AN INVESTIGATION OF THE MEASUREMENTS OF VITAMIN STATUS AND OUTCOME IN PATIENTS WITH CRITICAL ILLNESS

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Based on work conducted in the University Departments of Anaesthesia and Surgery and Department of Clinical Biochemistry

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Abstract

This thesis describes a prospective observational longitudinal study that examines the relationship between the systemic inflammatory response, vitamin concentrations and outcome in patients with critical illness. Venous blood samples were collected from patients admitted in the Intensive Care Unit of Glasgow Royal Infirmary on admission (n=126) and on follow-up (n=77). The concentrations of vitamins B2, B6, C, A, E, lutein, lycopene, α-carotene and β-carotene in plasma, red cells and white cells and plasma total and free malondialdehyde, that was used as a marker of lipid peroxidation, were assessed by high performance liquid chromatography (HPLC).

In Chapter 3, vitamin B2 was examined. The results of this study showed that the ratio of plasma FAD to riboflavin in critically ill patients on admission and on follow-up was much lower than that of the plasma ratio in the controls. There were also similar findings with respect to the red cell FAD to riboflavin ratios in controls and critically ill patients. However, on admission the reduction of the FAD to riboflavin ratio was greater in the plasma (83%) compared with the red cells (49%). These results showed perturbation of the relationship between plasma FAD and riboflavin in patients with critical illness. Moreover, this appeared to be primarily due to a relative reduction in plasma and intracellular FAD concentrations although red cell FAD concentrations were all within the reference interval.

In Chapter 4, vitamin B6 was examined. The results of this study showed that, in plasma, red cells and white cells, PLP is strongly and directly associated with the concentrations of PL in patients with critical illness. The ratio of plasma PLP to PL in critically ill patients on admission and on follow-up was much lower than that of the plasma controls. There were also similar findings with respect to the red cell PLP to PL ratios in controls and critically ill patients. However, on admission the reduction of the PLP to PL ratio was

greater in the plasma (55%) compared with the red cells (18%). These results report that the relationship between plasma PLP and PL in controls is similar to that found in red and white cells, in patients with critical illness. Given that, compared to plasma PLP, concentrations of plasma PL were more strongly correlated with those in the red or white cells and that red cell and white cell PL concentrations were strongly and similarly correlated with their respective PLP concentrations, demonstrates the importance of PL in the intracellular metabolism of PLP in normal subjects and critically ill patients. One interpretation of these results could suggest that plasma PL may be a good surrogate measure of intracellular PLP concentrations. However, PL is not the physiologically active form of vitamin B6 and therefore, the clinical relevance of measuring plasma PL concentrations is not clear. Therefore, the present intracellular measurements of PLP, compared with plasma, may be a more accurate reflection of vitamin B6 status.

In Chapter 5, vitamin C was examined. The results of this study showed that plasma ascorbic acid concentrations in critically ill patients were below reference intervals whereas white cell ascorbic acid concentrations were within the reference intervals. Moreover, plasma and intracellular concentrations of both vitamin C and α-tocopherol were poorly correlated and did not increase with supplementation in the ICU. Taken together these results would suggest that plasma ascorbic acid concentrations may poorly reflect intracellular concentrations in patients with critical illness.

In Chapter 6, vitamin E was examined. The results of this study showed that, compared with control subjects, the critically-ill patient group had lower plasma α-tocopherol concentrations and also when expressed per mmol of triglycerides. In contrast, α-tocopherol concentrations were higher when expressed per mmol of cholesterol despite these patients receiving no vitamin E supplementation in ICU. Moreover, neither plasma α-tocopherol nor plasma α-tocopherol expressed per mmol of lipids was strongly correlated with red cell α-tocopherol concentrations in the critically-ill patients. Finally,

median red cell α-tocopherol concentrations were the same in the healthy subjects and patients with critical illness and systemic inflammatory response syndrome. Taken together the results indicate that plasma α-tocopherol alone or expressed per mmol of lipids (using either cholesterol or triglycerides) may be a less reliable marker of vitamin E status than red cell α-tocopherol in patients with a systemic inflammatory response.

In Chapter 7, lipid soluble antioxidants A, E and carotenoids and lipid peroxidation, as measured by malondialdehyde (MDA, total and free), were examined. The results of this study showed that, on admission to ICU, the free MDA fraction was increased and carotenoid concentrations were low in patients with critical illness. The majority of patients had plasma retinol concentrations below the reference interval on admission to ICU and these were directly and significantly related to α-tocopherol and the carotenoids. Moreover, this relationship was maintained on follow-up. Both albumin and C-reactive protein were consistently correlated with these lipid soluble antioxidant concentrations. Therefore, the reduction of lipid soluble antioxidant concentrations, in particular carotenoids, is likely to be multifactorial and not solely dependent on consumption during lipid peroxidation.

In Chapter 8, the behaviour of plasma and intracellular B2, B6 and C vitamin concentrations during supplementation in patients with critical illness was studied. The results of the present study show a discrepancy in the behaviour of plasma and intracellular B2, B6 and C vitamin concentrations during supplementation in patients with critical illness. Indeed, on admission to ICU, almost 1/3 of the patients had plasma vitamin B2, B6 and C concentrations below the reference interval. In contrast, on admission to ICU, red cell FAD, red cell PLP and white cell ascorbic acid concentrations were significantly higher in the patients with records of prior supplementation compared with the patients without. The longitudinal findings were in agreement with this observation and taken together, this study may suggest that cellular concentrations of vitamins B2, B6 and C may

be more accurate markers of their respective vitamin status and may be expected to be a more true reflection of the presence of supplementation than their respective plasma concentrations.

In Chapter 9, the relationship between lipid peroxidation, water and lipid soluble vitamins and severity of illness or hospital outcome in critically ill patients was examined. The results of this study showed that lipid peroxidation, as evidenced by an elevated free MDA fraction, was most significantly associated with death in hospital. The concentrations of retinol and the carotenoids in the plasma were well below the reference intervals and although correlated with the free MDA fraction, had no independent prognostic value. Albumin was directly associated with the lipid soluble vitamins in the plasma, in particular retinol, α-tocopherol, lutein and lycopene. Albumin was shown to have prognostic value independent of APACHE II and when albumin was removed from the multivariate model, lycopene became an independent predictor of hospital death. None of the intracellular vitamin concentrations were related to severity of illness in critically ill patients. Taken together these results would suggest that low plasma levels of lipid soluble vitamins may be due to redistribution in the same way that albumin concentrations fall as part of the systemic inflammatory response syndrome.

In summary, the current thesis showed that, during the systemic inflammatory response, intracellular vitamin concentrations may be more accurate than plasma concentrations as indicators of vitamin status in patients with critical illness.

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Author's Declaration

The work presented in this thesis was performed entirely by the author except as acknowledged below. More specifically, the author has performed the following work:

1. Participated in the design of the study together with the supervisors of the study.

2. Wrote and submitted the ethics application form for this study.

3. Identified, recruited and consented the 126 critically ill patients of the study.

4. Collected longitudinal medical, anthropometric, nutritional, biochemical and outcome information for the critically ill patients consented in the study. As part of this, individually calculated the SOFA scores of the study.

5. Allocated, stabilised and stored longitudinal patient blood samples for plasma and red cell vitamin analysis.

6. Stabilised a novel white cell extraction method from 1 mL of whole blood. Prepared and stored longitudinal white cell alliquotes for vitamin analysis.

7. Performed red and white cell vitamin B2 and B6 and white cell vitamin C analysis and trained and supervised the students for red cell vitamin E and total and free malondialdehyde analysis.

8. Added all data on the statistical database and performed all statistical analysis with the assistance of Prof. DC McMillan.

Blood sampling was performed by the medical and nursing staff of the Intensive Care Unit, Royal Infirmary, Glasgow.

Routine laboratory analysis of plasma vitamins B6, C, retinol, α-tocopherol, lutein, lycopene, α- and β-carotene were performed by the laboratory staff of the Scottish Trace Element and Micronutrient Reference Laboratory, Royal Infirmary, Glasgow.

Laboratory analysis of red cell vitamin E was performed with the assistance of Dimitra Leivaditi, postgraduate student of the department of Human Nutrition, University of Glasgow.

Laboratory analyses of total and free malondialdehyde were performed with the assistance of Linda White, intercalated student of the faculty of Medicine, University of Glasgow.

This thesis has not been previously submitted for a degree or diploma at this or any other institution.

Aikaterini Vasilaki

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Publications

The work presented in this thesis has resulted in the following abstracts and presentations;

Vasilaki, A.T., McMillan, D.C., O'Reilly, D.S., Kinsella, J. & Talwar, D. (2006) *'Assessment of vitamin B6 (pyridoxal phosphate and pyridoxal) concentrations in patients with critical illness: Plasma or red cell?'*, E-poster presentation, BAPEN, Brighton, UK.

Vasilaki, A.T., Talwar, D., Kinsella, J., Duncan, A., O'Reilly, D.S. & McMillan, D.C. (2007) '*The impact of vitamin supplementation on plasma and white cell vitamin C concentrations'*, Poster presentation, 29th ESPEN Congress, Prague, Czech Republic.

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Dedication

Dedicated to my father Theofilos and my partner Colin for their love and support.

Abbreviations

ALP	Alkaline phosphatase
APACHE	Acute Physiology and Chronic Health Evaluation
ARDS	Acute respiratory distress syndrome
BW	Body weight
CRP	C-reactive protein
CV	Coefficient of Variation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EN	Enteral nutrition
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Mono Nucleotide
GI	Gastrointestinal
GRI	Glasgow Royal Infirmary
Hb	Haemoglobin
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
ICU	Intensive Care Unit
IL-1	Interleukin 1
ITU	Intensive Therapy Unit
IV	Intravenous
KCAL	Kilocalories
KG	Kilogram

LDL	Low Density Lipoprotein
MDA	Malondialdehyde
MODS	Multiple Organ Dysfunction Syndrome
NF-κB	Nuclear transcription factor-kappa B
PBS	Phosphate buffered saline
PL	Pyridoxal
PLP	5'-Pyridoxal phosphate
ROS	Reactive oxygen species
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential Organ Failure Assessment
TBARS	Thiobarbituric acid-reactive substances
TCA	Trichloroacetic acid
TNF	Tumour necrosis factor
TRAP	Total radical-trapping antioxidant parameter
UV	Ultraviolet
VLDL	Very Low Density Lipoprotein
WC	White cells (counts)

1 Introduction

The importance of antioxidant concentrations in the tissues and their relationship to the prevention of multiple organ dysfunction syndrome (MODS) and the increased mortality that accompanies it has been of considerable interest in the last two decades. During the course of critical illness a number of metabolic changes affecting micronutrient utilization and distribution have been documented. Micronutrient deficiencies have been associated with morbidity and mortality in this compromised group and thus it is of little surprise that researchers have focused on provision of optimal nutrition and micronutrient supplements, either enterally or parenterally, in order to mitigate organ dysfunction and mortality in patients with critical illness.

The assessment of micronutrient status and their provision to such patients has been monitored either by using functional tests (enzymatic activity) or by measuring micronutrient concentrations in plasma. However, since most micronutrients behave as acute phase reactants, the value of such plasma measurements has been questioned (Galloway et al. 2000).

Recent advances in methods of micronutrient assessment allow the direct measurement of their concentrations within the blood plasma and red cells (Talwar et al. 2003a). For example, based on these direct methods it has been shown that redistribution of B-vitamins between plasma and blood cells takes place during elective surgery (Gray et al. 2004) and critical illness (Talwar et al. 2003b; Quasim et al. 2005). This evidence questions the existence of micronutrient deficiency in these patients as well as the accuracy of micronutrient biochemical assessment methods used previously to assess these deficiencies.

1.1 Critical illness in Scotland

1.1.1 Demographics

The critically ill are the patients with the most deranged physiology of the health care setting. Due to their need for intensive nursing and their high mortality rates (31% hospital mortality for the critically ill patients admitted in ICUs in 2007) (Scottish Intensive Care Society Audit Group 2008), these patients are treated in a special designed area, the intensive care unit (ICU). There were 348 admissions in the Glasgow Royal Infirmary ICU in 2007. Having different pathologies (Ridley et al. 1997), the critically-ill patients are a heterogeneous group admitted in the ICU either from the hospital wards (25%) or as high-risk post-surgery patients (42%) with established or potential organ failure (Scottish Intensive Care Society Audit Group 2008).

Patients admitted to ICU suffer from organ dysfunction (or organ failure) of one or more organs with the most common failure being that of the respiratory system. Organ failure is one of the variables independently associated with an increased risk of mortality (Vincent et al. 1995). A single organ system failure lasting more than one day results in a mortality rate approaching 40%, where for three or more organ system failures for more than three days the death rates increase to 98% (Knaus et al. 1985a). It seems that at the final stage of multiple organ dysfunction syndrome (MODS) an immunologic dissonance occurs, characterized by an imbalance between the pro- and anti-inflammatory mediators (Bone 1996). It has been shown that organ failure, prolonged ICU stay, and high infection and mortality rates are associated with the presence of the systemic inflammatory response syndrome (Lobo et al. 2003).

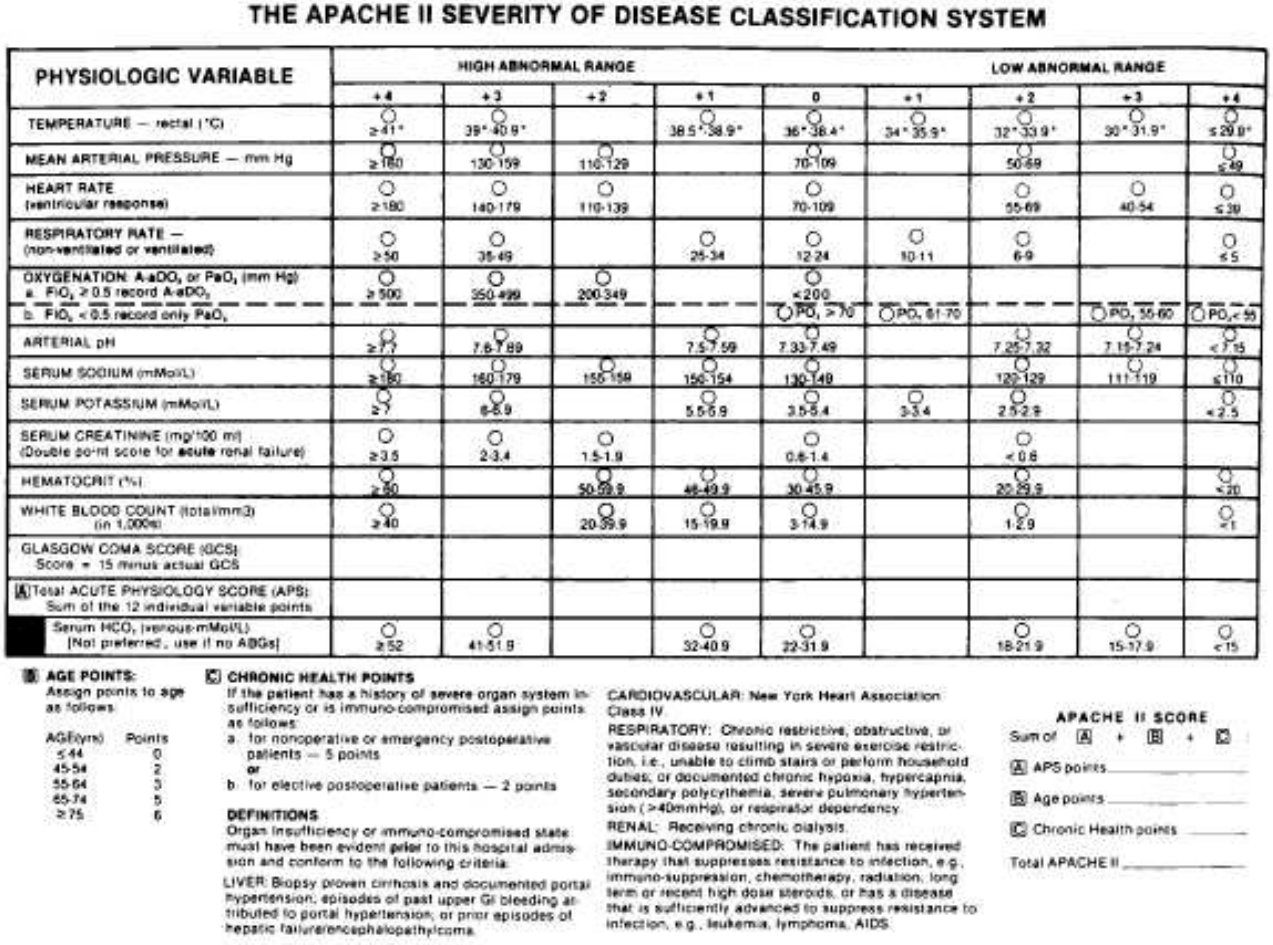
1.1.2 Illness severity scoring systems

1.1.2.1 APACHE II score

Using the Acute Physiology And Chronic Health Evaluation II (APACHE II) diagnostic classification (Knaus et al. 1985b, Figure 1-1), patients can be grouped into nine categories according to the primary organ system failure leading to ICU admission. Previous research (Beck et al. 1997; Pappachan et al. 1999; Woods et al. 2000) has shown that the APACHE II score is the most reliable scoring system for assessing severity of disease in the ICU for the U.K. In Glasgow, the overall five–year mortality rate for patients being admitted in the ICU was 47%, 3.4 times higher than that of the general population (Wright, Plenderleith & Ridley 2003).

1.1.2.2 SOFA score

The need for producing the Sequential Organ Failure Assessment (SOFA) score resulted because of the fact that outcome prediction scores, such as APACHE II, rely on ICU admission values and could ignore many factors that can influence patient outcome during their ICU stay (Vincent et al. 1996, Figure 1-2). The total maximum SOFA score (adding the highest value for each variable in the duration of stay) and the difference between total maximum and admission SOFA scores reflect the degree of organ failure during ICU stay (Moreno et al. 1999). The initial and highest SOFA score of more than 11 and mean SOFA score of more than 5, independent of length of stay, have been found to correlate well with mortality in ICU (Ferreira et al. 2001). Regardless of the initial value, when the score increased in the first four days the mortality rate was greater than 50%, 27-35% if it remained unchanged and less than 27% when it decreased (Ferreira et al. 2001).



THE APACHE II SEVERITY OF DISEASE CLASSIFICATION SYSTEM

PHYSIOLOGIC VARIABLE	HIGH ABNORMAL RANGE					LOW ABNORMAL RANGE			
	+4	+3	+2	+1	0	+1	+2	+3	+4
TEMPERATURE — rectal (°C)	≥41°	39°-40.9°		38.5°-38.9°	36°-38.4°	34°-35.9°	32°-33.9°	30°-31.9°	≤29.9°
MEAN ARTERIAL PRESSURE — mm Hg	≥160	130-159	110-129		70-109		50-69		≤49
HEART RATE (ventricular response)	≥180	140-179	110-139		70-109		55-69	40-54	≤39
RESPIRATORY RATE — (non-ventilated or ventilated)	≥50	35-49		25-34	12-24	10-11	6-9		≤5
OXYGENATION: A-aDO$_2$ or PaO$_2$ (mm Hg) a. FIO$_2$ ≥ 0.5 record A-aDO$_2$	≥500	350-499	200-349		≤200				
b. FIO$_2$ < 0.5 record only PaO$_2$					PO$_2$ > 70	PO$_2$ 61-70		PO$_2$ 55-60	PO$_2$ < 55
ARTERIAL pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	< 7.15
SERUM SODIUM (mMol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
SERUM POTASSIUM (mMol/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		< 2.5
SERUM CREATININE (mg*100 ml) (Double point score for **acute** renal failure)	≥3.5	2-3.4	1.5-1.9		0.6-1.4		< 0.6		
HEMATOCRIT (%)	≥60		50-59.9	46-49.9	30-45.9		20-29.9		< 20
WHITE BLOOD COUNT (total/mm3) (in 1,000s)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		< 1
GLASGOW COMA SCORE (GCS) Score = 15 minus actual GCS									
A Total ACUTE PHYSIOLOGY SCORE (APS): Sum of the 12 individual variable points									
Serum HCO$_3$ (venous-mMol/L) [Not preferred, use if no ABGs]	≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	< 15

B AGE POINTS:
Assign points to age as follows:

AGE(yrs)	Points
≤44	0
45-54	2
55-64	3
65-74	5
≥75	6

C CHRONIC HEALTH POINTS
If the patient has a history of severe organ system insufficiency or is immuno-compromised assign points as follows:
a. for nonoperative or emergency postoperative patients — 5 points
or
b. for elective postoperative patients — 2 points

DEFINITIONS
Organ insufficiency or immuno-compromised state must have been evident **prior** to this hospital admission and conform to the following criteria:

LIVER: Biopsy proven cirrhosis and documented portal hypertension; episodes of past upper GI bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma.

