Hypocholesterolemia and Plasma Amino Acids in Sepsis

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1 Introduction

Sepsis is characterized by an adaptation of plasma lipoprotein patterns which commonly results in hypocholesterolemia (Fraunberger et al., 1999; Giovannini et al., 1999; Chiarla et al., 2010) and the degree of hypocholesterolemia is generally related to severity of septic illness. The real pathophysiologic role of hypocholesterolemia is not yet adequately explained. It seems to be an adaptive response among the host defense manifestations of sepsis, however it may also reflect inadequate disposal of substrate for stress hormone, lipoprotein synthesis and for other important synthetic processes (Fraunberger et al., 1999; Giovannini et al., 2005; Marik, 2006; Vyroubal et al., 2008). The relationship between changes in cholesterol and septic metabolic abnormalities has not been satisfactorily evaluated. More extensive assessment would certainly improve our understanding of the patterns of septic acute phase response, and of evolution toward metabolic decompensation, as already demonstrated by study of the correlations with other plasma fat and protein components, and with several amino acids (Chiarla et al., 2004, 2006, 2010; Giovannini et al., 2005). The panel of amino acid (AA) abnormalities may reflect adequacy of biochemical, metabolic, organ function processes, evolution of disease and even gene expression (Siegel et al., 1979; Roth & Druml, 2011), and a thorough appraisal of the relationships with cholesterol abnormalities would certainly enhance our understanding of sepsis. These have never been evaluated in detail, at least to our knowledge, and we have performed this evaluation over an extended frame of reference of AA abnormalities.

2 Materials and Methods

2.1 Patients

Data from 504 plasma AA measurements with the corresponding metabolic and clinical variables, recorded in our patient AA database, were analyzed in detail. These were taken from 19 trauma patients (3 women, 16 men) who developed sepsis (Table 1). All patients had acute injury, caused by road accidents in 16 cases and gunshot wounds in 3 cases, with combinations of abdominal, chest, head and limb lesions. The cause of sepsis was intra-abdominal, pulmonary or extensive soft tissue infection. The diagnosis of sepsis was based on the occurrence of a temperature > 38.3°C, white blood cell count >12×10^9/L or < 3×10^9/L and clear evidence of infection verified by positive cultures from blood, surgical drainage of infected areas or sputum in the case of pulmonary sepsis. Median sepsis severity score (Skau, 1985) upon diagnosis of sepsis was 24 (range 11 – 75). Most patients survived, some of them after evolving through near-fatal illness, one patient died of septic metabolic and cardiorespiratory decompensation from multiple organ dysfunction, and one died suddenly without multiple organ dysfunction. The patients were receiving total parenteral nutrition (34 ± 14 kcal/kg/24 h, about three fourths glucose and one fourth fat, and 1.4 ± 0.6 g/kg/24 h amino acids).

2.2 Measurements

Serial AA determinations were performed every 8 to 12 hours during sepsis until the clinical criteria for the diagnosis of sepsis persisted, for a total number of 504 determinations (median per patient 23, interquartile range 15). The Fischer AA ratio (Freund et al., 1979) was calculated according to the ratio (leucine + isoleucine + valine)/(phenylalanine + tyrosine). The amino-acidograms were available together...
with the corresponding plasma cholesterol and alkaline phosphatase measurements. The urinary 3-methylhistidine excretion, the respiratory index (the ratio of alveolar-arterial $O_2$ tension gradient to arterial $O_2$ tension) and the Sepsis-related Organ Failure Assessment score (SOFA score) (Vincent, 2006) were also available in a smaller number of cases. The study protocol complied with the 1964 Helsinki declaration.

### 2.3 Statistical Analysis

Medians and ranges were used as indices of centrality and dispersion of the distributions. The obtained measurements provided a continuous distribution of observations over a wide range of conditions, extending from moderate to extremely severe septic illness, which was well suited to assessing the correlates of cholesterol over an ample area of pathophysiologic abnormalities. The relationships existing between cholesterol, the AA levels and the other variables were explored on two- or three-dimensional graphical displays. Further assessments and validations of the results were performed by least-square regression and covariance analysis, and analyzed for the Pearson correlation coefficient ($r$), with skewness and kurtosis control, and analysis of residuals (Statgraphics Plus, Manugistics, Rockville, MD). Significance of covariance was assessed by Scheffé criteria (based on confidence intervals and differences in slope and intercept) (Seber, 1977) and with the selection of the simplest possible regression yielding the best control of variability of cholesterol.
# Results

## 3.1 Basic Results

Median plasma cholesterol was 88 mg/dL, range 28 – 220 (corresponding to 2.28 mmol/L, range 0.72 – 5.70), and median alkaline phosphatase was 126.5 U/L, range 11 – 784 (n.v. 25 – 100). Plasma AA values are reported in Table 2.

