Apolipoproteins C-II and C-III as nutritional markers unaffected by inflammation

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Abbreviations: AP1, activator protein 1; Apo, apolipoprotein; CAPD, continuous ambulatory peritoneal dialysis; C/EBPs, CCAAT/enhancer-binding proteins; CRP, C-reactive protein; CV, coefficient of variation; Hb, hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HNF-1, hepatic nuclear factor 1; Ht, hematocrit; IL, interleukin; LCAT, lecithin-cholesterol acyltransferase; LDL-C, low-density lipoprotein-cholesterol; MCH, mean corpuscular
hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NFkB, nuclear factor κB; NST, nutrition support team; PPARs, peroxisome proliferator-activated receptors; RBC, red blood cell; RBP, retinol binding protein; RTP, rapid turnover protein; RXRα/RARα, retinoid X receptor/retinoic acid receptor; SAA, serum amyloid A protein; SD, standard deviation; SGA, Subjective Global Assessment; STAT, signal transducer and activator of transcription; TC, total cholesterol; Tf, transferrin; TG, triglyceride; TIBC, total iron binding capacity; TLC, total lymphocyte count; TNF-α, tumor necrosis factor-alpha; TP, total protein; TTR, transthyretin
Abstract

Background: Rapid turnover proteins (RTPs), such as transthyretin (TTR), retinol binding protein (RBP), and transferrin (Tf), provide an accurate assessment of nutritional status but are susceptible to inflammation. Lipid-related markers, which have short half-lives in serum, may be better suited for nutritional assessment. This study sought to identify sensitive nutritional markers unaffected by inflammation.

Methods: Fasting serum samples were collected from 30 malnourished inpatients and 25 healthy volunteers. Malnourished inpatients were divided into two groups: a low-C-reactive protein (CRP) group (CRP < 20 mg/L, n = 15) and a high-CRP group (CRP ≥ 20 mg/L, n = 15). Lipid-related markers, traditional nutritional markers, RTPs, micronutrients, and ketone bodies were measured and compared among the groups.

Results: Apolipoprotein (Apo)C-II and ApoC-III concentrations were lower in malnourished inpatients than in the control group. There was no significant difference in ApoC-II and ApoC-III between the low- and high-CRP groups. Carnitine transporters and ketone bodies did not show a significant difference among the three groups. Albumin, TTR, RBP, and Tf concentrations were lowest in the high-CRP group, intermediate in the low-CRP group, and highest in the control group.

Conclusions: These results indicate that ApoC-II and ApoC-III are appropriate nutritional biomarkers unaffected by inflammation.

Keywords: albumin, lipoprotein, malnutrition, nutrition support team, rapid turnover protein
1. Introduction

Malnutrition is one of the leading causes of bedsores, poor wound healing, and severe infection. As a result of these serious complications, malnutrition often leads to prolonged hospitalization and increased medical costs [1–6]. Even without overt infection, malnourished but otherwise healthy subjects exhibit impaired immune profiles [7], which put them at high risk for a variety of severe complications. Previous studies have shown that nutritional intervention for malnourished patients improves nutritional states as well as clinical outcomes [3, 8, 9]. Precise evaluation of nutritional state is essential for effective intervention by the nutrition support team (NST). The most common anthropometric parameters measured by NST members include body weight, triceps skinfolds, mid-arm circumference, and mid-arm muscle circumference [10]. However, these parameters are subject to individual variation and they change gradually depending on the nutritional state of the patients.