CARDIOVASCULAR: New York Heart Association Class IV.

RESPIRATORY: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, i.e., unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40mmHg), or respirator dependency.

RENAL: Receiving chronic dialysis.

IMMUNO-COMPROMISED: The patient has received therapy that suppresses resistance to infection, e.g., immuno-suppression, chemotherapy, radiation, long term or recent high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection, e.g., leukemia, lymphoma, AIDS.

APACHE II SCORE
Sum of A + B + C

A APS points _____

B Age points _____

C Chronic Health points _____

Total APACHE II _____

Figure 1-1 The Acute Physiology And Chronic Health Evaluation (APACHE) II score system. (Knaus et al. 1985b)

SOFA score	1	2	3	4
Respiration PaO_2/FiO_2 mmHg	< 400	< 300	< 200 with respiratory support	< 100 with respiratory support
Coagulation Platelets x $10^3/mm^3$	< 150	< 100	< 50	< 20
Liver Bilirubin, mg/dL (μmol/L)	1.2–1.9 (20–32)	2.0–5.9 (33–101)	6.0–11.9 (102–204)	> 12.0 (> 204)
Cardiovascular Hypotension[a]	MAP < 70 mm Hg	Dopamine ≤ 5 or Dobutamine (any dose)	Dopamine < 5 or epinephrine ≤ 0.1 or norepinephrine ≤ 0.1	Dopamine > 1.5 or epinephrine > 0.1 or norepinephrine > 0.1
Central Nervous System Glasgow coma score	13–14	10–12	6–9	< 6
Renal Creatinine, mg/dL (μmol/L) or urine output	1.2–1.9 (110–170)	2.0–3.4 (171–299)	3.5–4.9 (300–440) or < 500 mL/day	> 5.0 (> 440) or < 200 mL/day

[a] adrenergic agents administered for at least one hour (doses given are in μg/kg · min)

Figure 1-2 Sequential Organ Failure Assessment (SOFA) score system.
(Vincent et al. 1996)

1.2 Nutritional management in critical illness

1.2.1 Nutritional support

'The aims of nutrition support in critical ill patients are to minimise nutritional losses and provide basic nutrient requirements to sustain life' (Webster-Gandy, Madden & Holdsworth 2006).

In the presence of infectious disease, inadequate nutrition not only reduces the availability of endogenous stores of nutrients but it may also lead to increased susceptibility to secondary infection through reduced synthesis of pro-inflammatory cytokines and impaired cell-mediated immunity (Wan, Haw & Blackburn 1989). The European Prevalence of Infection in Intensive Care (EPIC) study showed that in a sample of 10,038 critically ill patients suffering trauma, sepsis, or major surgery, there is a high risk of developing nosocomial infection (21%) resulting in increased mortality (r^2 =0.68) (Vincent et al. 1995). Indeed, one out of four severely ill patients have been assessed as malnourished (Edington et al. 2000) and in addition, mechanically ventilated critically ill patients are more likely to receive <67% of energy and protein than non–ventilated patients (Kyle et al. 2006). Thus, feeding this group of patients seems to be of great importance.

Feeding and supplementing the compromised patient has been common practice over the last two decades. Nutritional requirements of the critically ill patient focus on their energy, protein, carbohydrate, lipid, fluid balance, electrolyte and micronutrient needs. Nevertheless, evidence on the actual amounts of nutrients that these patients require for optimal recovery can be limited in most cases. Thus, the clinicians and dietitians focus mainly in providing nutrition that aims to prevent further nutritional deterioration and on

the other hand avoid metabolic and non-metabolic complications of overfeeding, such as diarrhoea, metabolic acidosis, hyperglycaemia, increased respiratory quotient, etc.

Generally in critically ill patients enteral nutrition may result in fewer infectious complications compared with parenteral nutrition (Simpson & Doig 2005; Gramlich et al. 2004). However a statistically significant mortality benefit in favour of parenteral nutrition was evident in a recent meta–analysis of 11 trials (OR=0.56, p=0.03) and this benefit was greatest in trials in which enteral nutrition was delayed for more than 24 hours (Simpson & Doig 2005).

Enteral nutrition is initially considered for the newly admitted patients to the intensive care units. In 2006, the European Society of Parenteral and Enteral Nutrition (ESPEN) summarised the existing evidence on enteral nutrition in the intensive care unit and published guidelines for its use by dietitians working in the intensive care setting (Kreymann et al. 2006). Table 1-1 summarises these results.

Table 1-1 ESPEN guidelines for patients with critical illness. (Kreymann et al. 2006)

Indications	All patients who are not expected to be on a full oral diet within 3 days should receive enteral nutrition (EN).
Application	There are no data showing improvement in relevant outcome parameters using early EN in critically ill patients. Nevertheless, the expert committee recommends that haemodynamically stable critically ill patients who have a functioning gastrointestinal tract should be fed early (< 24 h) using an appropriate amount of feed. No general amount can be recommended as EN therapy has to be adjusted to the progression/ course of the disease and to gut tolerance.
Exogenous energy supply	During the acute and initial phase of critical illness: in excess of 20–25 kcal/kg BW/day may be associated with a less favourable outcome. During the anabolic recovery phase and in patients with severe undernutrition, the aim should be to provide 25–30 kcal/kg BW/day. If these target values are not reached supplementary parenteral nutrition should be given.
Route	Use EN in patients who can be fed via the enteral route. Avoid additional parenteral nutrition in patients who tolerate EN and can be fed approximately to the target values. Use supplemental parenteral nutrition in patients who cannot be fed sufficiently via the enteral route. Consider careful parenteral nutrition in patients intolerant to EN at a level equal to but not exceeding the nutritional needs of the patient.
Type of formula	Whole protein formulae are appropriate in most patients because no clinical advantage of peptidebased formulae could be shown. Immune-modulating formulae (formulae enriched with arginine, nucleotides and n-3 fatty acids) are superior to standard enteral formulae: in elective upper GI surgical patients (see guidelines surgery), in patients with a mild sepsis (APACHE II <15), in patients with severe sepsis, however, immune-modulating formulae may be harmful and are therefore not recommended, in patients with ARDS (formulae containing n-3 fatty acids and antioxidants). ICU patients with very severe illness who do not tolerate more than 700 ml/day of enteral formulae should not receive an immune-modulating formula enriched with arginine, nucleotides and ω-3 fatty acids. There are not sufficient data to support glutamine supplementation in surgical or heterogenous critically ill patients.

1.2.2 Immunonutrition

More recently, the concept of immunonutrition, i.e. nutrition that boosts the immune response to illness, has been introduced to critical care, and this appears to show beneficial effects such as improved immune function and reduced frequency of infectious complications (Beale, Bryg & Bihari 1999; Heys et al. 1999). The most common immune–enhancing substrates contained in these type of diets are arginine, nucleotides, branched–chain amino acids, glutamine or/and ω–3 fatty acids.

A randomised trial (Kudsk et al. 1996) compared a small group of critically ill trauma patients (n=16) who received an immune-enhancing diet (containing glutamine, arginine, ω-3 fatty acids and nucleotides) with critically ill patients on an isonitrogenous standard diet. This study showed that the immuno-enhancing diet significantly reduced major infectious complications (by 35%, $p<0.05$), hospital stay (by 16 days, $p<0.05$) and therapeutic antibiotics requirements (by 4 days, $p<0.05$). For burn patients immuno-enhancing diet administration (without glutamine) did not appear to show similar benefits (Saffle et al. 1997).

The effects of the immune–enhancing diets can be different depending on the subset of patients analyzed or on the methological quality of the studies included in the meta–analysis (Heyland et al. 2001). When it comes to subgroups of patients it seems that enteral immunonutrition benefits the surgical patients more (Beale, Bryg & Bihari 1999; Heyland et al. 2001; Montejo et al. 2003).

Certainly, it appears that the routine administration of immuno-enhancing nutrition in critically ill patients should be judged after the development of understanding the patient's patho-physiological situation and development of methods for monitoring immune responses and status to avoid adverse effects in recovery (Suchner, Kuhn & Fürst 2000).

Indeed, in critically ill patients with severe sepsis, enteral immunonutrition containing L-arginine, ω-3 fatty acids, vitamin E, β-carotene, zinc and selenium appears to show higher ICU mortality rates (44% vs 14%, $p<0.05$) compared with the patients that received parenteral nutrition (Bertolini et al. 2003). Furthermore, immune-enhanced enteral nutrition has been shown to be superior to parenteral nutrition in terms of episodes of sepsis or septic shock (5% vs 13%) and ICU length of stay (4d shorter) in critically ill patients without septic shock or severe sepsis (Radrizzani et al. 2006). Other studies have shown either benefit from the administration of similar immuno-enhancing formulas, e.g. in hospital length of stay (Bower et al. 1995), or no benefits (Mendez et al. 1997) compared with standard formulas.

A recent meta-analysis (24 studies) has reported an improved outcome, i.e reduced mortality, secondary infections and length of stay, in medical ICU patients receiving immuno-enhancing diets with fish oil while supplementation with arginine with or without additional glutamine or fish oil did not appear to provide any advantages compared with standard enteral formulas in trauma and burn patients admitted in ICU (Marik & Zaloga 2008).

1.3 The systemic inflammatory response

The systemic inflammatory response syndrome (SIRS) is a complex coordinated response to acute injury and has been associated with a number of clinical conditions, such as infection, pancreatitis, ischemia, burns, multiple trauma and tissue injury, hemorrhagic shock, and immunologically mediated organ injury (Bone et al. 1992; Levy et al. 2003) in areas located far from the initial invasion. Inflammation may generally be beneficial to the host until its clinical manifestations become unmanageable by the body (Bone 1996). Previous work has shown that almost all critically–ill patients satisfy the criteria for systemic inflammatory response syndrome (Pittet et al. 1995). This generalized tissue injury may lead to progressive organ failure, which has a high mortality rate (Lobo et al. 2003).

The SIRS is manifested by two or more of the following clinical conditions (Bone et al. 1992; Levy et al. 2003): (1) temperature > 38º C or < 36º C; (2) heart rate > 90 beats per minute; (3) respiratory rate > 20 breaths per minute or PaCO2 <32 mm Hg; and (4) white blood cell count >12,000/ cu mm, <4,000/ cu mm, or >10% immature (band) forms.

1.3.1 Inflammatory response and SIRS in health and critical illness

1.3.1.1 SIR and acute phase proteins

It is recognised that, in healthy conditions, the protein concentrations in plasma are determined by their synthesis and degradation rate. However, during the systemic inflammatory response, a number of changes in these liver-derived proteins occurs resulting in altered protein concentrations in blood (Gabay & Kushner 1999, Figure 1-3). Interleukin-1 (IL-1) or tumour necrosis factor (TNF) or both appear to participate in the initiation of the systemic inflammatory response (Dinarello 1984a; 1984b, 1988). More

particularly, IL-1 has been shown to be involved in triggering the transcription and synthesis of the acute-phase proteins by the liver (Ramadori et al. 1985).

The increase in the inter–cellular endothelial gaps (McDonald, Thurston & Baluk 1999) allows the translocation of proteins with small molecular weight, such as albumin (molecular weight=66,000), from the circulation to the extravascular space (Raines et al. 1984; Fleck et al. 1985). Indeed, in acute infectious disease, albumin concentrations in plasma significantly correlate with the albumin transcapillary escape rate ($r=0.83$, $p<0.05$) (Ballmer, Ochsenbein & Schutz-Hofmann 1994). As albumin is the main carrier protein for the majority of vitamins and trace elements, its decrease in plasma might be expected to affect the micronutrient concentrations in this blood compartment.

This mechanism is reflected in the first 2–4 hours of inflammation with a decrease in the negative inflammatory proteins' concentrations (albumin, transferrin, retinol–binding protein and pre–albumin), and an increase in the concentrations of the positive inflammatory proteins (fibrinogen, α1–antitrypsin, caeruloplasmin, and C-reactive protein) (Colley et al. 1983; Myers et al. 1984).

One of the most sensitive positive acute phase proteins is C-reactive protein (CRP), which was originally identified due to its peculiar property of precipitating with the C polysaccharide derived from the cell wall of pneumococcus (Tillet & Francis 1930). It contains 187 amino acids in a single polypeptide chain with a minimal molecular weight of 20,949 daltons (Oliveira, Gotschlich & Liu 1979). In serum, CRP has a variable molecular weight ranging from 110,000 to 144,000 (Gotschlich & Edelman 1965). Early observations (MacLeod & Avery 1941) showed that CRP was present in many acute illnesses but could not be detected in the plasma or serum of normal individuals. During SIRS a 1000-fold increase in CRP concentrations has been reported (Figure 3; Gabay &

Kushner 1999), primarily due to increased hepatic synthesis, a peak that is observed 48 hours after the initial insult (Colley et al. 1983).

CRP has many pathophysiologic roles in the inflammatory process. It induces the production of inflammatory cytokines and tissue necrosis factor from monocytes (Cermak et al. 1993) thus acting as a pro-inflammatory molecule. It has recently been reported that 98% of the mechanically ventilated ICU patients have CRP concentrations greater than 30 mg/L and 36% of this group have albumin concentrations lower than 30 g/L (Kyle et al. 2006) indicating the inverse relationship between the two proteins during the systemic inflammatory response syndrome. A CRP decrease ≥50 mg/L between admission and day 4 has been found to be an independent predictor of recovery in critically ill patients and in combination with SIRS it provides the best model to diagnose infection at admission (Reny et al. 2002).

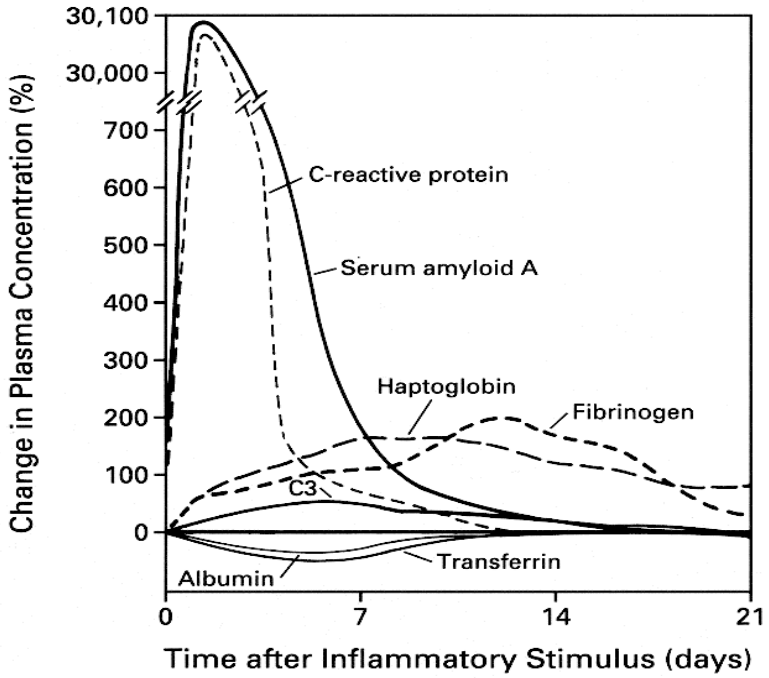


Figure 1-3 Acute phase proteins and the systemic inflammatory response. (From Gitlin & Cotlen 1987 as modified in Gabay & Kushner 1999)

1.4 The role of systemic inflammatory response in the development of multiple organ dysfunction syndrome (MODS)

1.4.1 Oxidative stress and MODS

1.4.1.1 Cytokine effect on reactive oxygen species production

There is mounting evidence that tissue injury is mediated at least in part by oxidative metabolites, or reactive oxygen species (ROS), derived from inflammatory cells and/or hypoxia (Hill & Hill 1998). ROS induce direct oxidative tissue injury by means of peroxidation of cellular membranes, oxidation of critical enzymatic and structural proteins and induction apoptosis. In addition, the activation of nuclear factor–kappa B (NF-κB) by ROS leads to the induction of genes critical to the initiation and establishment of the systemic inflammatory response (Ziegler-Heitbrock et al. 1993). As radicals may be produced via normal intracellular processes, but during inflammation in an exaggerated manner by inflammatory cells, a vicious cycle occurs. Any insult, if severe enough, may induce the release of proinflammatory mediators and may result in the formation of reactive oxygen species, possibly as following the activation of polymorphonuclear leukocyte and other reticulo-endothelial cells.

1.4.1.2 Increased oxidative stress, NF-κB cycle and development of MODS

The activity of ROS is normally limited by the antioxidant defence system of the body. In critically ill patients, the antioxidant capacity is likely to be compromised due to increased utilization in the face of increased ROS production (Goode & Webster 1993) and perhaps redistribution due to the acute–phase response (Louw et al.1992; Shenkin 1997).

Many critically ill surgical patients survive their initial physiologic insult only to die of infection or organ dysfunction over the ensuing days to weeks. The profound oxidative stress that occurs during critical illness is likely to lead to early depletion of many endogenous antioxidants. In general, plasma antioxidant vitamin concentrations seem to decrease and measures of oxidative stress seem to increase in critically ill populations (Oldham & Bowen 1998). It has been proposed that the critically ill patient has an increased need for antioxidants, due to hypermetabolism and increased losses. The relative decrease in antioxidant capacity appears to correlate with the severity of illness and suggests a causal relationship between antioxidant depletion and organ dysfunction (Goode et al. 1995; Borrelli et al. 1996). Micronutrient deficiency is often associated with defects in a number of organ systems, including the gastrointestinal, cardiovascular, haematological and musculoskeletal systems.

Antioxidant capacity may also be compromised in critically ill patients through redistribution of plasma–binding proteins, i.e. albumin, as part of the acute inflammatory response (Galloway, McMillan & Sattar 2000), poor nutritional state, and inadequate provision of optimal nutrition (O'Leary-Kelley et al. 2005). Given the apparent role oxidative stress and anti-oxidant mediated injury play in the development of acute respiratory distress syndrome and multiple organ failure, supplementation with antioxidants may augment endogenous antioxidant defences and serve to modulate the development of organ dysfunction.

There is some evidence that patients admitted to the ICU have reduced antioxidant capacity (Cowley et al. 1996; Alonso de Vega et al. 2002; Doise et al. 2008). It has been demonstrated that plasma redox status, as measured by the ratio of plasma total antioxidant capacity to lipoperoxides, significantly correlates to severity of disease, as measured by APACHE III (r^2=0.56, $p<0.001$), and survival in critically ill patients (Alonso de Vega et al. 2002). Moreover, survivors tend to have greater plasma antioxidant potential, by

approximately 8%, compared with nonsurvivors and, despite having an initial plasma antioxidant potential value lower than normal, survivors seem to be able to attain normal or supranormal values rapidly comparing to non-survivors that do not seem able to reach the normal values (Cowley et al. 1996).