<table>
<thead>
<tr>
<th>Alanine</th>
<th>272.5 (108-1013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>89 (29-280)</td>
</tr>
<tr>
<td>Asparagine</td>
<td>42 (15-610)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>6 (0-72)</td>
</tr>
<tr>
<td>Citrulline</td>
<td>11 (4-109)</td>
</tr>
<tr>
<td>Cystine</td>
<td>46.5 (18-110)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>453 (183-2438)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>54.5 (6-404)</td>
</tr>
<tr>
<td>Glycine</td>
<td>239 (74-1184)</td>
</tr>
<tr>
<td>Histidine</td>
<td>75 (3-427)</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>11.5 (0-76)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>73.5 (22-207)</td>
</tr>
<tr>
<td>Leucine</td>
<td>129 (64-296)</td>
</tr>
<tr>
<td>Lysine</td>
<td>163 (67-534)</td>
</tr>
<tr>
<td>Methionine</td>
<td>39 (4-411)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>69 (22-352)</td>
</tr>
<tr>
<td>Phosphoethanolamine</td>
<td>8 (0-63)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>114.5 (18-304)</td>
</tr>
<tr>
<td>Proline</td>
<td>187 (13-1445)</td>
</tr>
<tr>
<td>Phosphoserine</td>
<td>12 (5-41)</td>
</tr>
<tr>
<td>Serine</td>
<td>98 (25-301)</td>
</tr>
<tr>
<td>Threonine</td>
<td>107.5 (11-612)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>57 (25-94)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>56 (27-203)</td>
</tr>
<tr>
<td>Valine</td>
<td>253 (83-671)</td>
</tr>
</tbody>
</table>

**Multiple regression:**

\[
\text{Cholesterol} = 98.22 + 0.11 \text{ (alkaline phosphatase)} - 0.43 \text{ (phenylalanine)} + 0.14 \text{ (glutamate)} + 0.27 \text{ (ornithine)}
\]

Multiple $r = 0.74$, $r^2 = 0.55$, $p < 0.001$

**Table 2**: Medians (ranges) for plasma amino acids in µmol/L, with final regression.

## 3.2 Correlations with Amino Acids

The correlations between cholesterol and individual plasma AAs were assessed. The best correlate of cholesterol was phenylalanine, which was inversely related to it ($r = -0.46$, $p < 0.001$). Among the other aromatic and the branched chain AAs, tyrosine was also inversely related to cholesterol, although with a lower $r$ value ($r = -0.28$, $p < 0.001$), and the phenylalanine/tyrosine ratio was inversely related to cholesterol ($r = -0.27$, $p < 0.001$), while isoleucine was directly related to cholesterol ($r = 0.36$, $p < 0.001$). No
evident or significant correlation was found with the remainder. The inverse correlation with the phenylalanine/tyrosine ratio reflected the circumstance that the two AAs were directly correlated one to the other \((r = 0.44, p < 0.001)\) and both increased with decreasing cholesterol, however the increase in phenylalanine outweighed that of tyrosine. There was an inverse correlation with the Fischer AA ratio, which reached a higher \(r\) value \((r = -0.55, p < 0.001)\) most likely because the ratio cumulatively accounted for the impact of each individual AA. Among the other AAs, the main result was a direct correlation with glutamate \((r = 0.41, p < 0.001)\), while there were weaker correlations with ornithine (direct relationship), and with phosphoserine, hydroxyproline, proline and alanine (inverse relationships) \((\text{absolute } r < 0.24, p < 0.001 \text{ for all})\). In addition, there was an inverse correlation between cholesterol and 3-methylhistidine urinary excretion \((r = -0.34, p < 0.001)\) in 442 measurements which were selected on the basis of plasma creatinine < 1.7 mg/dL, corresponding to 150 µmol/L, to avoid confounding by the effect of kidney dysfunction, if present. The median 3-methylhistidine urinary excretion for these measurements was 537.2 µmol/24 h, range 100.8 – 2005.0. These correlations were also reconfirmed by evaluating individual patient trends on graphical displays.