Clinical laboratory tests used to assess nutritional states can overcome the drawbacks of anthropometric parameters. Rapid turnover proteins (RTPs) are typically used for nutritional assessment due to their short half-lives in serum. RTPs can be measured easily in hospital laboratories with the use of automated analyzers and commercial reagent kits. A rapid turn-around time allows physicians to evaluate the effectiveness of nutritional interventions on a timely basis. However, RTPs decrease with inflammation [11], which has also been observed with serum albumin, another commonly used biochemical marker for nutritional assessment [12, 13]. Since many malnourished inpatients suffer from infection or postoperative damage/wounds, it is necessary to assess nutritional status with markers not affected by inflammation. This study sought to identify potential markers that reflect nutritional state but are not influenced by inflammation. Lipid-related markers were selected...
because lipoproteins have short half-lives in serum and can be measured with routine laboratory tests in clinical settings.
2. Materials and Methods

2.1. Subjects

Study subjects were recruited from among inpatients referred to the NST for malnutrition at Juntendo University Hospital (Tokyo, Japan). In all subjects, nutritional status was first assessed with the Subjective Global Assessment (SGA) of nutritional status, a reliable and validated measure of nutritional status based on historical and physical exam findings [14, 15]. The patients were considered malnourished if they fulfilled both BMI < 22 and SGA rating B (moderately malnourished) or C (severely malnourished). Exclusion criteria included: disorders exhibiting severe hyperlipidemia with emaciation (anorexia nervosa, and generalized lipodystrophy); uncontrolled diabetes mellitus (hemoglobin A1c > 10.0%); end-stage renal disease requiring hemodialysis; and cholestatic liver disease or severe drug-induced liver dysfunction. A total of 30 patients were selected for further analysis (Fig.1). Causes of malnutrition noted were: malignancy, n = 17; systemic infections, n = 6; neurodegenerative disorders, n = 2; postoperative complication, n = 2; dysphagia, n = 2; and stroke, n = 1). The patients were divided into two subgroups based on C-reactive protein (CRP) concentration: a low-CRP group (CRP < 20 mg/L, n = 15) and a high-CRP group (CRP ≥ 20 mg/L, n = 15). A control group of 25 healthy volunteers was also examined. Anthropometric and comorbidity data were obtained from medical charts.

All participants provided informed consent prior to enrollment. The study was approved by the Juntendo University Hospital ethics committee, and complied with the dictates of the latest version of the Declaration of Helsinki (amended at the 64th WMA General Assembly, Fortaleza, Brazil, October 2013 [16]). The study protocol was registered in the UMIN Clinical Trials Registry (No. UMIN000026278; http://www.umin.ac.jp/ctr/index.htm).
2.2. Laboratory measurements

After fasting for at least 12 h, blood samples were taken from the malnourished group prior to nutritional intervention by the NST. Whole blood supplemented with ethylenediaminetetraacetic acid (EDTA) was used for hematology tests including red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), and total lymphocyte count (TLC), in a Sysmex XE-5000 hematology analyzer (Sysmex, Kobe, Japan).

Additional serum parameters were analyzed using a Hitachi LABOSPECT 008 (Hitachi, Tokyo, Japan). CRP concentrations were determined by latex immunoturbidimetry (Qualigent CRP; Sekisui Medical, Tokyo). Total protein (TP) and albumin were measured by a burette method and a modified bromocresol purple method, respectively, using commercial kits (Kainos Laboratories, Tokyo). As representative parameters of RTPs, transthyretin (TTR) and transferrin (Tf) were measured using a turbidimetric immunoassay. Retinol binding protein (RBP) was measured by latex immunoturbidimetry with commercial kits (Nittobo Medical, Koriyama, Japan).

Total cholesterol (TC) and triglyceride (TG) were measured by enzymatic methods. High-density lipoprotein (HDL)-cholesterol (HDL-C) and low-density lipoprotein (LDL)-cholesterol (LDL-C) were measured directly, which was expected to provide satisfactory accuracy [17]. Apolipoproteins were measured by turbidimetric immunoassay with commercial kits (Apo Auto N “Daiichi” series; Sekisui Medical, Tokyo). The intra- and inter-assay coefficients of variation (CVs) were <4% and <2% for apolipoprotein (Apo)C-II, and <2% and <1% for ApoC-III, respectively. Iron (Fe) and zinc (Zn) were measured by a chelation method with commercial kits (Shino-test, Sagamihara, Japan). Total ketone body,
3-hydroxybutyric acid, total carnitine, and free carnitine were determined by an enzymatic cycling method with commercial kits (Kainos Laboratories, Tokyo). Acetoacetic acid concentrations were expressed as the difference between total ketone body and 3-hydroxybutyric acid. Acylcarnitine concentrations were expressed as the difference between total carnitine and free carnitine.