Indeed, the critically ill patients appear to have decreased concentrations of plasma total radical–trapping antioxidant parameter (TRAP) and its components, such as vitamin C, vitamin E, and plasma unidentified antioxidants (Tsai et al. 2000; Alonso de Vega et al. 2002) and increased lipid peroxidation as shown by elevated concentrations of thiobarbituric acid–reactive substances (TBARS; Motoyama et al. 2003). In addition, the magnitude of oxidative stress appears to be proportional to the severity of multiple organ dysfunction syndrome (Motoyama et al. 2003). Modulating the oxidative stress seems to be one important intervention to control the systemic inflammatory response associated with acute respiratory distress syndrome and sepsis (Zhang, Slutsky & Vincent 2000; Lang et al. 2002).

As oxidative stress, measured by markers like malondialdehyde (MDA) and F2 isoprostanes, has been found to be increased (1.2 and 1.3 times, respectively) in non-survivor critically ill patients compared to survivors (Mishra et al. 2005), antioxidant deficiency may be related to less optimal recovery of the critically ill patient. Indeed in critically ill patients, MDA and F2 isoprostanes have both been shown to be positively correlated with multiple organ dysfunction syndrome on admission to ICU (Mishra et al. 2005). In patients with severe burns, increased oxidative stress (TBARS, 2.5 times higher comparing to healthy controls) is consistent with decreased plasma concentrations of the antioxidant vitamin C by approximately 60% (Bertin-Maghit et al. 2000).

The role of malondialdehyde concentrations in critical illness is not well understood. However, increased free MDA (unbound to plasma proteins) has been previously been

described to be an important marker during lipid peroxidation (Lee & Csallany 1987; Carbonneau et al. 1991; Draper et al. 1993; Cighetti et al. 2005; Hong et al. 2000; Grotto et al. 2007) and therefore increased free MDA concentrations may reflect increased free radical damage. Furthermore, increased free MDA concentrations may also have the potential to directly damage cellular proteins and DNA (Fleming et al. 1982; Cross et al. 1987; Hruszkewycz 1988). Measurement of MDA concentrations in combination with micronutrient concentrations have been proposed to be one of the most reliable markers of oxidative stress in patients with critical illness (Grune & Berger 2007).

A proposed mechanism of how the production and circulation of free radicals can exacerbate the inflammatory response has recently been described. Free radicals may cause a cascade of intracellular events which appear to be involved in the liberation of nuclear transcription factor-kappa B (NF-kB) from its inhibitory protein IkB in the cytoplasm (Flohe et al. 1997; Bulger, Garcia & Maier 2002). This would allow its translocation into the nucleus and its binding with the DNA, enabling the initiation of the transcription process. NF-κB has been demonstrated to be a central transcription factor that controls the production of the acute phase mediators including cytokines and macrophage stimulating factors (Flohe et al. 1997; Bulger, Garcia & Maier 2002). These factors may in turn activate NF-kB in macrophages, amplifying the inflammatory cascade involved in the regulation of several inflammatory genes (Flohe et al. 1997; Bulger, Garcia & Maier 2002). Thus, activation of NF-κB may lead to the induction of genes integral to the systemic inflammatory response (Ziegler-Heitbrock et al. 1993; Blackwell & Christman 1997) leading to a viscous cycle of products that activate each other resulting in a difficult to control inflammatory status. It is therefore of interest that the activation of the NF-κB is blocked by several antioxidants including α-tocopherol (Suzuki & Packer 1993; Packer & Suzuki 1993; Schreck & Baeuerle 1994).

Taken together these results suggest that supplementing these patients with antioxidants might reverse the effect of oxidative stress, reduce the incidence of multiple organ failure and perhaps reduce mortality. Indeed, patients receiving antioxidants (α-tocopherol and ascorbate) were less likely to develop multiple organ failure and had a 1.2 day reduction in their ICU length of stay (Nathens et al. 2002).

However, micronutrient concentrations are recognized to decrease acutely as part of the systemic inflammatory response in apparently healthy subjects and patients undergoing elective surgery (Galloway et al. 2000; Gray et al. 2004; Gray et al. 2005) and therefore differentiation of the effect of reduced dietary intake, redistribution or increased consumption on antioxidant concentrations is problematical and raises a number of important issues:

How important is vitamin supplementation for patients with critical illness?

More specifically:

How do we assess and monitor vitamin status accurately and reliably so that we know when the critically ill patient is deficient and requires supplementation?

Are intracellular (red cell or white cell) concentrations of vitamins, compared with functional tests or plasma concentrations, firstly independent of the magnitude of injury, secondly more reliable indicators of vitamin status and thirdly closely associated with poor outcome in patients with critical illness?

1.5 The inflammatory response and outcome in critical illness

1.5.1 The prognostic role of C-reactive protein in critical illness

C-reactive protein concentrations have been found to be predictive of outcome in various chronic inflammatory diseases, such as advanced cancer (O'Gorman, McMillan & McArdle 2000; Mahmoud & Rivera 2002; Scott et al. 2002; Bromwich et al. 2004).

In critical illness, high C-reactive protein concentrations have been associated with organ dysfunction, longer ICU length of stay, and high mortality rates. Indeed, in patients with C-reactive protein concentrations >10 mg/dl, a decrease in C-reactive protein concentrations 48 hours after admission has been associated with a mortality rate of 15% while an increase has been associated with a mortality rate of 61% (Lobo et al. 2003). In addition, an increment in C-reactive protein concentrations by 10 mg/l has been associated with an increased odds ratio of post–ICU death (OR=1.09, 95% CI=1.03-1.16) independently of predictive scores such as APACHE II and SOFA scores (Ho et al. 2008). In contrast, a previous study failed to show such discrimination between hospital survivors and non-survivors, with suspected sepsis, after discharged from ICU (Pettila et al. 2002).

1.5.2 The prognostic role of albumin in critical illness

It is common knowledge that plasma albumin concentrations, in acute and chronic illness, are inversely related to risk of death. In a meta-analysis of 10 studies (Goldwasser & Feldman 1997) estimated that there is an increase in the odds of death ranges from 24-56% for each 2.5 g/l decrement in albumin concentrations. The association was persistent even when it was adjusted for other known risk factors, pre-existing illness, and exclusion of early mortality (Goldwasser & Feldman 1997). Another more recent meta-analysis showed similar results in 90 cohort studies, with each 10 g/l albumin decline to

significantly raise the mortality odds by 137% independent of both nutritional status and inflammation (Vincent et al. 2003).

In order to correct hypoalbuminaemia, replace volume and support colloid oncotic pressure (Soni 1995) the use of albumin has been advocated in the past. However, no beneficial effect on the administration of albumin has been found on mortality and there is a strong suggestion that it may increase the risk of death by 6% in patients with hypovolaemia, burns, or hypoproteinaemia compared with controls (Roberts 1998). In the SOAP study, albumin administration was associated with decreased survival in ICU with approximately 7% greater ICU and hospital mortality rates compared with controls (Vincent et al. 2005). In contrast, the SAFE study, which included more than six thousand ICU patients, showed that the outcome of resuscitation of whether albumin or saline is independent of the baseline albumin concentrations (Finfer et al. 2006). Finally, the meta-analysis by Vincent and coworkers (2003) suggested that in order for albumin replacement to become effective, albumin concentrations should exceed 30 g/l.

Possible reasons for this adverse effect of albumin replacement therapy may be the increased anticoagulant activity and the increased oedema that albumin administration may cause (Fleck et al. 1985; Soni 1995), which could both be detrimental for the compromised critically ill patient.

1.6 Assessment of vitamin status in hospitalised patients

Vitamin status can be defined as the extent to which an individual's physiologic need for vitamins is being met. It is in other words the state of vitamin sufficiency or deficiency of any person. Optimal vitamin status is a balance between vitamin intake and vitamin requirements and a balance in these two factors is crucial in determining an individual's state of health. Vitamin intake depends on actual food consumption which can be influenced by various factors such as economic status, eating behaviour, emotional status, cultural influences, disease, and the ability to absorb the vitamins consumed. Vitamin requirements are also influenced by many factors, including infection, disease, fever or trauma, growth, pregnancy, etc.

1.6.1 Malnutrition

Malnutrition is a major clinical problem in the UK and is often unrecognized and untreated in hospitals. Indeed, around 70% of the malnourished patients go unrecognised following their hospital admission (Kelly et al., 2000). Malnutrition predisposes to disease, delays recovery from illness, and adversely affects body function, well-being and clinical outcome. Approximately 40% of the patients admitted to Scottish hospitals are already undernourished while the percentage of patients at risk of being malnourished is even greater (McWhirter & Pennington, 1994). In 2000, one out of four patients admitted to four hospitals in England were malnourished and this adversely affected their length of stay, the numbers of new prescriptions needed, prevalence of infections and disease severity (Edington et al., 2000). It is even more worrying that nutritional status is known to deteriorate over the course of the hospital stay, with 75% of the malnourished patients on admission having lost even more weight upon discharge, reclassifying 37% of those initially assessed as moderately undernourished to severely undernourished (McWhirter & Pennington, 1994). It has been estimated that the length of hospital stay for patients at risk

of malnutrition is 50% longer and costs are 36% higher than for those of well nourished patients (Chima et al., 1997) and thus it is noteworthy that malnutrition costs the UK more than £ 7.3 billion of actual expenditure each year while obesity costs less than half of this amount (BAPEN 2005).

One of the reasons why malnutrition is still such a major clinical problem in hospitals is the lack of consistent and universally accepted criteria which can clearly distinguish between malnourished and well – nourished patients. Generally the diagnosis of malnutrition is based on objective and subjective measurements of nutritional status, including assessments of oral energy intake, weight loss, anthropometric measurements, determination of cell - mediated immunity, biochemical indices and body composition analysis (Pablo et al., 2003). As the criteria used to define undernutrition vary, studies that try to address the problem use different methods to assess nutritional status and thus comparison between the studies is almost impossible. Revision of guidelines to give specific directions on the anthropometric, clinical and biochemical components that should be included in the nutritional assessment (Corish & Kennedy, 2000) and also a practical definition of malnutrition (Edington et al., 2000) seem to be an on-going challenge. This is a necessity if we are to address properly the problem of malnutrition through scientific research (Corish & Kennedy, 2000) and/or education of the health care professionals in hospitals and in the community (Edington et al., 2000).

Weight may be not an accurate measurement of nutritional status in critical illness, as it can change due to oedema and fluid retention. Lately, it has become evident that during acute illness the amount of weight loss generally is not as important for outcome as the fat-free mass that has been lost. A low fat free mass was noted in 37% of patients hospitalized 1-2 days and this increased to 56% of patients hospitalized > 12 days (Pichard et al., 2004). Patients with lower fat – free mass index (fat free mass corrected for height) had twice longer lengths of hospital stay comparing to patients with normal or high fat –free mass

index (Pichard et al., 2004). Also, 1 in 4 of acutely ill and 1 in 3 of chronically ill patients admitted to the hospital have been shown to have fat free mass below the 10th percentile as assessed by BIA measurements and these did not correlate with their BMI measurements (approximately 16% of acutely ill and 19% of chronically ill with BMI ≤ 20 kg/m2) (Kyle et al., 2002).

1.6.2 Clinical assessment of vitamin deficiency

Clinical evidence of vitamin deficiency is rare in modern Western medical practice. In addition, clinical symptoms occur at the final stages of vitamin deficiency thus their value as markers of vitamin deficiency and predictors of outcome in patients with critical illness comes possibly too late for both the clinician and the patient.

In critical illness where requirements for anti-oxidant and other vitamins may be increased, subclinical deficiency of many vitamins could have a negative impact on survival. Identifying patients likely to have relative deficiencies of important vitamins may be helpful in guiding the need for replacement and in anticipating the response to supplementation. The first step in identifying such patients is awareness of possible underlying conditions, co-morbidities or pre-existing illness that may predispose to clinically relevant vitamin deficiency.

1.6.3 Assessment of comorbidity which might lead to vitamin deficiency

In Scotland there were 42,430 alcohol related discharges from general hospitals in 2007/2008 with 13, 495 discharges in Greater Glasgow and Clyde specifically (Alcohol Statistics Scotland 2009). Subclinical thiamine deficiency develops in alcoholics who tend to have low thiamine intake and impaired absorption of the vitamin (Gallacher 2004). In addition, thiamine is important for the metabolism of ethanol so it may be consumed more rapidly in alcoholics (Jolliffe, Colbert & Joffe 1936; Gallacher 2004). Wernicke-

Korsakoff syndrome is a type of encephalopathy, which usually occurs when deficiency persists, and its signs range from mild confusion to coma.

Other diseases or comorbidities that might lead to vitamin deficiency are:

1. Diseases/ Comorbidities that have a negative impact to appetite and food intake such as prolonged illness and hospitalisation with inadequate nutritional support, old age, some cancers that can obstruct the GI tract such as head and neck cancers, cancer treatment such as radiotherapy.

2. Diseases/ Comorbidities that have a negative impact to vitamin absorption, such as Crohn's disease, short gut syndrome, liver disease and pancreatic disease, or an increased impact in vitamin excretion such as renal disease.

3. Diseases that have an increased demand of specific vitamins and antioxidants such as burns, trauma, inflammatory and septic states.

4. A combination of the above may occur such as the common scenario in Glasgow Royal Infirmary of alcoholics with acute pancreatitis admitted to ICU 2-3 weeks into the illness having developed infected pancreatic necrosis.

1.6.4 Assessment of vitamin intake

Nutritional and more specifically vitamin requirements increase due to different reasons in specific illnesses, reasons which may vary between dysphagia to malabsorption. This would also indicate that consideration of the route of supplementation, i.e. via oral, enteral or parenteral routes, is an essential step in prevention or, at a further stage, correction of any vitamin deficiencies. The response of supplementation should then be assessed taking

into consideration the extent of pre-existing deficiency, the amount and frequency of the supplementation and the requirements of the underlying illness.

When there is inadequate intake, impaired absorption and/ or increased nutrient losses, this can lead to body stores (tissue) depletion which has as a consequence a series of states such as biologic dysfunction, physiologic dysfunction, cellular dysfunction, clinical signs and symptoms of deficiency, morbidity and finally mortality as the deficiency progresses. As a first approach of nutritional assessment a weight and dietary history could be obtained to assess adequacy of intake. The second level of vitamin assessment is biochemical measurements in order to identify store depletion when inadequate intake, compromised absorption and/or increased vitamin losses progress. At a further stage assessment of clinica signs and symptoms could identify cellular dysfunction and morbidity. Nevertheless, most of the above methods of assessing nutritional status are often of limited value in the critically ill patient, bringing challenge to the assessment of vitamin status (Manning & Shenkin 1995; Winkler & Malone 2004). The critically ill patients are a heterogenous group with different medical backgrounds, and are usually unable to provide a dietary history due to sedation/confusion, weight measurements may be erroneous after fluid resuscitation and/ or oedema, and anthropometric measurements are not easily attainable, may be erroneous due to oedema, have been shown to be less sensitive to acute changes of status (Harvey et al. 1981; Winkler & Malone 2004).

1.7 Vitamin concentrations and biochemical assessment during the systemic inflammatory response and critical illness

For most watersoluble vitamins, biochemical measurements were performed using functional tests. This is routinely assessed by measuring activity in red cell enzymes that require the vitamins as cofactors (Tomkins 2003). However, such functional tests are indirect, prone to error and maybe affected by factors other than vitamin status (Talwar et al. 2000). It is now generally accepted that direct measurements of vitamins, usually in plasma, are more reliable for assessing status (Talwar et al. 2000). In the patient with critical illness, this presents a problem since plasma or serum concentrations of many vitamins are substantially changed as part of the systemic inflammatory response. Indeed, it has been proposed that vitamin concentrations are only of value in these patients when used sequentially, and when interpretation is linked to changes in the magnitude of the acute–phase response, e.g. by measuring C-reactive protein concentrations (Shenkin 2000).

1.7.1 Vitamins B1, B2, B6

In the critically-ill patient, vitamin B deficiencies have been associated with the serious complications of Wernicke – Korsakoff syndrome (Denny–Brown 1958), compromised immune and antioxidant status (Grimble 1997) and poor survival (Cruickshank, Telfer & Shenkin 1988; Shenkin, Cruickshank & Shenkin 1989). For example, the role of B2 and B6 vitamins on immune function, by maintenance of glutathione status, has been well–described (Grimble 1997). Vitamin B2 has shown a suppressive effect on the production of tissue inflammatory mediators and also decreases plasma elevated nitric oxide levels (Kodama et al. 2005). Vitamin B6 status appears to positively relate to lymphocyte proliferation (Kwak et al. 2002; Cheng et al. 2006). Therefore, the accurate assessment of B-vitamin status in patients with critical illness is of considerable importance.

1.7.1.1 Functional tests

Functional tests have been used extensively in the past and continue to be used for the assessment of B-vitamin status for both healthy and critically ill patients. In healthy patients undergoing elective surgery, for example, erythrocyte riboflavin coefficient concentrations were not reported to change significantly (Louw et al. 1992). Also, in patients on long–term parenteral nutrition and evidence of inflammatory response functional tests for both vitamins B1 and B2 were normal (Labadarios et al. 1988). In contrast, low levels of vitamins B1 (Cruickshank, Telfer & Shenkin 1988) and B2 status (Shenkin, Chruickshank & Shenkin 1989), as assessed by erythrocyte trasketolase activity and erythrocyte glutathione reductase activation respectively, have both been shown to be associated with worse outcome in patients with critical illness. Indeed, of the patients who died, 29% had low vitamin B1 status compared with 11% in those who survived ($p<0.01$; Cruickshank, Telfer & Shenkin 1988).

The effect of supplementation appears to be reflected in enzymatic activity measurements. Bradley and coworkers (1978) reported that, in critically ill patients, red cell B1, B2 and B6 status, as measured by methods based on red cell enzyme saturation, was increased by the daily administration of a parenteral multivitamin supplement containing 50 mg vitamin B1–chloride hydrochloride, 10 mg vitamin B2–5–phosphate sodium salt and 15 mg vitamin B6 hydrochloride. Moreover, this increased and normalised vitamin status was associated with improved clinical outcome (Bradley et al. 1978).

1.7.1.2 Plasma measurements

In plasma, B–vitamin concentrations are lowered in the presence of a systemic inflammatory response in both healthy subjects and critically ill patients. For vitamin B6, for example, a significant decrease, approximately 50%, has been reported in subjects undergoing elective surgery (Louw et al. 1992; Gray et al. 2004), critically ill patients

(Huang et al. 2005) and in elderly healthy subjects (Friso et al. 2001). The inverse effect of inflammation on plasma vitamin concentrations agrees with the observations of Labadarios and co-workers (1988) who observed low plasma B6 concentrations in the great majority of their patients who had evidence of inflammation. In the same manner, in 46 critically ill patients, Huang and co-workers (2002) showed that vitamin B6 supplementation (15 mg) was unable to increase their plasma pyridoxal 5'-phosphate and pyridoxal concentrations. Indeed, plasma concentrations for both markers decreased by approximately 20%.

1.7.1.3 Red cell measurements

Hustad and coworkers (2002) have reported that plasma flavine adenine dinucleotide (FAD) is possibly an inappropriate indicator of vitamin B2 status and that the measurement of plasma riboflavin and erythrocyte flavine mononucleotide (FMN) could be more appropriate for population studies. There might be a problem with hydrolysis of FAD to FMN within the first minutes of sampling (Akimoto et al. 2006).