3.3 Correlation with Alkaline Phosphatase

Because a basic procedure, when assessing the variability of cholesterol in multiple measurements, is to also account for the simultaneous effect of cholestasis, if present (Giovannini et al., 1999; Chiarla et al., 2010), the analysis was repeated including alkaline phosphatase as an independent variable together with the plasma AAs in multiple regressions. In fact, cholestasis is known to independently increase cholesterol, therefore masking or confounding the effect of the factors which cause hypocholesterolemia (Giovannini et al., 1999, Chiarla et al., 2010). The analysis showed that cholesterol was directly related to alkaline phosphatase \((r = 0.47, p < 0.001)\), and reconfirmed the significance of the previous panel of AA correlations.

3.4 “Simplest best fit”

Selection of the “simplest best fit”, explaining the largest possible variability of cholesterol by using the minimum possible number of variables, yielded a multiple regression which included alkaline phosphatase, phenylalanine, glutamate and ornithine, and these accounted for 55% of the variability of cholesterol \((\text{multiple } r = 0.74, r^2 = 0.55, p < 0.001, \text{ Table 2})\). This regression was not improved by including as independent variables other plasma AAs, or the parenteral AA infusion rate. Indeed the analysis showed that, although cholesterol in itself was directly related to AA infusion rate \((p < 0.001)\), in the multiple regression this effect was already accounted for by the impact of AA infusion rate on both glutamate and ornithine \((\text{direct correlations, } p < 0.001 \text{ for both})\).

Apart from the described statistical analyses and validations, many of the listed correlations were already evident when assessing individual patient trends (Figure 1), or all measurements pooled together on graphical displays.

3.5 Correlations with Respiratory Dysfunction and SOFA Score

Finally, although this study was performed to assess the relationship between hypocholesterolemia and plasma AA changes, a peculiar complementary finding was the tendency of signs of respiratory dysfunction to be associated with decreasing cholesterol and increasing phenylalanine. Indeed, in 251 measurements in which the respiratory index was simultaneously available (median 1.36, range 0.19 – 4.78), this
Figure 1: Evolution of plasma cholesterol and phenylalanine in a surviving trauma patient included in the study, who developed transient septic shock, with evident inverse correlation between cholesterol and phenylalanine. The fall in cholesterol, paralleled by increasing phenylalanine, preceded the development of septic shock, with mean blood pressure down to 74 mmHg, followed by improvement of the pattern. The downhill path was paralleled by respiratory dysfunction with respiratory index increasing up to a value of 4.78. Distance between data points 24 h. To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259.

was found to be inversely related to cholesterol ($r = -0.41, p < 0.001$) and directly related to phenylalanine ($r = 0.39, p < 0.001$). More detailed analysis showed that these correlations were supported by the tendency for a multi-systemic involvement, also implicating a variable degree of respiratory dysfunction, which paralleled the worsening of hypocholesterolemia and of septic metabolic patterns.

This aspect was further verified in correlations with the SOFA score. The score (median 8, range 2–16) was inversely and significantly correlated to cholesterol for any given alkaline phosphatase level (multiple $r = 0.72, p < 0.001$). Regression analysis on SOFA score and AA levels showed direct correlations between SOFA and phosphoserine, hydroxyproline ($r = 0.60$ for both), phenylalanine, tyrosine ($r = 0.56$ for both), methionine ($r = 0.56$) and cystathionine ($r = 0.50$), and inverse correlations with the Fischer AA ratio ($r = -0.45$), glutamate ($r = -0.40$) and aspartate ($r = -0.32$) ($p < 0.001$ for all). For many other AAs, including alanine and proline, there was a roughly direct correlation, however the distribution of measurements was unsuitable for regression analysis because the transition to the highest SOFA scores was often associated with abrupt hyperaminoacidemia without gradual continuity with previous measurements.

4 Discussion

4.1 Relevance and Determinants of Hypocholesterolemia

Although most of the interest in cholesterol abnormalities regards the implications of hypercholesterolemia, an intriguing issue for several categories of patients is hypocholesterolemia. This is particularly true for surgical septic and critically ill patients, in whom severity of hypocholesterolemia may parallel severi-
ty of illness: indeed factors such as acute phase response to trauma, host defense against infectious agents, liver dysfunction and hemodilution from hemorrhage, if present, cumulatively concur to decrease cholesterol (Giovannini et al., 1999; Vyroubal et al., 2008; Chiarla et al., 2010; Hrabovský et al., 2012). In common practice the only adverse factor increasing cholesterol, or moderating the decrease caused by the other factors, is cholestasis. This generally results in a quantifiable direct relationship between cholesterol and alkaline phosphatase, which needs to be taken into account when assessing other determinants of cholesterol level (Chiarla et al., 2010) to avoid confounding. Our present study substantiates and implements the available information on metabolic patterns associated with hypocholesterolemia, with particular regard to the associated panel of amino acid (AA) correlations. As already mentioned, this panel may reflect adequacy of biochemical, metabolic, organ function processes, evolution of disease and even gene expression (Siegel et al., 1979; Roth & Druml, 2011).