2.3. Statistical analysis

Laboratory data are presented as means (standard deviations) or medians (interquartile ranges). Statistical analyses were performed using Statcel 4, an add-in software for Excel (OMS, Tokorozawa, Japan) and Statmate III (ATMS, Tokyo, Japan). Student’s t-test, Welch’s t-test and the Mann-Whitney U-test were used to compare mean or median values between groups, according to the presence or absence of a Gaussian distribution. Chi-square tests were used in the analysis of contingency tables. Values of p<0.05 were considered statistically significant.
3. Results

3.1. Baseline characteristics of study subjects

Although the malnourished and control groups were of similar age, gender and height, the malnourished group had much lower body weight and BMI than the control group. Neither body weight nor BMI differed significantly between the low- and high-CRP groups (Table 1). Although the median CRP concentration in the low-CRP group was higher than in the control group, it was below the upper limit of the reference range.

3.2. Comparison of traditional nutritional markers and RTPs between the malnourished and control groups

All biochemical markers commonly used for nutritional assessment are reduced by malnutrition. TLC and TP concentrations were significantly lower in both the low- and the high-CRP groups (malnourished patients) than in the control group. However, concentrations of TLC and TP did not differ between the low- and high-CRP groups (Table 2). TLC values varied widely in both the low- and the high-CRP groups.

Albumin and RTPs (TTR, RBP, and Tf) were markedly lower in the malnourished group than in the control group, and the reductions were greater than those observed with TP. These nutritional markers were 23% to 50% lower in the high-CRP group than in the low-CRP group. For the high-CRP group, the reduction was 1.7- to 2.2-fold greater for RTPs than for albumin.
3.3. Lipids and apolipoproteins

Lipid-related markers were classified into three categories according to their response to malnutrition and inflammation. The first category included TG, ApoB, and ApoE; concentrations were similar between the groups, independent of malnutrition and inflammation (Table 3). The second category included HDL-C, ApoA-I, and ApoA-II; concentrations were significantly lower in the malnourished patients than in the control group. Additionally, significantly larger reductions were observed in the high-CRP group than in the low-CRP group. The third category included TC, LDL-C, ApoC-II, and ApoC-III; serum concentrations were significantly lower in the malnourished patients, with no significant difference between the low- and high-CRP groups.

3.4. Anemia-related markers

Lower concentrations of several anemia-related markers were observed in malnourished inpatients, and this was more evident in patients with high CRP. Concentrations of RBC, Hb, and Ht were ~17% and 30% lower in the low- and high-CRP groups, respectively, compared with those in the control group (Table 4). Total iron binding capacity (TIBC) was similarly reduced in the low-CRP group, although its reduction in the high-CRP group was nearly double that of RBC, Hb, and Ht. In contrast, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were similar among the low-CRP, high-CRP, and control groups.

Fe and Zn concentrations in the malnourished group were lower than those in the control group. In the malnourished group, these markers were lower in the high-CRP group than in the low-CRP group. The difference between groups was only statistically significant for Fe.
3.5. Markers related to fatty acid metabolism

Fatty acid metabolism markers (total carnitine, free carnitine, and acylcarnitine) were not significantly associated with malnutrition or inflammation (Table 5). No significant differences in ketone body parameters (total ketone body, 3-hydroxybutyric acid, and acetoacetic acid) were observed, although some patients with high CRP had very high concentrations of ketone bodies.
4. Discussion

ApoC-II and ApoC-III were identified as accurate nutritional biomarkers that are not affected by inflammation. ApoC-II and ApoC-III concentrations were 30%–45% lower in malnourished patients than in the control group, but did not differ significantly between the low- and high-CRP groups (Table 3). Traditional nutritional markers, such as RTPs, were lower in the malnourished group, but were further reduced in the presence of inflammation (Table 2). Other candidate markers, such as carnitine transporters, had similar concentrations in the malnourished and control groups.