Also, Talwar and colleagues (2003b) reported that during the presence of a systemic inflammatory response, plasma pyridoxal 5'-phosphate (PLP) but not red cell PLP concentrations were decreased by approximately 50% in critically ill patients compared with healthy subjects. This would suggest that vitamin B6 status, as measured by PLP, may not reflect supplementation of vitamin B6 in critically ill patients. In agreement it has been reported that, on supplementation, PLP concentrations were increased in red cells but not in plasma in critically ill patients (Talwar et al. 2003b; Quasim et al. 2005). It is not clear whether red cell and functional tests of B vitamins correlate in critically ill patients as they do in healthy subjects (Talwar et al. 2000; Hustad et al. 2002).

1.7.1.4 White cell measurements

White cell B6 vitamin concentrations correlate well with either plasma or functional tests in healthy subjects (Kwak et al. 2002). However, from the literature the relationship between plasma, red cell and white cell B–vitamin concentrations and outcome in patients with critical illness has not been examined.

1.7.2 Vitamin C

The role of vitamin C in alleviating the oxidant stress in vitro is well documented (Cross et al. 1990; Dhariwal, Washko & Levine 1990; Downing et al. 1993). However, there is conflicting evidence on whether supplementation with vitamin C is of any benefit for patients with critical illness. Supplementation of ascorbic acid (500mg/d) and α–tocopherol (400 IU/d) was reported to reduce oxidative stress, as measured by plasma TBARS and plasma PGF2α isoprostanes, and to reduce 28-day mortality in critically ill patients approximately by 20% (45.7% in the antioxidant group and 67.5% in the control group, $p<0.05$; Crimi et al. 2004).

However, vitamin C supplementation (12.5 mg/kg body weight) immediately after injury was found to act as a pro-oxidant by increasing oxidative stress and tissue damage probably because of its role in mobilization of metals, such as iron, from their stores (Childs et al. 2001).

Therefore, it is important to accurately and reliably assess vitamin C status and the effect of supplementation especially in patients with critical illness, as oxidative stress may contribute to multiple organ dysfunction syndrome. Assessment of vitamin C status is usually carried out in plasma or white cells since red cell vitamin C is analytically difficult to measure in presence of oxygen and the potential of oxidation of vitamin C by the haemoglobin iron (Jacob, Scala & Omaye 1987; Fell & Talwar 1998).

1.7.2.1 Plasma measurements

Plasma vitamin C concentrations have been found to be negatively correlated with plasma C-reactive protein concentrations in patients with gastrointestinal adenocarcinoma, and in addition, weight-losing patients had lower concentrations of the vitamin compared with the weight-stable patients (Georgiannos, Weston & Goode 1993). In patients on prolonged parenteral nutrition, 25 out of 30 had lower than normal plasma vitamin C concentrations (Labadarios et al. 1988). In critically ill patients, the severity of acute phase response has also been reported to be in part responsible for the lowering of plasma vitamin C concentrations. For example, plasma vitamin C concentrations were 25% lower in critically ill patients compared with control subjects, and were related to increased length of ICU stay and the severity of acute phase response, as measured by C–reactive protein (Schorah et al. 1996).

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 (Rumelin et al. 2002). In the same manner, supplementation of intravenous 1,000 mg vitamin C for 3 days was unable to prevent the fall of plasma concentrations to undetectable levels by day 12 in alcoholic critically ill patients (Mishra et al. 2005).

In healthy subjects, parenteral supplementation with large amounts of ascorbic acid (500mg four times in 12 hours and then 500mg twice a day) post–operatively, is suggested to be able to sustain the preoperative plasma concentrations (Rumelin et al. 2005). In critically ill surgical patients, a combined parenteral supplement of a-tocopherol (1000 IU/d) and ascorbic acid (1000 mg/d) eight times per day was able to increase plasma concentrations of these vitamins and reduce the incidence of organ failure, shorten the duration of mechanical ventilation and ICU length of stay (Nathens et al. 2002).

1.7.2.2 White cell measurements

In white cells, vitamin C measurements are considered to be the best marker of status however it is analytically too difficult to be suitable for use routinely. White cell vitamin C has been found to decrease during the systemic inflammatory response by approximately 40% in patients undergoing orthopaedic surgery (Louw et al. 1992). To date the relationship between plasma and white cell vitamin C concentrations and outcome in patients with critical illness has not been examined.

1.7.3 Vitamin E

Patients admitted to the ICU may have reduced total antioxidant capacity, with low plasma concentrations of vitamin E (α-tocopherol) (Goode et al. 1995; Borrelli et al. 1996; Metnitz et al. 1999). Low plasma concentrations of α-tocopherol have been shown in patients with sepsis and secondary organ dysfunction (Goode et al. 1995). This depletion appears to correlate with the severity of illness and has a causal relationship between antioxidant depletion and increasing levels of organ dysfunction (Goode et al. 1995; Borrelli et al. 1996; Cowley et al. 1996; Mishra et al. 2005). In addition, these patients appear to have a substantial increase of nitrite excretion (Goode et al. 1995) as well as of products of lipid peroxidation all markers of increased free radical activity. For example, patients with trauma or sepsis appear to have increased lipid peroxidation and decreased circulating endogenous antioxidants including vitamin E (Goode et al. 1995; Metnitz et al. 1999).

Vitamin E (α-tocopherol) is one of the most important lipid soluble antioxidants with its biological function in biomembranes. It has been described as the major chain- breaking antioxidant, interrupting the chain of membrane lipid peroxidation. Vitamin E is also present in lipoproteins, protecting polyunsaturated fatty acids from peroxidation. In case of inflammation, vitamin E is thought to be necessary for the maintenance of an appropriate immune response to infection. Vitamin E supplementation has been shown to

reduce organ failure and infectious complications in patients with critical illness (Nathens et al. 2002).

The depletion of circulating antioxidants, such as vitamin E is likely to have a considerable impact on the antioxidant reserve in critical illness. However, the presence of antioxidant vitamins deficiency in plasma may not reflect cellular deficiency. Systemic inflammatory response syndrome is associated with a redistribution of vitamins and trace elements from the circulating compartment to tissues and organs, which are involved in protein synthesis and immune cell production (Galloway, McMillan & Sattar 2000).

1.7.3.1 Plasma measurements

Being one of the most important antioxidants, the role of vitamin E in critical illness has long been of interest. Goode and coworkers (1995) showed that plasma α-tocopherol (vitamin E) was well below the reference ranges for all critically ill patients. Borrelli and coworkers (1996) however, did not find a difference in plasma α-tocopherol concentrations between critically ill patients who developed or not multiple organ failure. However, both of these studies investigated only a small number of patients (n=16) and did not express their plasma vitamin E concentrations per mmol of cholesterol, that act as a carrier in the plasma and fall in the presence of the inflammatory response (Quasim et al. 2003; Gray et al. 2005). Therefore, the role of plasma concentrations of vitamin E in outcome in patients with critical illness is not clear.

1.7.3.2 Red cell measurements

Red cell α-tocopherol is thought to be a reliable marker of vitamin E status in biomembranes (Kitagawa, Nakagawa & Mino 1983). Although there is a positive correlation between plasma and red cell α-tocopherol concentrations in non-inflamed subjects (Kitagawa, Nakagawa & Mino 1983), this relation is not as high as would be

expected in vitro studies, which show α-tocopherol freely exchanged between plasma and blood cells (Silber, Winter & Kayden 1969). Moreover, there are strong correlations between red cell and liver α-tocopherol, whilst this correlation did not occur for plasma vitamin E concentrations (Mino, Kasugai & Shimizu 1985). In addition, studies by Simon and coworkers (1997; 1998) in hypercholesterolemic men showed that red blood cell vitamin E content was decreased although their α-tocopherol plasma concentrations, expressed per mmol of lipids, were unchanged compared to normocholesterolemic men.

Lehmann and coworkers (1988) in a study of healthy subjects reported that plasma α-tocopherol was weakly correlated with red cell concentrations. Moreover, red cell α-tocopherol concentration was more sensitive to supplementation.

There are studies that have used red cell α-tocopherol concentrations as a marker of vitamin E status instead of plasma; these measurements have been used to assess the potential changes within and between groups in cystic fibrosis (Winklhoferroob et al. 1992), in smoker and non smoker groups (Brown, Morrice & Duthie 1997), in type I diabetic children (Jain, McVie & Smith 2000) and in early stages of atherosclerosis (Bonithon-Kopp et al. 1997). Results from these studies showed significant correlations between red cell vitamin E status and markers of oxidative stress, or a dose response relationship in cases of supplementation.

A study by Simon and coworkers (2001) in subjects at cardiovascular risk found a negative correlation between of carotid intima-media thickness indicator, an indicator of early atherosclerosis, and red cell α-tocopherol concentrations while no such correlation was found in plasma, expressed or not per HDL concentrations, α-tocopherol concentrations.

1.7.4 Carotenoids

1.7.4.1 Plasma measurements

Recently, there is some evidence that carotenoids may be implicated in the oxidative stress observed during the acute phase response in critically ill patients (Quasim et al. 2003). Lycopene seems to be the carotenoid affected in a greater scale comparing to the others during this increased breakdown (McMillan et al. 2002) and it has also been shown that β-carotene, the most active of the carotenoids, is also inversely related to systemic markers of inflammation (Erlinger et al. 2001). On the other hand, when expressed per mmol of cholesterol (i.e. the concentrations of the carotenoids were expressed per mmol of cholesterol to correct for the effect of the systemic inflammatory response on lipids; Thurnham et al. 1986; Thurnham et al. 2008; Doise et al. 2008) , neither α–tocopherol, lutein, lycopene, α–carotene nor β–carotene plasma concentrations decrease following elective surgery, showing that the redistribution that occurs during the systemic inflammatory response may falsely be interpreted as overconsumption of these antioxidants (Gray et al. 2005). Therefore, the role of plasma concentrations of carotenoids in outcome in patients with critical illness is not clear.

Table 1-2 The effect of SIRS on vitamin B, C, A, E and carotenoids' concentrations in critically patients with or without vitamin supplementation.

Study	Type of study and number of subjects	Vitamins assessed	Method of measurement	Markers affected	Effect	Nutrition
Bradley et al. 1978	Surgical critically ill patients (N=26) Non–randomised controlled trial	Vitamin B1 Vitamin B2 Vitamin B6 Vitamin C	Red cell enzymatic saturation White cell vitamin C	Vitamin B1, vitamin B6 and vitamin low on baseline, normalized by the end of the treatment period.	12% decrease in enzymatic saturation for vitamin B1 17% decrease in enzymatic saturation for vitamin B6 29% increase in white cell vitamin C concentrations	IV supplementation of 50 mg vitamin B1, 10 mg vitamin B2, 15 mg of vitamin B6 and 500 mg of vitamin C
Cruickshank, Telfer & Shenkin 1988	Critically ill patients (N=158) Retrospective study	Vitamin B1	Erythrocyte transketolase activity	Lower levels in non – survivors compared with survivors Supplementation unable to correct abnormal levels	Non – survivors: median enzyme activation = 15% Survivors : median enzyme activation = 10% (Normal activity: <25%)	Full continuous IV with 1.2 mg thiamine per day
Shenkin, Cruickshank & Shenkin 1989	Critically ill patients (N=152) Retrospective study	Vitamin B1 Vitamin B2	Erythrocyte transketolase activation Erythrocyte glutathione reductase	Lower levels in non – survivors compared with survivors for both of the vitamins Levels for B2 were normal for both groups although significantly lower for non – survivors	Non – survivors: median enzyme activation B1 = 15%, B2 = 29% Survivors : median enzyme activation B1=10%, B2=12%	IV supplementation with 1.8 mg riboflavin per day
Huang et al. 2002	Critically ill patients (N=46) Prospective, observational study	Vitamin B6	Plasma PLP and PL EALT–AC EAST–AC	Low concentrations even with supplementation No change in functional tests	Plasma PLP and PL decreased by 19%	EN, TPN or IV supplementation of 15 mg vitamin B6

Table 1-2 The effect of SIRS on vitamin B, C, A, E and carotenoids' concentrations in critically patients with or without vitamin supplementation (continued).

Study	Type of study and number of subjects	Vitamins assessed	Method of measurement	Markers affected	Effect	Nutrition
Talwar et al. 2003b	Critically ill patients (N=18) Prospective, observational study	Vitamin B6	Plasma PLP Red cell PLP	Decrease in plasma but not in red cells	49% lower plasma PLP concentrations compared with controls	
Huang et al. 2005	Critically ill patients (N=40) Cross–sectional observational study	Vitamin B6	Plasma PLP and PL EALT–AC EAST–AC	Marginal PLP deficiency compared with controls No change in functional tests	Decreased immune response markers	
Quasim et al. 2005	Surgical critically ill patients (N=41) Cross–sectional observational study	Vitamin B2 Vitamin B6	Plasma and red cell FAD Plasma and red cell PLP	Plasma concentrations not increased with supplementation Red cell concentrations increased with supplementation	Non – supplemented patients: 60% lower plasma FAD and 70% lower plasma PLP concentrations compared with healthy controls. Red cell PLP concentrations were almost two – fold higher compared with the non–supplemented group.	4 mg riboflavin and 150 mg pyridoxine
Cheng et al. 2006	Critically ill patients (N=51) Single–blind intervention study	Vitamin B6	Plasma PLP and PL EALT–AC EAST–AC	Increase of plasma PLP and PL concentrations with supplementation	50 mg pyridoxine supplementation: Plasma PLP increased by 250% and PL by 5000% 100 mg supplementation: Plasma PLP increased by 170% and PL by 2500%	IV 50 mg or 100 mg vitamin B6 for 14 days
Schorah et al. 1996	Critically ill patients (N=62) Prospective, observational study	Vitamin C	Plasma ascorbic acid	Plasma concentrations significantly lower compared with healthy controls.	More than 25% of the critically ill patients had plasma ascorbic acid concentrations 82% lower compared with healthy controls.	

Table 1-2 The effect of SIRS on vitamin B, C, A, E and carotenoids' concentrations in critically patients with or without vitamin supplementation (continued).

Study	Type of study and number of subjects	Vitamins assessed	Method of measurement	Markers affected	Effect	Nutrition
Tsai et al. 2000	Patients with evidence of SIRS (N=135) Prospective, observational study	Vitamin E Vitamin C Oxidative stress	Plasma α–tocopherol Plasma ascorbate TRAP	Plasma vitamin C and TRAP concentrations significantly lower by day 14 in non – survivors compared with survivors. TRAP showed to be related to MODS and CRP.	Plasma vitamin C was 64% less in non–survivors compared to survivors. Plasma vitamin E was 18% more in non–survivors compared with survivors. TRAP concentrations were significantly correlated to MODS ($r=0.431$, $p<0.001$) and CRP ($r=0.288$, $p=0.03$).	IV supplementation with 10 mg α–tocopherol and 100 mg of vitamin C.
Nathens et al. 2002	Trauma/surgical critically ill patients (N=595) Randomised, prospective trial	Vitamin E Vitamin C	Plasma α–tocopherol Plasma ascorbate	There was no change in controls while decreased plasma concentrations normalized in the supplemented group. Reduced incidence of organ failure, duration of mechanical ventilation and ICU LOS for the supplemented group.	Organ failure incidence decreased by 57% Duration of mechanical ventilation decreased by 0.7 days ICU LOS decreased by 1 day Pulmonary mortality decreased by 19%	Oral and IV supplement of 1000 IU α–tocopherol and 1000 mg ascorbic acid respectively per day, on admission and for 28 days
Alonso de Vega et al. 2002	Critically ill patients (N=73) Prospective, cohort study	Plasma redox status	Plasma antioxidant capacity/ lipoperoxides	Plasma redox status on admission significantly correlated with severity of illness and a relationship with survival was shown.	Negative correlation of plasma antioxidant capacity ($r^2=0.53$) and positive correlation of lipoperoxides ($r^2=0.71$) with APACHE III. In comparison with survivors (100%), non–survivors had increased plasma lipoperoxides (147%) and decreased total antioxidant capacity (84%).	Measurements on admission before feeding administration

Table 1-2 The effect of SIRS on vitamin B, C, A, E and carotenoids' concentrations in critically patients with or without vitamin supplementation (continued).

Study	Type of study and number of subjects	Vitamins assessed	Method of measurement	Markers affected	Effect	Nutrition
Crimi et al. 2004	Critically ill patients (N=216) Randomised, double–blind, placebo–controlled supplementation trial	Vitamin E Vitamin C Oxidative stress	Plasma and LDL α–tocopherol Plasma ascorbate TBARS Isoprostanes	Reduced oxidative stress, days on ventilator and 28–day mortality	Plasma and LDL α–tocopherol increased by 40% TBARS reduced by 55% Isoprostanes reduced by 37% Duration of mechanical ventilation decreased by 4.5 days 28-day mortality decreased by 22%	Enteral supplement of 400 IU α–tocopherol and 500 mg ascorbic acid per day for 10 days
Mishra et al. 2005	Critically ill patients (N=60) Prospective, observational study	Vitamin C	Plasma ascorbic acid	Plasma concentrations decreased despite supplementation	Plasma ascorbic acid concentrations fell to undetectable levels by 12.	IV supplementation of 1000 mg vitamin C per day for 3 days
Doise et al. 2008	Critically ill patients (N=56) Prospective, observational study	Total antioxidant capacity (TAC) Vitamin A Vitamin E Vitamin C	Plasma TAC Plasma retinol Plasma α-tocopherol Plasma ascorbic acid	Vitamins A, E and C were decreased on baseline in the critically ill patients compared to controls. TAC values statistically declined over 10d of ICU stay. Vitamins A and E increased over time and vitamin C concentrations decreased over time.	Vitamins A, E and C were decreased on baseline in the critically ill patients compared to controls by 67%, 46% and 71% respectively.	
Goode et al. 1995	Critically ill patients with septic shock (N=16) Prospective, observational study	Vitamin E Carotenoids	Plasma α-tocopherol Lycopene β-carotene	Plasma antioxidant concentration low compared with healthy subjects	Plasma antioxidant concentrations below the reference range.	

Table 1-2 The effect of SIRS on vitamin B, C, A, E and carotenoids' concentrations in critically patients with or without vitamin supplementation (continued).

Study	Type of study and number of subjects	Vitamins assessed	Method of measurement	Markers affected	Effect	Nutrition
Borrelli et al. 1996	Surgical critically ill patients (N=16) Prospective, observational study	Vitamin C Vitamin E	Plasma ascorbic acid Plasma α-tocopherol	Low vitamin C concentrations in patients going into multiple organ failure compared with those that were not.	Approximately 2-3 times lower plasma vitamin C concentrations in patients with multiple organ failure compared with those of the non multiple organ failure group. No difference was observed for plasma vitamin E concentrations.	
Quasim et al. 2003	Surgical critically ill patients (N=43) Prospective, cross-sectional study	Vitamin E Carotenoids Oxidative stress	Plasma α-tocopherol Lutein Lycopene α-carotene β-carotene Malonyldialdehyde (MDA)	Compared with healthy subjects, plasma antioxidant concentrations were significantly lower in critically ill patients. Compared with healthy subjects, plasma MDA concentrations were significantly higher in critically ill patients.	Lutein and lycopene concentrations decreased by 65% and 80% respectively in critically ill patients when compared with controls. α-carotene and β-carotene were also significantly decreased. After expressing per mmol of cholesterol, only α – tocopherol concentrations were higher in critically ill patients when compared with controls or longitudinally. Only lycopene concentrations remained in low levels when expressed per mmol of cholesterol.	