4.2 Dominant Amino Acid Correlations

The results have shown that, in spite of the heterogeneous conditions of the patients and of the many uncontrolled factors affecting cholesterol, alkaline phosphatase and three dominant AA variables accounted for as much as 55% of the variability of cholesterol (Table 2, Regression, multiple r = 74, r² = 0.55, p < 0.001). The selected best simultaneous AA correlates of cholesterol were phenylalanine (inverse relationship), glutamate and ornithine (direct relationships).

The association between decreasing cholesterol and increasing phenylalanine is consistent with the role of cholesterol as a marker of severity of septic illness, because high phenylalanine mostly reflects the increased load of this AA from endogenous protein catabolism and/or its inadequate metabolic handling, both of which are well known metabolic consequences of deteriorating sepsis (Siegel et al., 1979). This was substantiated by the inverse correlation found with the phenylalanine/tyrosine ratio, which is a substrate/byproduct ratio whose increase reflects impaired phenylalanine metabolism (Wannemacher et al., 1976), and by the inverse correlation found between cholesterol and urinary 3-methylhistidine excretion, which reflected increased endogenous proteolysis at low cholesterol levels. The most likely pathophysiologic explanation is that the cytokine pattern which is held responsible for the amplification of septic acute phase response and severe hypocholesterolemia (Fraunberger et al., 1998; Fraunberger et al., 1999; Gordon et al., 2001; Giovannini et al., 2005; Vyroubal et al., 2008), simultaneously inhibits use of AAs in muscular protein synthesis and enhances proteolytic drive, as reflected by rising urinary 3-methylhistidine excretion and plasma phenylalanine. The rise of the latter is further enhanced by liver dysfunction and impaired phenylalanine hydroxylase activity (Wannemacher et al., 1976), and is often associated with more intense inflammation, encephalopathy, reduction of vascular tone and shock (Eggers et al., 2003; Ploder et al., 2009; Bozza et al., 2013). This situation differs from what may be found in phenylketonuria, where an inverse relationship between cholesterol and phenylalanine may at least in part be explained by an inhibitory effect of phenylalanine on cholesterol synthesis (Colomé et al., 2001; Hargreaves, 2007).

4.3 Additional Amino Acid Correlations

With regard to the other AA correlations found in our septic patients, while some relationship between decreasing cholesterol and increasing proline or alanine may be a predictable pattern depending on severity of illness, the relationship which emerged in this study with increasing phosphoserine is a completely new finding. This is consistent with a previous generic report of higher phosphoserine in lethal compared to non-lethal sepsis (Roth et al., 1982), and with recent evidence suggesting that intensity of
acute phase response and septic multiorgan dysfunctions alter the balance between plasma phosphoserine and serine, therefore resulting in higher phosphoserine and phosphoserine-serine ratios in cases with a worse degree of illness (unpublished observations). This topic deserves further specific investigation, to assess the mechanisms which increase phosphoserine, and the performance of this AA as a new biomarker of severity of illness.

4.4 Hypcholesterolemia and Organ Dysfunction

The tendency of hypocholesterolemia to precede, or to be associated with the development of organ dysfunction, was confirmed in our study by the highly significant relationships found between decreasing cholesterol, or increasing phenylalanine, and increasing respiratory index, although an incomplete number of measurements was available for this purpose (n = 261). Of note, some degree of pulmonary dysfunction from ventilation/perfusion mismatch may be a component of the septic multisystemic involvement even in the absence of true parenchymal lesions (Siegel et al., 1979). In these measurements, the tendency of decreasing cholesterol to be associated with organ dysfunctions was substantiated by its simultaneous correlation with increasing SOFA score. Moreover, the relationships observed between AAs and SOFA score basically reconfirmed that the AA abnormalities which were associated with decreasing cholesterol were also associated with increasing SOFA score. Within these abnormalities, the hyperammonacidemia which characterized the highest SOFA scores was consistent with impaired use of AAs in synthetic processes in the more advanced stages of septic metabolic dysregulation (Cerra et al., 1980).