Inflammation changes some serum protein concentrations through the actions of pro-inflammatory cytokines. Interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α) are the main cytokines responsible for the acute-phase response [18, 19]. These cytokines modulate the DNA-binding activity of various transcription factors. For example, hepatic synthesis of positive acute-phase proteins is enhanced by the increased binding to target gene promoters of transcription factors in the nuclear factor κB (NFκB), signal transducer and activator of transcription (STAT), activator protein 1 (AP1), and CCAAT/enhancer-binding protein (C/EBP) families [19]. On the other hand, hepatic synthesis of negative acute-phase proteins is downregulated by reduced binding of other transcription factors, such as hepatic nuclear factor-1 (HNF-1) and retinoid X receptor/retinoic acid receptor (RXRα/RARα) heterodimers [19]. However, the promoters of ApoC-II and ApoC-III genes do not have binding sites for the abovementioned transcription factors. Instead, these genes are regulated by nuclear receptors associated with glucose and lipid metabolism, including peroxisome proliferator-activated receptors (PPARs) [20]. Therefore, it is not surprising that serum ApoC-II and ApoC-III concentrations are unaffected by inflammation.
In addition to their insensitivity to inflammation, ApoC-II and ApoC-III are good clinical nutritional markers, for several reasons. First, ApoC-II and ApoC-III have short half-lives in serum. Huff et al. performed a turnover study of apolipoproteins by injecting $^{125}$I-labeled very low-density lipoprotein in human subjects [21]. The average half-lives of ApoC-II, ApoC-III$_1$, and ApoC-III$_2$ were 26, 28, and 28 hours, respectively. These values are greater than the half-life of RBP (0.5 days), but less than those of TTR (2 days) and Tf (8.8 days) [22–25].

Second, ApoC-III concentration is stable within a day [26]. For ApoC-II, the within-day variation is minimal (unpublished observation). Furthermore, a previous study reported no significant postprandial changes in ApoC-II and ApoC-III concentrations, even after a fat-rich meal containing 63g fat (P/S ratio of 0.08), 25 g carbohydrate, and 35 g protein [27]. This allows malnourished patients to avoid the need to fast prior to blood sampling to assess their nutritional status. In addition, ApoC-II and ApoC-III are the main components of the TG-rich lipoproteins that are synthesized in the liver and small intestine. According to the Human Protein Atlas project, the gene expression of ApoC-II and ApoC-III are much lower in the small intestine than in the liver [28]. These data strongly suggest that serum concentrations of ApoC-II and ApoC-III are mainly influenced by hepatic protein synthesis. Moreover, ApoC-III can be transferred from HDL to TG-rich lipoproteins during the meal absorption phase, and can move back from TG-rich lipoproteins to HDL during the postprandial lipolytic phase [29]. These findings can explain why serum ApoC-III concentration remains stable throughout the day.

Although TC and LDL-C concentrations have features that favor their use as nutritional markers, they also have several shortcomings. TC and LDL-C concentrations were low in the malnourished group, and did not differ significantly between the low- and high-CRP groups.
(Table 3). A fractional catabolic rate of LDL-C is short enough for a clinical nutritional marker (0.33–0.45 per day) [30]. However, factors unrelated to nutritional status affect TC and LDL-C concentrations. For example, patients with anorexia nervosa often exhibit hypercholesterolemia [31–33]. Anorexia nervosa patients have very low serum albumin concentrations [34], indicative of severe malnourishment. Patients with anorexia nervosa were excluded from our study. Another confounding factor is ApoE phenotype, which is a common determinant of LDL-C concentrations in the general population. E4 carriers have high LDL-C concentrations while E2 carriers have low LDL-C concentrations [35–38]. Additionally, the effect of fatty acids on TC concentration varies according to fatty acid species or ApoE phenotype [39]. Changes in TC and LDL-C induced by nutritional intervention may not be indicative of nutritional status in patients with different ApoE phenotypes.