1.8 Aims of the thesis

From the introduction there is evidence of the transient changes of plasma vitamin concentrations during the systemic inflammatory response. Therefore the aims of the thesis were;

A) To examine whether, compared with plasma, intracellular vitamin concentrations are a more reliable measure of status in patients with critical illness. And,

B) To examine the relationship between plasma and intracellular vitamin concentrations and hospital mortality in patients with critical illness.

In order to address these aims a prospective longitudinal study of the systemic inflammatory response, plasma and intracellular vitamin concentrations and outcome in patients with critical illness was undertaken.

2 Materials and Methods

2.1.1 Controls, patients and study design

Blood samples for population references values were obtained from laboratory staff, from local health centres and from people attending a cardiovascular risk clinic. None of the subjects were taking any vitamin supplements or had any significant medical history or evidence of a systemic inflammatory response (serum C-reactive protein <10 mg/ l). No formal diet histories were taken (Talwar et al. 2003a; Quasim et al. 2005).

Patients in the Intensive Care Unit (ICU) of the Royal Infirmary, Glasgow, who had respiratory failure requiring ventilatory support, were ≥ 18 years old, and who had evidence of the systemic inflammatory response syndrome as per Bone's criteria (1992), were studied. Venous blood samples (EDTA) were withdrawn on admission (day 1) and on follow–up (days 2-7) for the analysis of plasma, red and white cell vitamin analysis. APACHE II score (Knaus et al. 1985b) and associated predicted mortality, SOFA score individual value components (Vincent et al. 1996) and vitamin supplementation were recorded from the database system of the ICU (CareVue and WardWatcher softwares). Hospital mortality data were recorded from the ICU database system (WardWatcher software) or, when data were not available on Carevue, from the discharges of the main hospital database system.

Enteral feeding was usually instituted on the second day in ICU and provided RDA levels of vitamin B1, B2, B6, A, E, C and carotenoids in 1500kcal. More specifically, the dietary (energy, macronutrient and fluid) requierements for each patient were calculated by the specialist dietitian and continuous feed of 1 kcal per mL was initiated slowly (30 mL/hr) for the first 4 hours to increase to maximum of 50-60 mL/hr by the first 24 hours when there are

aspirates <150mL. Target is the patient to receive approximately 50-75 mL/hr by day 3, always taking into consideration patient tolerance to the feed. In the Intensive Care Unit of Glasgow Royal Infirmary, the following enteral formulas were used: Osmolite® (standard feed without fibre, post gastric surgery patients and patients with gut issues; Abbott Laboratories), Jevity® (standard feed with fibre, burns and medical patients; Abbott Laboratories) and Peptisorb® (elemental feed, patients with pancreatitis; Nutricia) via usually a nasogastric tube unless there were aspirates of more than 150 mLs after 48 hours of feed initiation. In such a case the tube would be replaced with a nasojejunal tube to avoid feed aspiration and associated pneumonia.

Patients received vitamin supplementation in ICU if they were considered clinically to be malnourished, had a history of excessive alcohol intake or were considered to have a general requirement for additional vitamin intake. Vitamin supplementation was recorded from the drug cardex and given parenterally as Pabrinex® (Link Pharmaceuticals Ltd, West Sussex, UK) one dose of which contains 500mg ascorbic acid, 160mg nicotinamide, 50mg pyridoxine hydrochloride, 4mg riboflavin, 250mg thiamine hydrochloride. In those patients who received vitamin B supplementation a single dose of Pabrinex was given on the morning of day 2 and daily during their ICU stay. Some patients received additional doses such that the median number of doses received was 3 per day in those who had follow-up measurements.

With respect to the critically ill patients, the study was approved by the ethics committees of the North Glasgow NHS Trust and Multicentre Research Ethics Committee (MREC) Scotland. When patients were unable to give signed informed consent, consent was obtained from the patients' next of kin or welfare guardian in accordance with the requirements of the Adults with Incapacity Scotland (2000) Act.

2.1.2 Analytical methods

2.1.2.1 White cell preparation

One mL of EDTA whole blood was transferred into a 15ml (16 x 100mm) conical tube and the red blood cells were lysed, within 4-6 hours of blood sampling, with 9mL of cold ammonium chloride (83g/L) containing EDTA solution (372 mg/L). EDTA has been shown to prevent platelet binding to leukocytes (Milne, Ralston & Wallwork 1985). In order to minimise damage to white cells, resulting in low leucocytes yields, the pH of the lysing solution was adjusted to 7.4 (Hinks, Colmsee & Delves 1982). The samples were gently mixed for 2 minutes and kept at -15 ^{o}C for 10 minutes, to avoid the creation of clumps and simplify the elimination of haemoglobin (Hinks, Colmsee, Delves 1982).

The samples were then centrifuged (200g, 4 ^{o}C, 10mins) and the red supernatant containing haemoglobin were discarded. The remaining pellet containing enriched white cells was washed with 10mL of cold Dulbecco's Phosphate Buffered Saline (PBS, Sigma Chemical). This process was performed twice. The samples were centrifuged again but at 100g in order to keep the lighter platelets in the supernatant so they could be removed. The supernatant was removed and the resulting pellet was dispersed in 1mL of PBS and the number of cells/L counted using a hemocytometer (KX–21N, Sysmex, UK Ltd). In addition, white blood cells and lymphocyte numbers were measured in whole blood by the same counter. The method provided a white cell extraction yield of 63% (n=76, 50-93%) containing mainly neutrophils (>90%; Appendix 5).

Following a final brief centrifugation (5000g, 3mins), the supernatant was removed and the resulting pellet was dispersed in 250µL of deionised water. The cell suspension was stored at -

80°C for vitamin analysis. Before analysis, the cell suspension was sonicated for 10 min to ensure complete lysis of the cells.

2.1.2.2 Laboratory measurement of whole blood and plasma proteins

Total protein, albumin, C-reactive protein, alkaline phosphatase, cholesterol and triglycerides were measured, in accordance with the manufacturers' instructions, by routine laboratory procedures using an automated analyser (Architect, Abbott Diagnostics, USA). The inter-assay coefficient of variation was less than 5% over the sample concentration range for total protein, alkaline phosphatase, cholesterol and triglycerides. Globulin concentrations were calculated from the difference between total protein and albumin concentrations.

Albumin was measured by a BCP dye-binding method and C-reactive protein was measured using an automated analyser (Architect, Abbott Diagnostics, USA). For C-reactive protein the limit of detection was 5 mg/l. The inter-assay coefficient of variation was less than 3% and 5% over the sample concentration range for albumin and C-reactive protein respectively. The limit of detection for albumin was 10 g/L.

Haemoglobin estimation was performed using Drabkins Reagent (Sigma Diagnostics, UK). Haemoglobin is oxidised and converted to stable cyanmethaemoglobin and the absorbance measured at the main wavelength of 546nm using automatic analyser (Sapphire 350, Audit Diagnostics, Ireland). The within batch imprecision (CV%) was 0.95% at 6.9g/dL. The between imprecision (CV%) was 4.7% at 7.1 g/dL.

2.1.2.3 Laboratory measurement of TDP

Since thiamine diphosphate (TDP) is present almost entirely within red cells, vitamin B1 status was assessed by measuring TDP in whole blood by HPLC using post-column

ferricyanide derivatisation and fluorimetric detection as previously described (Talwar et al. 2000). The within batch imprecision for red cell TDP was 5.1% at 380ng/gHb. Plasma TDP concentrations are extremely low accounting for less than 5% of the plasma TDP concentration and were below the detection limits of the assay.

TDP concentrations in red cells were expressed per gram of haemoglobin (Hb) rather than per volume of packed red cells because of the difficulty in accurately pipetting packed red cells, due to high viscosity. The reference intervals for the above assays as established in our laboratory were as follows: whole blood TDP 275–675 ng/ g Hb.

2.1.2.4 Laboratory measurement of FAD, FMN and riboflavin

FAD, FMN and riboflavin were measured in plasma, red and white cells by HPLC with reverse phase C18 column and detected by fluorescence based on the method of Speek and coworkers (Speek et al. 1982). Plasma or diluted red cell haemolysates are precipitated with methanol, centrifuged and the supernatant injected for HPLC analysis. FAD, FMN and riboflavin were separated on an isocratic HPLC system. The within batch imprecision for plasma FAD was 5.9% at 33 nmol/l and 4.4% at 79 nmol/l, for red cell FAD was 4.8% at 2.8 nmol/gHb and for white cell FAD was 3.4% at 55 pmol/ 10^6 cells. The within batch imprecision for plasma FMN and riboflavin was 7.1% and 5.6% respectively. The corresponding values for red cell FMN and riboflavin were 5.3% and 8.9% respectively. Within batch imprecision for white cell riboflavin was 4.2% at 2.95 pmol/ 10^6 cells.

FAD concentrations in red cells were expressed per gram of haemoglobin (Hb) rather than per volume of packed red cells because accurate pipetting of packed red cells is difficult, due to high viscosity. This was the haemoglobin measured in the blood sample which reflects the haemoglobin of the patient also. The critically ill patient haemoglobin cannot be used as a

nutritional marker as it changes constantly, e.g. due to resuscitation, reflecting fluid balance rather than nutritional status. White blood cell vitamin B2 concentrations were not measured in the controls as this was not part of the original protocol for establishing reference intervals in a healthy population and therefore could not be compared with values in the critically ill group.

2.1.2.5 Laboratory measurement of PLP and PL

PLP and PL concentrations were measured in plasma, red cells and white cells by high performance liquid chromatography (HPLC) using pre-column semi-carbazide derivatisation and fluorescent detection (Talwar et al. 2003a; 2003b). Plasma (500uL) diluted red cell haemolysates (300uL of red cell in 700uL of water) or white cell preparation (250uL) were derivatized with semicarbazide. The mixtures were then deproteinized with perchloric acid, stabilized with sodium hydroxide and injected on the HPLC column via an autosampler (Waters, Watford, UK) (Talwar et al. 2003a).

The within batch imprecision for plasma PLP was 4.9% at 59nmol/l and 6.3% at 16nmol/l. The within batch imprecision for red cell PLP 5.2% at 367pmol/ gHb (Talwar et al. 2003a) and for white cell PLP was 3.4% at 1.64 nmol/ 10^9cells (Vasilaki A, McMillan DC, Kinsella J, Duncan A, O'Reilly DS, Talwar D, unpublished data). The within batch imprecision for plasma PL was 4.6% at 36 nmol/l and 3.0% at 144 nmol/l, for red cell PL 4.6% at 36 pmol/gHb and for white cell PL 2.8% at 0.92 nmol/ 10^9cells. White blood cell vitamin B6 concentrations were not measured in the controls as this was not part of the original protocol for establishing reference intervals in a healthy population and therefore could not be compared with values in the critically ill group.

PLP concentrations in red cells were expressed per gram of haemoglobin (Hb) rather than per volume of packed red cells because accurate pipetting of packed red cells is difficult, due to high viscosity. This was the haemoglobin measured in the blood sample which reflects the haemoglobin of the patient also. The critically ill patient haemoglobin cannot be used as a nutritional marker as it changes constantly, e.g. due to resuscitation, reflecting fluid balance rather than nutritional status. The 95% reference intervals for the above assays established in our laboratory were as follows: plasma PLP 17–135 nmol/ l, plasma PL 5-26 nmol/ l, red cell PLP 250–680 pmol/ gHb, and red cell PL 25-195 pmol/ gHb.

2.1.2.6 Laboratory measurement of plasma vitamin C

Samples were analyzed for vitamin C in the same assay to minimize the effect of interassay variation.

Plasma and white cell samples were stabilised to prevent oxidation and deproteinised with metaphosphoric acid. Following centrifugation and separation, an aliquot of the supernatant was injected on to a C18 reverse phase chromatographic column and the ascorbic acid concentration assayed using an electrochemical detector.

Ascorbic acid concentrations in white cells were expressed per number of white cells. The reference interval as established in our laboratory for plasma ascorbic acid was15-90 umol/L. The reference interval for white cell ascorbic acid was taken as 1.5-2.5 umol/10^9 cells as reported by Lee and coworkers (1982). The within batch imprecision for plasma ascorbic acid was 1.0% at 31.66 μmol/l and between batch was 3.7% at 38.16 μmol/l. White blood cell vitamin C concentrations were not measured in the controls as this was not part of the original protocol for establishing reference intervals in a healthy population and therefore could not be compared with values in the critically ill group.

2.1.2.7 Measurement of plasma and red cell vitamin E

Laboratory measurement of plasma and red cell α-tocopherol concentrations were determined by a high-performance liquid chromatography (HPLC) method (Talwar et al. 1998). Plasma and red cell α-tocopherol measurements in samples from the same patient were carried out in the same batch to minimise intra-patient measurement error. Plasma was deproteinised with alcohol containing tocopherol acetate as an internal standard and extraction was performed using hexane. HPLC analysis was carried out using a reverse-phase analytical column (5 μm C18; 3.2 x 250mm, Nucleosil, Phenomenex, Macclesfield, UK) with UV monitoring at 295nm. The limit of sensitivity for plasma α-tocopherol was 3 umol/ l. The intra-assay coefficient of variation was less than 9% over the sample concentration range.

Analysis of red cell α-tocopherol was performed using a modified procedure of Talwar et al. (1998). Eight hundred μl of the ascorbic acid stabilised red cell sample was thawed and after vortex mixing, 400μl was used for α-tocopherol measurement with the remainder retained for haemoglobin (Hb) estimation. Since in red cells vitamin E is bound to cell membranes, it was important to ensure that the ascorbic acid red cell sample was vortex mixed thoroughly before taking it through the extraction procedure. For extraction of α-tocopherol from red cell membranes, 400μl of the vortex mixed red cell sample was diluted with an equal volume of ascorbic acid (1%) and 100μl of internal standard (α-tocopherol nicotinate 50umol/l) added. This mixture was deproteinised with 1.5ml of ethanol and the α-tocopherol was extracted twice with 3ml of hexane. The hexane layer was removed and evaporated to dryness under a stream of air at 40ºC. The residue was reconstituted with 100μl ethanol and 50μl injected onto the column via an autosampler. The chromatographic conditions were as described above for plasma α-tocopherol (Talwar et al. 1998). Red cell concentration of α-tocopherol was based

on a standard curve prepared by extracting ethanolic α-tocopherol standards as described above for red cells.

The concentration of α-tocopherol in red cells was also expressed as a ratio to haemoglobin concentrations (to improve the precision of the assay since the accurate pipetting of packed red cells is difficult, due to high viscosity, Talwar et al. 2000; 2003a). This was the haemoglobin measured in the blood sample which reflects the haemoglobin of the patient also. The critically ill patient haemoglobin cannot be used as a nutritional marker as it changes constantly, e.g. due to resuscitation, reflecting fluid balance rather than nutritional status. The intra-assay coefficient of variation was <7% over the sample concentration range.

2.1.2.8 Measurement of lipid soluble antioxidants

Laboratory measurements of plasma retinol, lutein, lycopene, α-carotene and β-carotene concentrations were determined by a high-performance liquid chromatography (HPLC) method (Talwar et al. 1998). Plasma was deproteinised with alcohol containing internal standards and extraction of the analytes of interest was performed using hexane. Analysis was carried out using reversed-phase HPLC (5 mm microbore, Phenomenex, Macclesfield, UK) and dual wavelength monitoring (Waters, MA, USA). The limit of sensitivity for retinol, lutein, lycopene, α-carotene and β-carotene was 5 umol/ l. The intra-assay coefficient of variation was less than 9% for all analytes over the sample concentration range.

2.1.2.9 Measurement of Malondialdehyde (MDA)

The majority of MDA exists in plasma in bound form (approximately 85%, Pilz, Meineke & Gleiter 2000), to a number of different macromolecules, but primarily to plasma proteins (Esterbauer, Schaur & Zollner 1991). Critically ill patients often have low circulating plasma

proteins so both total MDA and free (unbound) MDA and the free fraction in EDTA-treated plasma were studied.

Total malondialdehyde was measured using reversed-phase HPLC with fluorometric detection as described by Young and Trimble (1991). Fifty micro litres of EDTA plasma sample or standard (tetramethoxypropane) was hydrolysed with orthophosphoric acid (100°C). The MDA released from plasma proteins and the unbound MDA reacted with thiobarbituric acid (TBA) to form the MDA-TBA adduct and this was measured using reverse-phase HPLC using fluorimetric detection to increase sensitivity and specificity (Young & Trimble 1991). The intra-assay coefficient of variation was 9% over the sample concentration range.

For the measurement of free MDA, 30µl of 50% trichloroacetic acid (TCA) was added to 200ul of plasma sample to precipitate the proteins. After centrifugation 50ul of supernatant was then hydrolysed as described above for total MDA. The remaining steps in the procedure were carried out identically to those described in total MDA analysis, to achieve consistency and minimise possible sources of confusion and error.

Total MDA was also expressed per gram of total protein as total protein concentrations fall during the systemic inflammatory response. The free MDA fraction was defined as the ratio of free MDA to total MDA concentration.

3 The relationship between riboflavin, flavin mononucleotide and flavin adenine dinucleotide concentrations in plasma and red cells in patients with critical illness

3.1 Introduction

It has long been recognised that physiological active coenzyme form of vitamin B2, FAD, is required for normal immune function, by maintenance of glutathione status (Grimble 1997). Vitamin B2 has shown a suppressive effect on the production of tissue inflammatory mediators and also decreases plasma elevated nitric oxide levels (Kodama et al. 2005). Shenkin and coworkers (1989) reported that in patients with critical illness suboptimal concentrations of vitamin B2 were linked to adverse mortality outcomes. Riboflavin is an essential constituent of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which carry out redox reactions in a variety of metabolic pathways (McCormick et al. 1988). Therefore, the accurate assessment of B-vitamin status in patients with critical illness is of considerable importance to identify critically-ill patients with 'true' deficiency of vitamin B2 who may benefit from supplementation and also to avoid inappropriate supplementation of vitamins which brings the risk of tissue accumulation and toxicity.

In contrast to functional tests, direct measurements of FAD concentrations in blood are thought to more reliably reflect nutritional status of vitamin B2 (Bates 1997; Tietz 2006). Recently, Hustad and coworkers (2002) proposed that direct measurements of plasma riboflavin followed by red cell FMN and FAD were the most sensitive markers of low dose riboflavin supplementation in apparently healthy subjects. However, it is recognised that the

physiologically active form of vitamin B2 in plasma and tissue is FAD (Massey 2000; Tietz 2006). Furthermore, Stripp (1965) reported that following oral supplementation of 500 mg FMN, in apparently healthy subjects, there was a 1.7-fold increase in red cell FAD concentrations.

Recent work has shown that, as part of the systemic inflammatory response, plasma FAD concentrations, in contrast to red cells, are reduced such that the relationship between plasma and red cell FAD concentrations is disturbed (Gray et al. 2004; Quasim et al. 2005). Louw and coworkers (1992) reported in patients undergoing elective surgery that vitamin B2 concentrations in the plasma were affected by albumin redistribution due to the systemic inflammatory response. It is recognised that, in plasma, riboflavin is extensively bound to proteins mainly albumin and globulins, primarily immunoglobulins (Innis, McCormick & Merrill 1986). More recently, in a small study of patients with critical illness, low dose supplementation with riboflavin was associated with an increase in concentrations of FAD in the red cell, but not in the plasma (Quasim et al. 2005). However, the basis of this observation is as yet unclear.

Therefore, the aim of the present study was to examine the cross sectional and longitudinal inter-relationships between riboflavin, FMN and FAD concentrations in plasma and red cells in patients with critical illness. This information is required to assess reliability of these measurements as indicators of vitamin B2 status in the critically ill patient.