4.5 Role of Hypcholesterolemia and Clinical Implications

The real pathophysiologic role of hypocholesterolemia is not yet adequately explained. As previously mentioned, it seems to mostly represent an adaptive lipoprotein response among the host defense manifestations of sepsis (Fraunberger et al., 1999; Giovannini et al., 2005; Vyroubal et al., 2008), however it may also reflect inadequate disposal of substrate for important synthetic processes, including lipoprotein, new cell membrane and stress hormone synthesis (Giovannini et al., 2005; Marik, 2006; Vyroubal et al., 2008). Within these considerations, a direct relationship between amino acid supply and cholesterol remains intriguing and unexplained (Giovannini et al., 2005), as remains the direct relationship found between cholesterol and glutamate or ornithine (which should to some extent reflect the wealth of AA pool and disposal) that emerged as the main driving correlations in this study. These results reconfirm and expand some findings which were previously noted in smaller and less heterogeneous groups of measurements included in our same database (Chiarla et al., 2004, 2006). Of course the significance of the described correlations was verified in the remainder of the measurements, therefore strengthening the evidence relating increases in cholesterol (or, more precisely, moderation of the decrease due to sepsis) to availability of amino acid substrate.

In clinical practice the role of hypocholesterolemia as a marker of sepsis should be emphasized more strongly. As a general concept, transient hypocholesterolemia may depend on clearly evident causes such as surgical or nonsurgical trauma, hemodilution from hemorrhage, or easily diagnosable sepsis. However, given the difficulties which may often occur in diagnosing sepsis (Assunção et al., 2010), especially the occult form presenting with light or deceitful manifestations, unexplained hypocholesterolemia may motivate deeper patient assessment looking for concealed infectious sources.

Use of the absolute value of cholesterol as a single marker of severity of septic illness may be limited by some inter-patient variability (Giovannini et al., 1999, 2005; Kruger, 2009; Nuzzo & Giovannini, 2010; Hrabovský et al., 2012) and baseline pre-illness cholesterol should also be considered. In individu-
al patients an important source of variability is cholestasis which increases cholesterol, and therefore moderates the decrease caused by other factors, while the impact of exogenous AAs needs to be more precisely assessed. Nevertheless, these considerations do not prevent serial determinations of plasma cholesterol, in individual patients, from being an inexpensive adjunctive tool to monitor the tendency toward recovery or worsening of sepsis. This better emends and confirms principles and concepts which do not simply regard sepsis in the trauma patients in our study (Figure 2a), but also extend to other conditions of sepsis (Figure 2b).

**Figure 2a:** Evolution of hypocholesterolemia in a trauma patient with sepsis included in the study, showing initial transient improvement, relapse of sepsis and final progression toward recovery. To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259.

**Figure 2b:** Evolution of hypocholesterolemia in a different surgical patient, with nearly fatal and persistent abdominal sepsis. Sepsis was due to a complex surgical problem, a cryptic juxta-papillary duodenal fistula within obstructive duodenal scarring, causing recurrent peritonitis and paraduodenal abscesses. **A.** Referral with recurrent peritonitis: surgical, endoscopic and percutaneous treatments followed by unsteady improvement. **B.** Relapse of severe sepsis: surgical drainage of periduodenal abscesses, duodenal exclusion and complete drainage of the whole area, followed by steadier improvement and progressive recovery from sepsis (with transient mild relapses resolved by antibiotic/percutaneous treatments). To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259.
Another important point is that degree of hypocholesterolemia in itself (even as low as 11 mg/dL) may still be associated with good prognosis if hypocholesteremia is rapidly reversed by eradication of the septic focus; on the contrary, persistently severe hypocholesterolemia is more often associated with death (Giovannini et al., 2003, 2007).

4.6 Conclusions

Our study adds some new pieces to the mosaic of knowledge on septic metabolic and cardiorespiratory abnormalities, by quantifying the AA correlations associated with hypocholesterolemia over a large number of measurements in trauma patients who developed sepsis. The correlations found between decreasing cholesterol, changes in AA levels and SOFA score emphasize the role of hypocholesterolemia as a marker of severity of septic metabolic illness, although cholestasis, if present, may moderate degree of hypocholesterolemia. Further study is needed to confirm the correlation with increasing glutamate and ornithine and its clinical significance.

References