In contrast to TC and LDL-C concentrations, many studies have reported that HDL-C, ApoA-I, and ApoA-II concentrations decrease with inflammation [40–43]. In this study, these markers were significantly lower in the high-CRP group than in the low-CRP group (Table 3), caused by concomitant activation of inflammatory responses. Proinflammatory cytokines increase the hepatic synthesis of SAA. SAA displaces ApoA-I on HDL particles, thereby reducing HDL-C [40, 42, 43]. Lecithin-cholesterol acyltransferase (LCAT) activity plays a crucial role in increasing HDL size by causing the accumulation of hydrophobic cholesterol ester in the HDL core. LCAT activity is decreased during inflammation, which leads to reduced concentrations of HDL-C [40]. Inflammation enhances endothelial lipase activity, which may also contribute to reductions in HDL-C and ApoA-I [40].

Fatty acid-related markers were also assessed in this study, as malnourished patients are expected to use more fatty acids than carbohydrates. There were no significant differences in
fatty acid transporter (carnitine) or ketone body concentrations between the malnourished and control groups (Table 5). Ketone body concentrations varied widely in the malnourished group, particularly in those with high CRP. Differences in adipose tissue reserve may explain the significant individual variability.

Several limitations of the present study deserve consideration. Serial changes in ApoC-II and ApoC-III concentrations could not be observed in study subjects until their nutritional status improved. Some patients were referred to the hospital for definitive diagnoses or operations, and returned to their previous medical facilities without any changes in their nutritional status. The average hospital stay was 12.1 days. Some seriously ill patients died of underlying diseases. A study designed to examine the response of ApoC-II and ApoC-III specifically to nutritional intervention in a large number of subjects may be needed. Moreover, specific conditions may limit the utility of ApoC-III as a nutritional marker. Patients undergoing continuous ambulatory peritoneal dialysis (CAPD) have elevated ApoC-III concentrations [44]. Additionally, increased ApoC-III concentration is a typical feature of renal dyslipidemia, and may result from a disturbance in insulin metabolism in chronic renal disease [45]. Patients with uncontrolled diabetes can exhibit severe chylomicronemia [46]. ApoC-III is found on chylomicrons, and plasma ApoC-III concentrations are elevated during both hyperlipidemia and diabetes [47, 48]. Despite these limitations, ApoC-II and ApoC-III are preferable to current nutritional markers.

ApoC-II and ApoC-III concentrations accurately reflect nutritional status and are not influenced by inflammation. ApoC-II and ApoC-III concentrations are sensitive and robust biomarkers for nutritional assessment in patients receiving oral, enteral, and intravenous alimentation, regardless of inflammatory status.
Acknowledgments

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Authors’ contributions: All authors confirm that they have contributed to the intellectual content of this article and have met the following three requirements: (1) significant contributions to the study conception and design, data acquisition, or analysis and interpretation of the data; (2) drafting or revising the article for intellectual content; and (3) final approval of the published article.
Figure Legend

**Figure 1: Flowchart of enrollment and allocation of the study subjects.**

Fifty subjects were recruited from among inpatients referred to the NST for malnutrition in Juntendo University Hospital. Twenty subjects were excluded as shown in Figure 1. A total of 30 patients were selected and divided into two groups, based on the absence or presence of inflammation.
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abnormalities in obese children particularly depending on apolipoprotein E phenotype.


Malnourished group

Initial screening by NST

(n = 50)

Malnourished?