3.2 Materials and Methods

3.2.1 Controls, patients and study design

See paragraph 2.1.1

3.2.2 Analytical methods

3.2.2.1 Collection and preparation of blood samples

The EDTA samples were centrifuged (500g, 4^{o}C, 10mins) and plasma was removed into another plastic tube for measurements of plasma FAD, FMN and riboflavin. After removing the buffy coat, the remaining packed red cells were kept for red blood cell vitamin B2 determination. All tubes were stored at -70^{o} C until analysis. All samples were protected from light and assayed in a single batch for each of the analytes to minimise interbatch analytical variation.

3.2.2.2 Laboratory measurements of FAD, FMN and riboflavin

See paragraph 2.1.2.4

3.2.2.3 Laboratory measurements of whole blood and plasma proteins

See paragraph 2.1.2.2

3.2.3 Statistics

Data from normal subjects and critically ill patients groups are presented as median and range. Comparison between the control and critically groups were carried out with the use of the Mann-Whitney U test. Correlations between variables in the control and critically-ill groups

were carried out using the Spearman rank correlation (r_s). Data from different time points in the patient groups were tested for statistical significance with the use of the Wilcoxon signed rank test. Because of the number of statistical comparisons, a p-value of <0.01 was considered to be significant. Analysis was performed with the use of SPSS software (version 15; SPSS Inc., Chicago, IL).

3.3 Results

The baseline characteristics of controls (n=119) and critically ill patients (n=125) studied are shown in Table 3-1. The majority of patients were men, >50 years, and similar to the control group. The patients' median APACHE II score was 21 and the associated median predicted mortality was 34%. The majority of patients had low concentrations of albumin and high concentrations of C-reactive protein.

The 95% reference intervals in the normal subjects for plasma and red cell FAD were 57-149 nmol/ l and 0.75-3.35 pmol/gHb respectively. Plasma FAD was significantly lower in the critically ill patients compared with the controls ($p<0.001$) with 63% of patients having concentrations below the reference interval (69 out of 125). Median red cell FAD concentrations were significantly lower in the critically ill patients compared with the controls group ($p<0.001$) however, all patients had red cell FAD concentrations within the reference interval. Compared with the control group, median plasma FMN concentrations were higher ($p<0.001$) and red cell FMN concentrations were similar in the critically ill group. Compared with the control group, median plasma riboflavin concentrations were higher ($p<0.001$) and red cell riboflavin concentrations were similar in the critically ill group. Compared with the control group, median plasma and red cell FAD to riboflavin ratio were significantly lower in the critically ill group (both $p<0.001$). In the control and critically ill patients' group plasma FAD and FMN were not significantly correlated with red cell FAD, red cell FMN or red cell riboflavin. In contrast, plasma riboflavin was correlated with red cell FAD ($r_s=0.52$, $p<0.001$), red cell FMN ($r_s=0.55$, $p<0.001$) and red cell riboflavin ($r_s=0.60$, $p<0.001$) in the critically ill patients' group but not in the control group.

The interrelationships between the concentrations of FAD, FMN and riboflavin in the plasma and red cells in the control population are shown in Table 3-2. Plasma FAD was directly associated with plasma FMN (r_s=0.51, p<0.001) and plasma riboflavin (r_s=0.49, p<0.001). Plasma FMN was directly associated with plasma riboflavin (r_s=0.55, p<0.001). Red cell FAD was directly associated with red cell FMN (r_s=0.44, p<0.001) but not with red cell riboflavin. Red cell FMN was directly associated with red cell riboflavin (r_s=0.52, p<0.001).

The interrelationships between the concentrations of FAD, FMN and riboflavin in the plasma and red cells in the critically ill patients on admission to ICU are shown in Table 3-3. Plasma FAD was inversely associated with C-reactive protein (r_s=-0.24, p<0.01) and directly associated with albumin (r_s=0.31, p<0.001) and alkaline phosphatase (r_s=0.35, p<0.001). Globulins were directly associated with albumin (r_s=0.24, p<0.001) and alkaline phosphatase (r_s=0.37, p<0.001). Plasma FMN was directly associated with plasma riboflavin (r_s=0.52, p<0.001). Plasma riboflavin was correlated with red cell FAD (r_s=0.52, p<0.001), red cell FMN (r_s=0.55, p<0.001) and red cell riboflavin (r_s=0.60, p<0.001). Alkaline phosphatase was directly associated with red cell FAD (r_s=0.31, p<0.001) and red cell riboflavin (r_s=0.32, p<0.001). Red cell FAD was directly associated with red cell FMN (r_s=0.83, p<0.001) and red cell riboflavin (r_s=0.72, p<0.001). Red cell FMN was directly associated with red cell riboflavin (r_s=0.79, p<0.001).

Of the 123 patients who were admitted in the ICU, 60 had longitudinal measurements of both plasma and red cell FAD concentrations (Table 3-4). In those 60 critically-ill patients with follow-up samples, 18 patients had recorded supplementation prior to admission to ICU and 38 patients had supplementation in ICU and 16 patients had recorded supplementation before and after admission to ICU. 22 patients had no recorded supplementation in ICU. The rest of the patients did not have a longitudinal measurement due to discharge (n=59) or death (n=4).

The time between admission and follow-up samples was median 4 (2-12) days. There was a significant decrease in albumin concentrations ($p<0.001$) between the admission and follow-up measurements. There was a significant increase in red cell FAD ($p<0.001$) and red cell FMN ($p<0.01$) concentrations between admission and follow-up measurements.

The interrelationships between the changes in FAD, FMN and riboflavin concentrations in the plasma and red cells in the critically ill patients are shown in Table 3-5. The change in plasma FAD was directly associated with the change in plasma albumin ($r_s=0.40$, $p<0.01$). The change in plasma FAD was directly associated with the change in plasma riboflavin ($r_s=0.42$, $p<0.01$), red cell FAD ($r_s=0.37$, $p<0.01$), red cell FMN ($r_s=0.40$, $p<0.01$) and red cell riboflavin ($r_s=0.45$, $p<0.01$). The change in plasma riboflavin was directly associated with the change in alkaline phosphatase ($r_s=0.43$, $p<0.01$), the change in red cell FAD ($r_s=0.60$, $p<0.001$), red cell FMN ($r_s=0.46$, $p<0.01$) and red cell riboflavin ($r_s=0.70$, $p<0.001$). The change globulins was directly associated with the change in alkaline phosphatase ($r_s=0.39$, $p<0.001$). The change in red cell FAD was directly associated with the change in red cell FMN ($r_s=0.83$, $p<0.001$) and the change in red cell riboflavin ($r_s=0.62$, $p<0.001$). The change in red cell FMN was directly associated with the change in red cell riboflavin ($r_s=0.59$, $p<0.001$).

3.4 Discussion

The results of the present study show that plasma riboflavin is directly associated with red cell concentrations of FAD, FMN and riboflavin in patients with critical illness. Moreover, red cell riboflavin was strongly and directly associated with red cell FAD and FMN. In contrast, although red cell FMN was associated with red cell FAD, plasma FMN was not associated with either plasma or red cell FAD. These results indicate that riboflavin, either in the plasma or in the cell, is the main determinant of FAD status in patients with critical illness. Given that, compared with plasma FAD and FMN, concentrations of plasma riboflavin are more strongly correlated with those in the red cells and that red cell riboflavin concentrations are strongly and similarly correlated with their respective FAD concentrations, this demonstrates the importance of riboflavin in the intracellular metabolism of FAD in critically ill patients.

In the present study, it was also of interest that the ratio of plasma FAD to riboflavin in critically ill patients on admission and on follow-up was much lower than that of the plasma ratio in the controls. There were also similar findings with respect to the red cell FAD to riboflavin ratios in controls and critically ill patients. However, on admission the reduction of the FAD to riboflavin ratio was greater in the plasma (83%) compared with the red cells (49%). To date, the present study shows, for the first time, perturbation of the relationship between plasma FAD and riboflavin in patients with critical illness. Moreover, this appears to be primarily due to a relative reduction in plasma and intracellular FAD concentrations although red cell FAD concentrations were all within the reference interval.

The basis of these results is not clear since few studies have examined the relationship between plasma and intracellular concentrations of FAD, FMN and riboflavin. However, it is recognised that FAD, FMN and riboflavin are bound to plasma proteins and that these fall as

part of the systemic inflammatory response (Gabay & Kushner 1999). Also, both FAD and FMN require to be hydrolysed in order to enter the cells (Aw, Jones & McCormick 1983; McCormick & Zhang 1993) and that this hydrolysis is carried out mainly by phosphatases. Indeed, in the present study, plasma alkaline phosphatase activity was greatly elevated in some patients with critical illness and there was a significant association with FAD in the red cell. It has also been reported that the increase in red cell FAD during infection is associated with a reduction of hepatic flavokinase (which convert riboflavin to FMN), an increase in FAD synthetase and FAD pyrophosphatase (which convert FMN to FAD) such that there is a loss of FAD from the liver (Brijlal et al. 1996). Furthermore, increased urinary loss of riboflavin during infection has been reported (Brijlal & Lakshmi 1999). Therefore, it would appear that during the systemic inflammatory response there is mobilisation of riboflavin from the liver to the cells, including red cells. Taken together, systemic inflammation might have accounted for the redistribution of FAD and FMN observed in the critically ill patient in the present study.

Hustad and coworkers (2002) reported that, in 124 healthy subjects, red cell FAD and FMN correlated with each other and with plasma riboflavin. Also in a subgroup of these subjects (n=46), they showed that supplementation (low dose riboflavin–1.6mg/d) increased concentrations of plasma FMN, plasma riboflavin, red cell FAD and red cell FMN, but not plasma FAD (they were unable to detect red cell riboflavin). One interpretation of these and the present results is that plasma riboflavin would be a good surrogate measure of intracellular FAD concentrations. However, it is recognised that the biological variation of plasma riboflavin is large (Hustad et al. 2002). Moreover, plasma riboflavin does not correlate with functional tests in apparently healthy subjects (Hustad et al. 2002). Also, given that riboflavin is not the physiologically active form of vitamin B2 (Massey 2000; Tietz 2006), the basis and

the clinical relevance of measuring plasma riboflavin concentrations is not certain. In contrast, the biological variation of red cell FAD is small (Hustad et al. 2002; Talwar et al. 2005), correlates with functional tests (Hustad et al. 2002) and is the physiologically active form of vitamin B2 (Massey 2000; Tietz 2006). Furthermore, the longitudinal red cell FAD measurements appeared to be the most responsive to low dose supplementation. Therefore, we believe that the red cell measurements of FAD are an accurate reflection of vitamin B2 status in patients with critical illness.

Patients admitted to intensive care are under severe metabolic stress and in a catabolic state and may have increased utilization and consumption of vitamin B2 and therefore some workers have advocated supplementation of vitamin B2 in these patients, which appears to have a beneficial effect on immune responses (Shenkin, Cruikshank & Shenkin 1989; Grimble 1997; Kodama et al. 2005). In the present study, the extreme high concentrations of plasma FAD and riboflavin were measured in those patients who had recorded supplementation. In contrast, all concentrations of red cell FAD both on admission and follow-up were within the normal range. Therefore, it is likely that the extreme values of plasma FAD, FMN and riboflavin reflect the effect of supplementation and recent intake and not analytical or methodological error and more importantly vitamin B2 status. Indeed, it was of interest that, on admission, a few patients had extremely high concentrations of plasma FAD, FMN and riboflavin suggesting that some patients had received supplementation prior to admission and would confirm the utility of red cell measurements. The question of whether red cell concentrations are more likely to detect deficiencies or toxicity in these patients cannot be definitively answered by the present study since intra-cellular concentrations were not measured in other tissues. Nevertheless, in future studies it will be important to establish

whether deficiencies in red cell FAD concentrations are related to outcome in the critically-ill patients.

In summary, the relationship between plasma FAD, FMN and riboflavin is disturbed in patients with critical illness. This is less pronounced in red cells. Therefore, red cell FAD concentrations are likely to be a more reliable measure of status in the critically ill patient. Also, from these data, critical illness does not seem to be related to vitamin B2 deficiency.

Table 3-1 Characteristics and B2 vitamin concentrations in healthy subjects and critically-ill patients on admission

	Control subjects (n=119)	Critically-ill patients (n=125)	p-value
Age (yr)	53 (35-73)	60 (18-100)	0.084
Sex (M/F)	67/ 52	82/ 43	0.137
APACHE II score		21 (3-38)	
Predicted mortality (%)		33.7 (0.2-92.6)	
SOFA score		7 (0-18)	
Medical/ Surgical		56/ 69	
ICU stay (days)		3.5 (0.2-76.4)	
ICU death (no/ yes)		101/ 24	
Hospital stay (days)		21.4 (0.40-508)	
Hospital death (no/ yes)		93/ 32	
C-reactive protein (mg/ l)	<6*	108 (<6-565)	
Albumin (g/ l)	32-45*	17 (9-47)	
Globulins (g/ l)	23-38*	27 (8-56)	
Alkaline phosphatase (IU/ l)	40-150*	83 (16-2326)	
Plasma FAD (nmol/ l)	101 (57-170)	45 (10-1916)	<0.001
Plasma FMN (nmol/ l)	6.3 (3.3-14.1)	17.0 (2.8-75.6)	<0.001
Plasma Riboflavin (nmol/ l)	11 (4-34)	33 (3-748)	<0.001
Plasma FAD/ Riboflavin ratio	8.8 (3.2-29.5)	1.5 (0.0-9.7)	<0.001
Red cell FAD (pmol/gHb)	1.9 (0.7-3.8)	1.5 (1.0-2.6)	<0.001
Red cell FMN (pmol/ gHb)	0.11 (0.04-0.44)	0.09 (0.05-0.48)	0.299
Red cell Riboflavin (pmol/ gHb)	0.02 (0.01-0.13)	0.03 (0.00-0.23)	0.037
Red cell FAD/ Riboflavin ratio	96.6 (13.2-280.0)	49.6 (9.5-423.6)	<0.001

Median (range)

*laboratory reference intervals

Table 3-2 Spearman correlations of characteristics and vitamin B2 concentrations in the control population (n=119)

	Plasma FMN	Plasma Riboflavin	Red cell FAD	Red cell FMN	Red cell Riboflavin
Plasma					
Plasma FAD	0.51***	0.49***	0.21*	-0.10	0.12
Plasma FMN		0.55***	0.11	-0.02	0.13
Plasma Riboflavin			0.20*	0.06	0.06
Intracellular					
Red cell FAD				0.44***	0.05
Red cell FMN					0.52***

*p<0.05, **p<0.01, ***p<0.001

Table 3-3 Spearman correlations of characteristics and vitamin B2 concentrations in critically-ill patients on admission to ICU (n=125)

	Plasma FMN	Plasma Riboflavin	CRP	Albumin	Globulins	Alkaline phosphatase	Red cell FAD	Red cell FMN	Red cell Riboflavin
Plasma									
Plasma FAD	0.25	0.07	-0.24**	0.31***	0.22*	0.35***	0.14	0.08	0.20*
Plasma FMN		0.52**	-0.06	0.28	0.11	0.18	0.03	0.05	0.02
Plasma Riboflavin			0.06	-0.02	0.12	0.24*	0.52***	0.55***	0.60***
CRP				-0.36***	0.12	0.13	0.16	0.10	0.09
Albumin					0.24**	-0.03	-0.16	-0.15	-0.12
Globulins						0.37***	0.14	-0.02	0.08
Alkaline phosphatase							0.31***	0.17	0.32***
Intracellular									
Red cell FAD								0.83***	0.72***
Red cell FMN									0.79***

*p<0.05, **p<0.01, ***p<0.001

Table 3-4 Characteristics and B2 vitamin concentrations in critically-ill patients on admission and follow-up

	Critically-ill patients (n=60)		
	Admission	Follow-up	p-value[b]
Age (yr)	61 (18-86)		
Sex (M/F)	43/ 17		
APACHE II	23 (7-38)		
Predicted mortality (%)	41.1 (4.3-92.6)		
SOFA score	7 (1-14)	7 (0-16)	0.085
Medical/ Surgical	26/ 34		
ICU stay (days)	9.3 (0.9-76.4)		
ICU death (no/ yes)	40/ 20		
Hospital stay (days)	21.4 (0.4-508)		
Hospital death (no/ yes)	36/ 24		
C-reactive protein (mg/ l)	97 (2-565)	131 (20-356)	0.340
Albumin (g/ l)	16 (9-45)	15 (9-29)	<0.001
Globulins (g/ l)	26 (8-56)	25 (9-41)	0.684
Alkaline phosphatase	91 (16-1089)	115 (17-659)	0.085
Plasma FAD (nmol/ l)	51 (10-1916)	55 (12-5479)	0.123
Plasma FMN (nmol/ l)[a]	11.5 (2.80-34.2)	6.9 (4.1-23.1)	0.075
Plasma Riboflavin (nmol/ l)	41.3 (3-748.1)	58.1 (3-910.8)	0.034
Plasma FAD/ Riboflavin ratio	1.2 (0.0-8.9)	0.6 (0.1-20.7)	0.016
Red cell FAD (pmol/gHb)	1.6 (1.0-2.6)	1.8 (1.0-2.6)	0.001
Red cell FMN (pmol/ gHb)	0.12 (0.05-0.33)	0.13 (0.05-0.25)	0.006
Red cell Riboflavin (pmol/ gHb)	0.04 (0.01-0.22)	0.06 (0.00-0.62)	0.043
Red cell FAD/ Riboflavin ratio	41.7 (9.5-226.4)	33.1 (4.1-242.7)	0.038

Median (range)

[a]n=11, [b]Wilcoxon signed rank test

Table 3-5 Spearman correlations of the changes of the characteristics and vitamin B2 concentrations in critically-ill patients between admission and follow-up to ICU

(n=60)

	Plasma FMN[a]	Plasma Riboflavin	CRP	Albumin	Globulins	Alkaline phosphatase	Red cell FAD	Red cell FMN	Red cell Riboflavin
Plasma									
Plasma FAD	0.50	0.42**	-0.19	0.40**	0.11	0.25	0.37**	0.40**	0.45**
Plasma FMN[a]		0.62*	0.16	0.13	0.20	-0.04	0.49	0.35	0.53
Plasma Riboflavin			-0.06	0.31*	0.21	0.43**	0.60***	0.46**	0.70***
CRP				-0.12	0.15	-0.22	0.14	0.14	0.12
Albumin					0.19	0.31*	0.06	-0.03	0.24
Globulins						0.39**	0.06	0.08	-0.07
Alkaline phosphatase							0.13	0.20	0.08
Intracellular									
Red cell FAD								0.83***	0.62***
Red cell FMN									0.59***

[a]n=11, *p<0.05, **p<0.01, ***p<0.001

4 The relationship between pyridoxal and pyridoxal phosphate concentrations in plasma, red cells and white cells in patients with critical illness

4.1 Introduction

Vitamin B6 is an essential precursor of pyridoxal (PL) and pyridoxamine phosphate coenzymes of a wide variety of enzymes of intermediary metabolism (Leklem 1991). It is recognised that the active form of vitamin B6 in plasma and tissue is pyridoxal phosphate (PLP). In health, plasma PLP appears to be determined primarily by intake of vitamin B6, its' binding to albumin and its' hydrolysis to PL, by alkaline phosphatase (Merrill et al. 1984; Brussaard et al. 1997).

