) (n=250) | 664 (201-2552) (n=209) | 766 (196-3650) (n=94) | |

**Median (range), where n<10 median and range not calculated.**

**Table 3. Distribution of red cell vitamin B2 according to C-reactive protein and albumin concentrations (n=251).**

| **Red cell B2 (nmol/g Hb** | **CRP ≤10 (mg/l)** | **11-80 (mg/l)** | **>80 (mg/l)** | **Red cell B2 according to albumin** |
|---|---|---|---|---|
| **Albumin ≥35 (g/l)** | 2.1 (1.2-5.9) (n=121) | 2.2 (1.9-3.1) (n=15) | - (n=0) | 2.1 (1.2-5.9) (n=136) |
| **34-25 (g/l)** | 2.4 (1.2-27.3) (n=38) | 2.4 (1.6-5.8) (n=38) | - (n=1) | 2.4 (1.2-27.3) (n=77) |
| **<25 (g/l)** | (n=6) | 2.3 (1.3-3.5) (n=21) | 2.5 (2.0-5.5) (n=11) | 2.3 (1.3-5.5) (n=38) |
| **Red cell B2 according to CRP** | 2.2 (1.2-27.3) (n=165) | 2.3 (1.3-57.8) (n=74) | 2.4 (1.6-5.5) (n=12) | |

**Median (range), where n<10 median and range not calculated.**

**Table 4. Distribution of red cell vitamin B6 (pmol/g Hb) according to C-reactive protein and albumin concentrations (n=312).**

| **Red cells B6 (pmol/g Hb)** | **CRP ≤10 (mg/l)** | **11-80 (mg/l)** | **>80 (mg/l)** | **Red cells B6 according to albumin** |
|---|---|---|---|---|
| **Albumin ≥35 (g/l)** | 478 (138-72374) (n=155) | 413 (181-2812) (n=18) | - (n=0) | 462 (138-72374) (n=173) |
| **34-25 (g/l)** | 818 (79-61870) (n=47) | 547 (164-9245) (n=42) | (n=2) | 644 (79-61870) (n=91) |
| **<25 (g/l)** | 1037 (206-3036) (n=12) | 822 (183-100755) (n=24) | 881 (291-23294) (n=12) | 840 (183-100755) (n=48) |
| **Red cells B6 according to CRP** | 534 (79-27374) (n=214) | 548 (164-100775) (n=85) | 767 (291-23294) (n=14) | |

**Median (range), where n<10 median and range not calculated**

**Table 5: Distribution of plasma vitamin C (ascorbic acid) (µmol/l) according to CRP and albumin concentrations (n=494).**

| Plasma vitamin C (ascorbic acid) (µmol/l) | CRP ≤10 (mg/l) | 11-80 (mg/l) | >80 (mg/l) | Plasma C (ascorbic acid) according to albumin |
|---|---|---|---|---|
| Albumin ≥35 (g/l) | 33 (0.99-235) (n=175) | 30 (0.99-79.0) (n=44) | 31 (17.0-66.0) (n=4) | 32 (0.99-235) (n=223) |
| 34-25 (g/l) | 12 (0.90-126) (n=61) | 15 (0.99-136) (n=66) | 10 (0.99-61.0) (n=19) | 13 (0.90-136) (n=146) |
| <25 (g/l) | 3 (0.90-40.0) (n=12) | 10 (0.90-262) (n=50) | 5.0 (0.90-102.0) (n=63) | 5 (0.90-262) (n=125) |
| Plasma C (ascorbic acid) according to CRP | 25 (0.90-235) (n=248) | 15 (0.90-262.0) (n=160) | 6.0 (0.90-102.0) (n=86) | |

Median (range) (number of observation)

**Table 6: Distribution of plasma α-tocopherol adjusted to cholesterol according to CRP and albumin concentrations (n=359).**

| Plasma α-tocopherol / cholesterol ratio | CRP ≤10 (mg/l) | 11-80 (mg/l) | >80 (mg/l) | Plasma α-tocopherol / cholesterol ratio according to albumin |
|---|---|---|---|---|
| Albumin ≥35 (g/l) | 6.2 (3.2-10.7) (n=131) | 5.4 (3.3-10.8) (n=18) | - (n=1) | 6.0 (3.2-10.8) (n=150) |
| 34-25 (g/l) | 5.2 (0.97-10.4) (n=48) | 6.0 (1.2-9.7) (n=38) | 4.6 (1.3-8.3) (n=12) | 5.5 (1.0-10.4) (n=98) |
| <25 (g/l) | 3.8 (1.1-7.6) (n=15) | 2.4 (0.9-17.0) (n=31) | 1.7 (0.4-19.4) (n=65) | 2.1 (0.4-19.5) (n=111) |
| Plasma α-tocopherol / cholesterol ratio according to CRP | 5.9 (1.0-10.7) (n=194) | 4.6 (0.9-17.0) (n=87) | 2.1 (0.4-19.5) (n=78) | |

Median (range) (number of observation)