Yes

(n = 37)

Exclusion criteria

Yes

7 excluded

Uncontrolled diabetes mellitus (n = 1)
End-stage renal disease on hemodialysis (n = 1)
Anorexia nervosa (n = 2)
Cholestatic liver disease (n = 2)
Severe drug-induced liver dysfunction (n = 1)

No

BMI ≥ 22 or SGA score A

(n = 11)

CRP < 20 mg/L

Yes

(n = 15)

Low-CRP group

No

High-CRP group

(n = 15)

Control group

Healthy volunteers

(n = 25)
Table 1: Baseline profiles of the malnourished and control groups

<table>
<thead>
<tr>
<th></th>
<th>Malnourished group (n = 30)</th>
<th>Control group (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-CRP group (n = 15)</td>
<td>High-CRP group (n = 15)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.9 (9.8)</td>
<td>70.3 (14.1)</td>
</tr>
<tr>
<td>Male/female</td>
<td>4/11</td>
<td>7/8</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>1.54 (0.08)</td>
<td>1.57 (0.11)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>40.6 (6.7) ***</td>
<td>42.2 (7.8) ***</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.1 (2.8) ***</td>
<td>17.2 (3.1) ***</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.8 (0.9, 10.9) ***</td>
<td>77.1 (52.3, 130.3) +++</td>
</tr>
</tbody>
</table>

BMI, body mass index; CRP, C-reactive protein

Data are presented as means (SD) or medians (interquartile ranges).

*** $p < 0.001$ vs. Control group.

+++ $p < 0.001$ vs. Low-CRP group.
Table 2: Traditional nutritional markers and rapid turnover proteins in the malnourished and control groups

<table>
<thead>
<tr>
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<th>Malnourished group (n = 30)</th>
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<tbody>
<tr>
<td></td>
<td>Low-CRP group (n = 15)</td>
<td>High-CRP group (n = 15)</td>
</tr>
<tr>
<td>TLC ($10^9$/L)</td>
<td>1.0 (0.6, 1.3) ***</td>
<td>0.7 (0.5, 1.1) ***</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>59.4 (7.0) ***</td>
<td>57.1 (9.3) ***</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>30.3 (4.3) ***</td>
<td>23.4 (7.0) *** ++</td>
</tr>
<tr>
<td>TTR (g/L)</td>
<td>0.16 (0.07) ***</td>
<td>0.08 (0.04) *** +++</td>
</tr>
<tr>
<td>RBP ($\mu$mol/L)</td>
<td>1.12 (0.52) **</td>
<td>0.64 (0.39) *** ++</td>
</tr>
<tr>
<td>Tf (g/L)</td>
<td>1.69 (0.47) ***</td>
<td>1.04 (0.38) *** +++</td>
</tr>
</tbody>
</table>

RBP, retinol binding protein; Tf, transferrin; TLC, total lymphocyte count; TP, total protein; TTR, transthyretin

Data are presented as means (SD) or medians (interquartile ranges).

** $p < 0.01$ and *** $p < 0.001$ vs. Control group.

++ $p < 0.01$ and +++ $p < 0.001$ vs. Low-CRP group.
Table 3: Lipids and apolipoproteins in the malnourished and control groups

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Low-CRP group (n = 15)</td>
<td>High-CRP group (n = 15)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.89 (0.83) ***</td>
<td>3.51 (0.85) ***</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.97 (0.37)</td>
<td>1.03 (0.28)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.99 (0.27) ***</td>
<td>0.78 (0.29) *** +</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.43 (0.75) *</td>
<td>2.07 (0.64) ***</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.05 (0.28) ***</td>
<td>0.81 (0.26) *** +</td>
</tr>
<tr>
<td>ApoA-II (g/L)</td>
<td>0.21 (0.07) ***</td>
<td>0.14 (0.05) *** ++</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.82 (0.16)</td>
<td>0.82 (0.21)</td>
</tr>
<tr>
<td>ApoC-II (mg/L)</td>
<td>26.1 (15.0) **</td>
<td>26.3 (19.2) **</td>
</tr>
<tr>
<td>ApoC-III (mg/L)</td>
<td>69.2 (19.5) ***</td>
<td>54.5 (23.7) ***</td>
</tr>
<tr>
<td>ApoE (mg/L)</td>
<td>42.0 (7.5)</td>
<td>38.1 (15.6)</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.