In contrast to functional tests, direct measurement of PLP concentrations in plasma is thought to most accurately reflect nutritional status of vitamin B6 (Vuilleumier et al. 1991; Bates 1997). In health, PLP concentrations in plasma and tissues are determined mainly by the intake and conversion of pyridoxine to PLP and then to PL and therefore, there is a strong association in the plasma and in the tissues between PLP and PL (Johansson, Lindstedt & Tiselius 1974; Talwar et al. 2003a). However, recent work has shown that, as part of the systemic inflammatory response, plasma PLP concentrations are reduced such that the relationship between plasma and red cell PLP concentrations is disturbed (Talwar et al. 2003b; Gray et al. 2004). For example, in patients with critical illness, supplementation with pyridoxine is associated with an increase in concentrations of PLP in the red cell, but not in the plasma (Quasim et al. 2005). The mechanisms underlying this observation are as yet unclear. Cheng and coworkers (2006) reported that, during pyridoxine supplementation in critically-ill patients, plasma PL concentrations increased 15-20 fold whereas plasma PLP

concentrations only increased approximately 3 fold. However, it is unclear whether the reduced plasma PLP concentrations in critically-ill patients are due to reduced binding of PLP to albumin, increased hydrolysis of PLP by alkaline phosphatase or both. Moreover, to date, the relationship between the intracellular PL and PLP concentrations has not been previously examined in the critically ill patient.

Therefore, the aim of the present study was to examine the cross sectional and longitudinal inter-relationships between PL and PLP concentrations in plasma, red cell and white cell in patients with critical illness. This information is required to assess reliability of these measurements as indicators of vitamin B6 status in the critically ill patient.

4.2 Materials and Methods

4.2.1 Controls, patients and study design

See paragraph 2.1.1.

4.2.2 Analytical methods

4.2.2.1 Collection and preparation of blood samples

The ethylenediaminetetraacetic acid (EDTA) samples were centrifuged (500g, 4^{o}C, 10mins) and plasma was removed into another plastic tube for measurements of plasma PLP and PL. After removing the buffy coat, the remaining packed red cells were kept for red blood cell B6 determination. All tubes were stored at -70^{o} C until analysis. All samples were protected from light and assayed in a single batch for each of the analytes to minimise interbatch analytical variation (Talwar et al. 2003a).

4.2.2.2 Laboratory measurements of PLP and PL

See paragraph 2.1.2.5

4.2.2.3 Laboratory measurement of whole blood and plasma proteins

See paragraph 2.1.2.2

4.2.3 Statistics

Data from normal subjects and critically ill patients groups are presented as median and range. Comparison between the control and critically groups were carried out using the Mann-Whitney U-test. Correlations between variables in the critically-ill group were carried out

using the Spearman rank correlation (r_s). Data from different time points in the patient groups were tested for statistical significance using the Wilcoxon signed rank test. Due to the number of statistical comparisons a p-value of less than 0.01 was considered to be significant. Analysis was performed using SPSS software (version 15, SPSS Inc., Chicago, Illinois, U.S.A.).

4.3 Results

The baseline characteristics of controls (n=126) and critically ill patients (n=96) studied are shown in Table 4-1. The majority of patients were male, over the age of 50 years, and similar to the control group. The patients' median APACHE II score was 20 and the associated median predicted mortality was 32%. The majority of patients had low haemoglobin and albumin concentrations, and high concentrations of C-reactive protein and alkaline phosphatase. There were 96 patients admitted in the ICU who had plasma PLP and also PL concentrations measured. Of these patients seventy four also had red cell and white cell PLP and PL concentrations measured. There were 43 out of the 69 patients with plasma PLP below the reference interval and 46 out of the 69 patients with red cell PLP be low the reference interval. Compared with the control group, median plasma PLP, PL and their ratio (PLP/ PL) were significantly lower in the critically ill group ($p<0.001$, $p<0.01$ and $p<0.001$ respectively). Compared with the control group, median red cell PLP and the ratio of red cell PLP to PL were significantly lower in the critically ill group ($p<0.001$ and $p<0.01$ respectively). White blood cell vitamin B6 concentrations were not measured in the controls as this was not part of the original protocol for establishing reference intervals in a healthy population and therefore could not be compared with values in the critically ill group. The correlations between plasma PLP and red cell PLP were 0.90 ($p<0.001$) and 0.46 ($p<0.001$) in the control and the critically ill group respectively. In the critically ill patients the plasma PLP to PL ratio was significantly lower compared with red cell PLP to PL ratio ($p=0.001$) and white cell PLP to PL ratio ($p=0.008$). In contrast, the red cell and white cell PLP to PL ratios were not significantly different ($p=0.515$).

The interrelationships between the concentrations of PLP and PL in the plasma and red cell in the control population are shown in Table 4-2. Plasma PLP was directly associated with

plasma PL (r_s=0.58, p<0.001). Plasma PL was directly associated with both red cell PLP (r_s=0.51, p<0.001) and red cell PL (r_s=0.36, p<0.001). Red cell PLP was directly associated with red cell PL (r_s=0.66, p<0.001).

The interrelationships between the concentrations of PLP, PL and proteins in the plasma, red cell and white cell in critically ill patients are shown in Table 4-3. Plasma PLP was directly associated with plasma PL (r_s=0.51, p<0.001). Plasma PL was directly associated with both red cell PL (r_s=0.73, p<0.001) and white cell PL (r_s=0.68, p<0.001). Red cell PL and white cell PL were directly associated with red cell PLP (r_s=0.82, p<0.001) and white cell PLP (r_s=0.68, p<0.001) respectively. The red cell PLP to PL ratio was significantly correlated with the white cell PLP to PL ratio (r_s=0.70, p<0.001).

Of the 96 patients who were admitted in the ICU, 48 had longitudinal measurements of both PL and PLP concentrations (Table 4-4). These patients had a higher APACHE II score (p<0.001) and predicted mortality score (p<0.01) and lower albumin concentrations (p<0.01) compared with those patients who did not have a follow-up sample. There were no significant differences in PL and PLP concentrations between the subgroups. In those 48 critically-ill patients with follow-up samples, 15 patients had recorded supplementation prior to admission to ICU and 31 patients had supplementation in ICU and 13 patients had recorded supplementation before and after admission to ICU. Of these patients thirty five also had longitudinal measurements of red cell and white cell PL and PLP concentrations. The rest of the patients did not have a longitudinal measurement due to discharge (n=45) or death (n=3). The time between admission and follow-up samples was median 4 (range 2-12) days. There was a significant decrease in albumin concentrations (p<0.01) between the admission and follow-up measurements.

The interrelationships between the changes in PLP and PL concentrations in the plasma, red cell and white cell are shown in Table 4-5. The change in plasma PLP was directly associated with the change in plasma PL (r_s=0.67, p<0.001). The change in plasma PL was directly associated with the change in both red cell (r_s=0.83, p<0.001) and white cell PL (r_s=0.67, p<0.001). The change in red cell PL and white cell PL were directly associated with the change in red cell PLP (r_s=0.95, p<0.001) and white cell PLP (r_s=0.72, p<0.001) respectively.

4.4 Discussion

It has long been recognised that the physiological active coenzyme form of vitamin B6, PLP, is required for normal nucleic acid and protein synthesis and for cellular multiplication. Vitamin B6 deficiencies cause a more profound effect on humoral and cell mediated immune function than deficiencies of any other B-group vitamins (Leklem 1991). Indeed, low vitamin B6 status appears to impair lymphocyte proliferation in normal subjects and patients with critical illness (Kwak et al. 2002; Cheng et al. 2006). Therefore, it is of considerable importance to identify critically-ill patients with 'true' deficiency of vitamin B6 and to avoid inappropriate supplementation of vitamins which brings the risk of tissue accumulation and toxicity.

Few studies have examined the relationship between plasma and intracellular concentrations of pyridoxal and pyridoxal phosphate. Two such studies have reported relationships in small numbers of healthy subjects (Hamfelt 1967; Shephard, van der Westhuizen & Labadarios 1989). Hamfelt (1967) reported that, in 10 healthy children and 37 adults, there were significant correlations between plasma, red cell and white cell pyridoxal phosphate concentrations. However, they noted that the correlation between plasma and white cell pyridoxal phosphate concentrations appeared to be weaker than the corresponding plasma and red cell concentrations (Hamfelt 1967). Also, Shephard and colleagues (1989) reviewed the concentrations of pyridoxal phosphate in plasma, red cells and white cells of healthy subjects. In the present study, we did not measure pyridoxal phosphate in the white cells of healthy subjects. However, the concentration of PLP in white cells was approximately twofold higher in the critically ill patients compared with previous concentrations reported in the literature (Shephard, van der Westhuizen & Labadarios 1989).

With respect to comparison of the present red cell PLP concentrations in healthy subjects to previous reports (Hamfelt 1967; Shephard, van der Westhuizen & Labadarios 1989), this is problematical due to methodological differences. For example, both these previous reports used postcolumn derivatisation whereas the present study used pre-column semicarbazide derivatisation. It is recognised that the acid precipitation in the postcolumn derivatisation method may lead to suboptimal extraction of PLP from red cells (Srivastava & Beutler 1973; Talwar et al. 2003a).

The results of the present study show that, in plasma, red cells and white cells, PLP is strongly and directly associated with the concentrations of PL in patients with critical illness. It was of interest that the ratio of plasma PLP to PL in critically ill patients on admission and on follow-up was much lower than that of the plasma controls. There were also similar findings with respect to the red cell PLP to PL ratios in controls and critically ill patients. However, on admission the reduction of the PLP to PL ratio was greater in the plasma (55%) compared with the red cells (18%). To date, the present study shows, for the first time, that the relationship between plasma PLP and PL in controls is similar to that found in red and white cells, in patients with critical illness. Given that, compared to plasma PLP, concentrations of plasma PL are more strongly correlated with those in the red or white cells and that red cell and white cell PL concentrations are strongly and similarly correlated with their respective PLP concentrations, this demonstrates the importance of PL in the intracellular metabolism of PLP in normal subjects and critically ill patients. One interpretation of the results of the present study might be to suggest that plasma PL would be a good surrogate measure of intracellular PLP concentrations. However, pyridoxal is not the physiologically active form of vitamin B6 and therefore, the clinical relevance of measuring plasma PL concentrations is not certain. Therefore, we believe that the present intracellular measurements of PLP, compared with

plasma, are a more accurate reflection of vitamin B6 status and should be used in routine assessment of the patient with critical illness.

The results of the present study are therefore, consistent with previous small cross-sectional studies, which questioned the use of plasma PLP as a marker of vitamin B6 status in subjects with evidence of a systemic inflammatory response (Talwar et al. 2003b; Gray et al. 2004). There are parallels between the present study of vitamin B6 and previous work on selenium and glutathione peroxidase activity (Fell & Talwar 1998). Also, it has recently been reported that the prognostic value of plasma PLP concentrations, as a marker of myocardial infarction risk, can be accounted for, in large part, by the presence of a systemic inflammatory response as evidenced by an elevated C-reactive protein concentration (Dierkes et al. 2007).

The basis of the relatively low plasma PLP concentrations in these patients is not clear. However, albumin, whose binding appears to protect PLP from hydrolysis, was low both on admission and on follow-up. Furthermore, albumin was directly associated with plasma PLP both in the cross-sectional measurements and longitudinal changes. Interestingly, Keniston and coworkers (1988; 1989) reported that the ratio of PLP concentration in deproteinised (bound and unbound PLP) and non-deproteinised (unbound PLP) plasma samples varies with clinical condition. Since albumin is the main binding protein in PLP in plasma, they concluded that albumin concentration affects PLP concentration in plasma. Indeed, since albumin is readily redistributed from plasma, as part of the systemic inflammatory response, this is consistent with the observation that plasma concentrations of vitamin B6 are transiently decreased in subjects undergoing elective surgery (Gray et al. 2004) and that vitamin-B6 supplementation in critically-ill patients was unable to increase plasma PLP concentration (Quasim et al. 2005; Louw et al. 1992; Huang et al. 2002).

Alkaline phosphatase is another potentially important determinant of plasma PLP concentrations in patients with critical illness. In contrast to albumin, alkaline phosphatase activity was not associated with plasma PLP either in the cross-sectional or longitudinal studies. However, the laboratory measurement of alkaline phosphatase might not indicate the true functional activity since the analytical method requires dilution of the serum sample and serum phosphate concentration in the clinically observed range inhibits ALP activity under physiological conditions (Coburn et al. 1998). Clearly, this may have contributed to the weak correlation observed in the present study between plasma PLP concentrations and measured ALP activity. These results are consistent with the concept that plasma PLP concentrations are determined, at least in part, by its binding to albumin, and that any free PLP is subject to hydrolysis to PL by alkaline phosphatase (Brussaard et al. 1997).

The results of the present study do not address the question of increased utilization and metabolic turnover of plasma PLP. However, the results of studies of Huang and colleagues show that the metabolic end product of PLP, pyridoxic acid, is significantly increased both in the plasma and in the urine of critically ill patients (Huang et al. 2005; Cheng et al. 2006). Taken together with the results of the present study would suggest that the low concentrations of vitamin B6 in plasma are influenced both by increased redistribution and catabolism.

Patients admitted to intensive care are under severe metabolic stress and may have increased utilization and consumption of vitamin B6 and therefore some workers have advocated supplementation of vitamin B6 in these patients, which appears to have a beneficial effect on immune responses (Huang et al. 2005; Cheng et al. 2006). In the present study the extreme high concentrations of red cell PL and PLP were measured in those patients who had recorded supplementation. Therefore, it is likely that the extreme values reflect the effect of supplementation and not analytical or methodological error. Indeed, it was of interest that, on

admission, a few patients had extremely high concentrations of red cell PLP and PL suggesting that some patients had received supplementation prior to admission and would confirm the utility of red cell measurements. Furthermore, it is not clear whether red or white cells should be used, in preference to plasma. However, taking into consideration the higher sensitivity and that red cells are simpler to separate and analyse, we would recommend red cell analysis for assessment of vitamin B6 status and to guide supplementation in patients with critical illness.

The question of whether red cell concentrations are more likely to detect deficiencies or toxicity in these patients cannot be definitively answered by the present study since intra-cellular concentrations were not measured in other tissues. With respect to red cell PLP, it has been proposed as a more relevant measure of vitamin B6 status because the site of PLP coenzyme function is intracellular (Leklem 1990; Vermaak et al. 1990). In addition, in health red cell PLP concentrations have been shown to be associated with the dietary intake of vitamin B6, vitamin B6 supplementation and with the functional tests used to assess vitamin B6 status (Heiskanen et al. 1994; Heiskanen et al. 1996; Hansen et al. 2001). This work showed that red cell values were less sensitive to acute changes compared with plasma values. Nevertheless, in future studies it will be important to establish whether deficiencies in red cell vitamin B6 concentrations are related to outcome in the critically-ill patients.

Although there is little evidence from the literature that supplementation with vitamin B6 is toxic, the doses given in the present study (median 150mg/day) are above the recommendations which have, on the basis of development of sensory neuropathy, set the tolerable upper intake level of 100mg/day (Food and Nutrition Board IOM 1998). Given that there is significant accumulation in the red cells (approximately 30 times the upper limit of normal values in some patients) and assuming that red cell PLP concentrations reflect the

concentrations in other tissues, there is evidence of significant accumulation in patients with critical illness. It would therefore be reasonable to adopt a cautious approach to vitamin B6 supplementation in these patients.

Therefore, given the results of the present study it would be important to monitor red cell PLP concentrations to identify patients with evidence of excessive cellular accumulation of vitamin B6 and to regulate subsequent vitamin B6 supplementation accordingly. In the Royal Infirmary, Glasgow, we routinely monitor for accumulation of vitamin B6 (PLP) in red blood cells in critically-ill patients. On the basis of these results we advise that there is significant tissue accumulation of PLP in red cells, when concentrations in red cells are above 4000 pmol/gHb (approximately 5 times the upper limit of normal), and the potential risk of toxicity.

In summary, the relationship between plasma PLP and PL is disturbed in patients with critical illness. This is less pronounced in both red cells and white cells. Therefore, intracellular PLP concentrations are likely to be a more reliable measure of status than plasma measurements in the critically-ill patient. Also, from these data, critical illness could be related to true vitamin B6 deficiency and supplementation with vitamin B6 could be important for some patients admitted in ICU.

Table 4-1 Characteristics and vitamin B6 concentrations in controls and critically-ill patients on admission to ICU

	Normal subjects (n=126)	Critically-ill patients Admission (n= 96)	p-value**
Age (yr)	53 (31-73)	60 (18-100)	0.113
Sex (M/F)	67/ 59	61/35	0.122
APACHE II		20 (3-38)	
Predicted mortality (%)		32.3 (0.9-92.6)	
Haemoglobin (g/dL)	11.5-17.7*	9.9 (6.7-16.2)	
C-reactive protein (mg/l)	<6	110 (<6-565)	<0.001
Albumin (g/l)	43 (38-49)	18 (9-45)	<0.001
Alkaline phosphatase (U/l)	40-150*	84 (16-1221)	
Plasma PLP (nmol/l)	52 (19–194)	20 (<2-333)	<0.001
Plasma PL (nmol/l)	10 (3-40)	9 (<2-1346)	0.004
Plasma PLP/PL	4.9 (2.5-44.7)	2.2 (0.0-61.8)	<0.001
Red cell PLP (pmol/gHb)	391 (234–815)	261 (104-25583)	<0.001
Red cell PL (pmol/gHb) [a]	72 (25-248)	44 (6-53480)	0.040
Red cell PLP/ PL [a]	6.1 (2.8-14.5)	4.9 (0.2-24)	0.003
White cell PLP (pmol/ 10^6 cells)[a]	NM	2.2 (0.4-8.2)	
White cell PL (pmol/ 10^6 cells) [a]	NM	0.5 (0.1-10.3)	
White cell PLP/ PL [a]	NM	4.4 (0.3-10.7)	

Median (range), [a]n=75, *laboratory reference intervals, **Mann-Witney U-test, NM=not measured

Table 4-2 The relationship between plasma and red cell vitamin B6 concentrations in the control population (n=126)

	Plasma PL	Red cell PLP	Red cell PL
Plasma			
Plasma PLP	r=0.58***	r=0.90***	r=0.57***
Plasma PL		r=0.51***	r=0.36***
Intracellular			
Red cell PLP			r=0.66***

***P<0.001, Correlations between variables were carried out using the Spearman rank correlation (r_s)

Table 4-3 The relationship between laboratory characteristics, plasma and red cell vitamin B6 concentrations in critically-ill patients on admission to ICU
(n=96)

	Plasma PL	Albumin	Alkaline phosphatase	CRP	Haemoglobin	Red cell PLP	Red cell PL[a]	White cell PLP[a]	White cell PL[a]
Plasma									
Plasma PLP	r=0.51***	r=0.38***	r=0.20	r=-0.37***	r=0.23	r=0.46***	r=0.54***	r=0.56***	r=0.55***
Plasma PL		r=0.20	r=0.24	r=-0.16	r=0.07	r=0.66***	r=0.73***	r=0.60***	r=0.68***
Albumin			r=0.02	r=-0.40***	r=0.50***	r=-0.06	r=0.00	r=0.31**	r=0.08
Alkaline phosphatase				r=0.12	r=0.00	r=0.29**	r=0.38***	r=0.38**	r=0.21
CRP					r=-0.28**	r=0.05	r=0.00	r=-0.40**	r=-0.01
Intracellular									
Haemoglobin						r=-0.21	r=-0.04	r=0.22	r=0.04
Red cell PLP							r=0.82***	r=0.48***	r=0.73***
Red cell PL[a]								r=0.61***	r=0.88***
White cell PLP[a]									r=0.68***

[a]n=74, **P<0.01, ***P<0.001, Correlations between variables were carried out using the Spearman rank correlation (r_s)

Table 4-4 Characteristics and B6 vitamin concentrations in critically-ill patients on admission and follow-up