Data are presented as means (SD).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. Control group.

+ $p < 0.05$ and ++ $p < 0.01$ vs. Low-CRP group.
### Table 4: Anemia-related markers in the malnourished and control groups

<table>
<thead>
<tr>
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<th>Malnourished group (n = 30)</th>
<th>Control group (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-CRP group (n = 15)</td>
<td>High-CRP group (n = 15)</td>
</tr>
<tr>
<td><strong>RBC (10^12/L)</strong></td>
<td>3.71 (0.5) ***</td>
<td>3.09 (0.3) ***+++</td>
</tr>
<tr>
<td><strong>Hb (g/L)</strong></td>
<td>111.1 (15.0) ***</td>
<td>92.7 (13.4) ***++</td>
</tr>
<tr>
<td><strong>Ht (L/L)</strong></td>
<td>0.34 (0.04) ***</td>
<td>0.28 (0.04) ***++</td>
</tr>
<tr>
<td><strong>MCV (fL)</strong></td>
<td>91.1 (6.8)</td>
<td>91.8 (6.1)</td>
</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>30.0 (2.7)</td>
<td>30.0 (2.3)</td>
</tr>
<tr>
<td><strong>MCHC (g/L)</strong></td>
<td>328.7 (8.9)</td>
<td>326.7 (10.5) *</td>
</tr>
<tr>
<td><strong>TIBC (µmol/L)</strong></td>
<td>45.2 (10.2) ***</td>
<td>25.2 (5.4) ***++</td>
</tr>
<tr>
<td><strong>UIBC (µmol/L)</strong></td>
<td>34.4 (11.8)</td>
<td>19.1 (6.8) ***+++</td>
</tr>
<tr>
<td><strong>Fe (µmol/L)</strong></td>
<td>10.7 (3.7) **</td>
<td>6.2 (5.4) ***+</td>
</tr>
<tr>
<td><strong>Zn (µmol/L)</strong></td>
<td>10.7 (2.6) *</td>
<td>8.6 (2.9) ***</td>
</tr>
</tbody>
</table>

Fe, iron; Hb, hemoglobin; Ht, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; Zn, zinc

Data are presented as means (SD).

* \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \) vs. Control group.

\( p < 0.05 \), ++ \( p < 0.01 \) and +++ \( p < 0.001 \) vs. Low-CRP group.
Table 5: Markers of fatty acid metabolism in the malnourished and control groups

<table>
<thead>
<tr>
<th></th>
<th>Malnourished group (n = 30)</th>
<th>Control group (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-CRP group (n =15)</td>
<td>High-CRP group (n = 15)</td>
</tr>
<tr>
<td>Total carnitine (µmol/L)</td>
<td>53.3 (24.1)</td>
<td>57.0 (30.8)</td>
</tr>
<tr>
<td>Free carnitine (µmol/L)</td>
<td>44.3 (20.4)</td>
<td>41.3 (22.5)</td>
</tr>
<tr>
<td>Acylcarnitine (µmol/L)</td>
<td>7.7 (3.8, 14.7)</td>
<td>12.2 (8.8, 16.6)</td>
</tr>
<tr>
<td>Total ketone body (µmol/L)</td>
<td>15.8 (8.1, 87.6)</td>
<td>102.3 (6.6, 466.7)</td>
</tr>
<tr>
<td>3-Hydroxybutyric acid (µmol/L)</td>
<td>7.4 (4.1, 66.6)</td>
<td>78.4 (3.8, 325.7)</td>
</tr>
<tr>
<td>Acetoacetic acid (µmol/L)</td>
<td>8.0 (4.0, 21.4)</td>
<td>25.7 (7.1, 177.3)</td>
</tr>
</tbody>
</table>

Data are presented as means (SD) or medians (interquartile range).