	Critically-ill patients (n=48)		
	Admission	Follow-up	p-value[b]
Age (yr)	61 (20-81)		
Sex (M/F)	32/ 16		
APACHE II	23 (7-38)		
Predicted mortality (%)	43.5 (4.3-92.6)		
Haemoglobin (g/dl)	9.6 (6.7-16.2)	9.0 (6.8-13.5)	0.085
C-reactive protein (mg/l)	105 (<6-565)	136 (20-356)	0.528
Albumin (g/l)	15 (9-32)	14 (9-29)	0.001
Alkaline phosphatase (U/l)	95 (16-1089)	118 (17-659)	0.067
Plasma PLP (nmol/l)	21 (<2-296)	23 (<2-357)	0.695
Plasma PL (nmol/l)	9 (<2-912)	16 (<2-1057)	0.042
Plasma PLP/ PL	2.2 (0.1-62)	1.1 (0.02-17)	<0.001
Red cell PLP (pmol/gHb)	300 (111-25583)	548 (51-33797)	0.415
Red cell PL (pmol/gHb)	65 (17-53480)	331 (12-28868)	0.694
Red cell PLP/ PL	4.5 (0.2-15)	2.1 (0.6-9)	0.012
White cell PLP (pmol/10^6 cells)[a]	2.2 (0.9-6.2)	2.2 (1.4-7.2)	0.574
White cell PL (pmol/10^6 cells)[a]	0.7 (0.2-9.8)	1.3 (0.2-10.3)	0.091
White cell PLP/ PL[a]	3.1 (0.3-10)	1.7 (0.3-8)	0.004

Median (range), [a]n=35, [b]Wilcoxon signed rank test

Table 4-5 The relationship between the changes in laboratory characteristics and vitamin B6 concentrations in critically-ill patients between admission and follow-up in ICU
(n=48)

	Change Plasma PL	Change Albumin	Change Alkaline phosphatase	Change CRP	Change Haemoglobin	Change Red cell PLP	Change Red cell PL	Change White cell PLP[a]	Change White cell PL[a]
Plasma									
Change Plasma PLP	r=0.67***	r=0.37**	r=-0.04	r=0.03	r=0.21	r=0.55***	r=0.57***	r=0.23	r=0.41
Change Plasma PL		r=0.28	r=-0.05	r=0.11	r=-0.03	r=0.83***	r=0.83***	r=0.43	r=0.67***
Change Albumin			r=0.27	r=-0.15	r=0.55***	r=0.12	r=0.27	r=-0.11	r=-0.09
Change Alkaline phosphatase				r=-0.22	r=0.07	r=-0.22	r=-0.20	r=-0.36	r=-0.54**
Change CRP					r=-0.37	r=0.34	r=0.29	r=-0.15	r=0.15
Intracellular									
Change Haemoglobin						r=-0.16	r=-0.06	r=-0.23	r=-0.19
Change Red cell PLP							r=0.95***	r=0.49**	r=0.79***
Change Red cell PL								r=0.41	r=0.81***
Change White cell PLP[a]									r=0.72***

[a]n=35, **P<0.01, ***P<0.001, Correlations between the changes in variables were carried out using the Spearman rank correlation (r_s)

5 Assessment of vitamin C status in patients with critical illness: Plasma or white cells?

5.1 Introduction

Vitamin C (ascorbic acid) has long been recognised to be one of the most effective water soluble antioxidants. Vitamin C is proposed to act in synergy with the fat soluble antioxidants such as vitamin E and β-carotene to protect cell membranes (Cross et al. 1990; Dhariwal, Washko & Levine 1990; Downing et al. 1993). Indeed, there has been continuing interest in its use in clinical conditions associated with increased generation of reactive oxygen species. For example, there is increasing evidence that the enhanced production of reactive oxygen species may be associated with adverse outcome in patients with critical illness (Cowley et al. 1996; Alonso de Vega et al. 2000). Therefore, there is considerable interest in the use of antioxidants, in particular ascorbic acid, in these patients.

The role of vitamin C in alleviating the oxidant stress and recycling the oxidized α-tocopherol is well recognised. Plasma α-tocopherol concentrations, in addition to being redistributed as part of the systemic inflammatory response, have been reported to be regenerated by vitamin C. Early work on fat autoxidation performed by Golumbic and Mattill (1941) reported the antioxygenic action of ascorbate in association with tocopherols. More recently, in vitro studies have was shown that ascorbic acid reduces the tocopheroxyl radical (Packer, Slater & Willson 1979) and thereby restores the radical-scavenging activity of tocopherol (Niki et al. 1982; Niki 1987; Wayner et al. 1987; Doba, Burton & Ingold 1985; Lambelet, Saucy & Lölliger 1985). It would appear that the tocopheroxyl radical, that forms in membranes, is thought to react with ascorbic acid to yield tocopherol and the ascorbyl radical, the result of which is to maintain radical scavenging potential within the membrane by regenerating

tocopherol and to transfer the oxidative challenge to the aqueous phase. However, although such a synergistic activity has been shown using model systems, direct and unambiguous evidence for its occurrence in biological systems has yet to be presented.

There is consistent evidence that plasma ascorbic concentrations are extremely low in patients with critical illness (Schorah et al. 1996; Nathens et al. 2002; Mishra et al. 2005). However, it has been recognised for some time that plasma ascorbic acid concentrations decrease as part of the systemic inflammatory response and its value as an assessment of status, during the systemic inflammatory response has been questioned (Galloway, McMillan & Sattar 2000). Following injury or critical illness, there is a profound and self-limiting systemic inflammatory response as evidenced by an increase in C-reactive protein up to 1000-fold and a decrease in albumin concentrations up to 2-fold (Gabay & Kushner 1999). Indeed, Louw and coworkers (1992) reported that, following elective orthopaedic surgery, both plasma ascorbic acid concentrations and white cell ascorbic acid concentrations fell significantly although the white cell concentrations did not fall as much. To date, no studies have examined the relationship between plasma and white cell ascorbic acid concentrations in patients with critical illness.

Therefore, the aim of the present study was to examine the longitudinal inter-relationship between plasma and white cell ascorbic acid in patients with critical illness.

5.2 Materials and Methods

5.2.1 Controls, patients and study design

See paragraph 2.1.1

5.2.2 Analytical Methods

5.2.2.1 Collection and preparation of blood samples

An EDTA tube containing approximately 6 mL of whole blood was taken for each patient on admission and follow-up. The EDTA sample was centrifuged (500g, 4^{o}C, 10mins) and 500 uL of plasma was removed into another plastic tube containing, metaphosphoric acid 6%, for plasma vitamin C determination. All tubes were stored at -70^{o} C until analysis. All samples were assayed in a single batch for each of the analytes to minimise interbatch analytical variation.

5.2.2.2 White cell preparation

See paragraph 2.1.2.1

5.2.2.3 Laboratory measurement of plasma vitamin C

See paragraph 2.1.2.6

5.2.2.4 Measurement of plasma and red cell vitamin E and plasma β-carotene

See paragraphs 2.1.2.7 and 2.1.2.8

5.2.2.5 Measurement of free and total MDA concentrations

See paragraph 2.1.2.9

5.2.2.6 Measurement of whole blood and plasma proteins concentrations

See paragraph 2.1.2.2

5.2.3 Statistics

Data from normal subject and critically ill patient groups are presented as median and range. Comparisons between the control and critically groups were performed with the use of the Mann-Whitney U test. Correlations between variables in the critically ill group were performed with the use of the Spearman's rank correlation (r_s). Data from different time points in the patient groups were tested for statistical significance with the use of the Wilcoxon's signed-rank test. Because of the number of statistical comparisons, a P value of<0.01 was considered to be significant. Analysis was performed with the use of SPSS software (version 15; SPSS Inc, Chicago, Illinois, U.S.A.).

5.3 Results

In total, thirty eight healthy controls and eighty three critically ill patients (medical n=36, surgical n=47) were studied (Table 5-1). The patients were not different in terms of age and sex compared with the controls. The patients had median APACHE II score of 20, predicted hospital mortality of 32% and SOFA score of 7.

Compared with controls, C-reactive protein concentrations were significantly higher in the critically ill patients group ($p<0.001$). Total protein and albumin concentrations were significantly lower in the critically ill patients group (both $p<0.001$). Plasma ascorbic acid, plasma α-tocopherol and plasma β-carotene were significantly lower in the critically ill patients compared with controls (all $p<0.001$). Sixty three out of 83 patients had plasma ascorbic acid concentrations below the reference interval and 10 out of 41 patients had white cell ascorbic acid concentrations below the reference interval. Total MDA/g total protein was higher in the critically ill patients compared with controls ($p<0.001$).

The interrelationships between plasma concentrations of ascorbic acid, plasma α-tocopherol, plasma β-carotene and MDA in the control subjects are shown in Table 5-2. Total MDA /g total protein was directly associated with free MDA ($r_s=0.77$, $p<0.001$).

The interrelationships between plasma and white cell concentrations of ascorbic acid, plasma and red cell concentrations of α-tocopherol, plasma β-carotene and proteins in the critically-ill patients are shown in Table 5-3. Plasma ascorbic acid was directly associated with white cell ascorbic acid ($r_s=0.48$, $p<0.01$). Plasma α-tocopherol was directly associated with total protein ($r_s=0.60$, $p<0.001$), and albumin ($r_s=0.56$, $p<0.001$) and inversely associated with C-reactive protein ($r_s=-0.32$, $p<0.01$). Plasma β-carotene was directly associated with albumin

(r_s =0.29, $p<0.01$), plasma α-tocopherol (r_s=0.50, $p<0.001$) and red cell α-tocopherol (r_s=0.36, $p<0.01$).

Of the 83 patients who were admitted to the intensive care unit, 44 had longitudinal measurements (Table 5-4). Those patients with a follow-up sample had a higher APACHE II score, predicted mortality, ICU length of stay, ICU death and total MDA/g of total protein than did those patients who did not ($p<0.01$). The patients who did not have a longitudinal measurement were either discharged from ICU (n=38) or dead in ICU (n=1). The time between admission and follow-up samples was a median of 3 days (range: 2–12 days). There were no significant differences in plasma and white cell ascorbic acid, plasma and red cell α-tocopherol, or plasma β-carotene concentrations between the admission values of those with and without a follow-up sample.

Between the admission and follow-up measurements there was a decrease in albumin concentrations ($p<0.001$).

There were no significant differences in age, sex, APACHE II, predicted mortality, patient type, and SOFA score between patients that received vitamin C supplementation (n=28) and those who did not (n=15). Similarly there were no significant differences in total protein, C-reactive, albumin, white cell counts, plasma and white cell ascorbic acid, plasma and red cell α-tocopherol, plasma β–carotene, total and free MDA between these two groups.

5.4 Discussion

In the present prospective longitudinal study plasma ascorbic acid concentrations in critically ill patients were below reference intervals whereas, white cell ascorbic acid concentrations were within the reference intervals. Moreover, plasma and intracellular concentrations of both vitamin C and α-tocopherol were poorly correlated and did not increase with supplementation in the ICU. Taken together these results would suggest that plasma ascorbic acid concentrations poorly reflect intracellular concentrations in patients with critical illness.

In contrast, there is a good correlation between plasma and white cell vitamin C concentrations in healthy subjects (Bates 1977; Jacob, Skala & Omaye 1987; Omaye et al. 1987). Moreover, it has been shown that both plasma and white cell vitamin C concentrations are increased on supplementation in healthy subjects (Jacob, Skala & Omaye 1987). Furthermore, previous studies in healthy subjects suggest that plasma vitamin C concentrations reflect recent dietary intake, whereas white cell concentrations reflect cellular stores and vitamin C status (Omaye et al. 1987).

Schorah and colleagues (1996) reported that plasma vitamin C concentrations were 25% lower in critically ill patients compared with control subjects, and were directly related to the severity of the systemic inflammatory response (as evidenced by C-reactive protein) and to the length of ICU stay. Interestingly, Mishra and coworkers (2005) using a similar dose of vitamin C to that in the present study (1,000 mg for 3 days) reported that they were unable to prevent the fall of plasma ascorbic acid concentrations to undetectable levels by day 12 in alcoholic critically ill patients. In contrast, Nathens and workers (2002) reported that, in patients with critical illness, supplementation with large doses (approximately 10 times that used in the present study) was associated with a normalisation of plasma vitamin C

concentrations. Therefore, these studies would confirm the concept that plasma vitamin C concentrations reflect intake poorly in patients with critical illness and are in agreement with the results of the present study.

To date, there no studies which have reported white cell vitamin C concentrations or the effect of vitamin C supplementation in critically ill patients. Therefore, the value of white cell vitamin C concentrations in critically ill patients remains unclear. A limitation of the present study was that the number of patients with white cell results was limited to approximately half of the total number of patients. This difference in numbers occurred because vitamin C has to be stabilised within 4-6 hours of sampling and some of the samples arrived during evening and night times, and secondly because a number of patients had very low white cell counts for the white cell extraction method to be of use.

There is some indirect evidence of the utility of white cell vitamin C concentrations such as in the present study the median of white cell vitamin C concentrations was within the reference interval and remained within the reference interval on follow up. In contrast, on admission, white cell vitamin C concentrations were poorly correlated with α-tocopherol concentrations and the lipid peroxidation product MDA, and they did not increase with supplementation in the ICU despite the recognised role of vitamin C in alleviating the oxidant stress and recycling the oxidized α-tocopherol. These results question whether the interrelationship between vitamin C and vitamin E and lipid peroxidation is important in inflammatory states but could also be confounded by the smaller numbers of white cell ascorbic acid observations. It may be that white cell vitamin C concentrations do not increase on supplementation in the critically ill patients due to increased consumption or urinary loss of vitamin C or both. In the present study, urine samples were not available thus future studies comparing the effect of vitamin C supplementation on vitamin C urine exretion in patients with critical illness could assess this

possibility. Another explanation could be that not all of these patients had recorded vitamin C supplementation in ICU and thus the results of the present study could be compromised by the data of the patients that did not have recorded supplementation. Also, the possibility of a saturation effect could be considered as an additional explanation as some of the patients had records of supplementation prior to ICU admission. The effect of supplementation on both the patients that had or did not have recorded ICU supplementation is discussed in Chapter 8.

In summary, the results of the present study indicate that plasma vitamin C concentrations are of limited value and suggest that white cell vitamin C concentrations may be of value in assessing status in patients with critical illness. Also, from these data, critical illness could be related to true vitamin C deficiency and supplementation could be important for some patients admitted in ICU.

Table 5-1 Characteristics and vitamin C in healthy subjects and critically-ill patients on admission to ICU

	Healthy subjects (n=38)	Critically–ill patients (n=83)	p-value**
Age (yr)	55 (43-73)	59 (20-86)	0.376
Sex (M/F)	21/ 17	51/ 32	0.522
APACHE II		20 (3-38)	
Predicted mortality (%)		32.2 (0.9-92.6)	
Medical/ Surgical		36/ 47	
SOFA score		7 (0-14)	
Alive/ Dead		68/ 15	
Total protein (g/ l)	73 (66-81)	46 (23-83)	<0.001
C-reactive protein (mg/ l)	6 (<6-8)	96 (1-565)	<0.001
Albumin (g/ l)	44 (38-50)	18 (9-45)	<0.001
White cell counts (x 10^9 cells)		12 (3.8-34.2)	
Plasma ascorbic acid (μmol/ l)	27 (2-82)	<5 (<5-136)	<0.001
Neutrophil ascorbic acid (μmol/ 10^9 cells)	1.5-2.5*	1.9 (0.5-8.8)[a]	
Plasma α-tocopherol (μmol/ l)	30 (14-42)	15 (5-41)	<0.001
Red cell α-tocopherol (pmol/ g Hb)	19 (12-28)*	18 (3-39)[b]	
Plasma β-carotene (μg/ l)	162 (57-708)	25 (<5-518)	<0.001
Total MDA/ total protein (μmol/ g)	0.01 (0.00-0.02)	0.01 (0.01-0.53)[c]	<0.001
Free MDA (μmol/ l)	0.08 (0.04-0.10)	0.09 (0.02-5.96)[c]	0.043

Median (range)

[a]n=41, [b]n=60, [c] n=70, *reference interval, **Mann-Whitney U test

Table 5-2 Spearman correlations between plasma concentrations of ascorbic acid, plasma α-tocopherol, plasma β-carotene and MDA in the control subjects
(n=38)

	Plasma α-tocopherol	Plasma β-carotene	Total MDA/ total protein	Free MDA
Plasma ascorbic acid	0.28	0.14	-0.04	-0.20
Plasma α-tocopherol		0.34*	0.15	0.11
Plasma β-carotene			-0.17	-0.22
Total MDA/ total protein				0.77***

*P<0.05, ** P<0.01, *** P<0.001

Table 5-3 Spearman correlations between plasma and white cell concentrations of ascorbic acid, plasma and red cell concentrations of α-tocopherol, plasma β-carotene and proteins in the critically-ill patients on admission to ICU (n=83)

	CRP	Albumin	Plasma ascorbic acid	White cell ascorbic acid	Plasma α-tocopherol	Red cell α-tocopherol	Plasma β-carotene	Total MDA/ total protein	Free MDA
Total protein	-0.18	0.74***	0.21	-0.02	0.60***	-0.01	0.28*	-0.37**	-0.07
CRP		-0.36**	0.14	0.16	-0.32**	-0.12	-0.25*	-0.16	0.05
Albumin			0.23*	0.04	0.56***	-0.02	0.29**	-0.20	-0.22
Plasma ascorbic acid				0.48**	-0.00	-0.05	0.04	-0.23	0.18
White cell ascorbic acid					0.13	0.12	0.07	-0.16	0.18
Plasma α-tocopherol						0.23	0.50***	-0.06	-0.13
Red cell α-tocopherol							0.36**	-0.20	0.10
Plasma β-carotene								-0.10	-0.11
Total MDA/ total protein									0.30*

*P<0.05, ** P<0.01, *** P<0.001

Table 5-4 Characteristics and vitamin C, E, β-carotene concentrations in critically ill patients on admission and at follow-up

	Critically–ill patients (n=44)		
	Admission	Follow-up	p-value*
Age (yr)	61 (20-86)		
Sex (M/F)	30/ 14		
APACHE II	23 (7-38)		
Predicted mortality (%)	41.1 (4.3-92.6)		
Medical/ Surgical	19/ 25		
SOFA score	7 (1-14)	6 (1-16)	0.035
Alive/ Dead	30/ 14		
Total protein (g/ l)	43 (23-82)	42 (27-62)	0.020
C-reactive protein (mg/ l)	103 (2-565)	149 (23-336)	0.316
Albumin (g/ l)	17 (9-45)	15 (9-29)	<0.001
White cell counts (x 10^9 cells)	11.5 (3.8-31.2)	12.3 (3.9-29.5)	0.095
Plasma ascorbic acid (μmol/ l)	<10 (<10-136)	<10 (<10-69)	0.242
Neutrophil ascorbic acid (μmol/ 10^9)[a]	2.0 (0.5-8.8)	2.2 (0.7-5.8)	0.563
Plasma α-tocopherol (umol/ l)	14 (5-41)	16 (8-34)	0.102
Red cell α-tocopherol (pmol/ g Hb)[b]	19 (3-39)	22 (8-48)	0.141
Plasma β-carotene (μg/ l)	23 (<5-402)	25 (<5-248)	0.052
Total MDA/ total protein (μmol/ g)[b]	0.02 (0.01-0.53)	0.01 (0.01-0.57)	0.898
Free MDA (μmol/ l)[b]	0.15 (0.04-5.96)	0.13 (0.03-9.41)	0.518

Median (range)

[a] n=23, [b] n=38, *Wilcoxon signed rank test

6 Assessment of vitamin E status in patients with systemic inflammatory response syndrome: Plasma, plasma expressed per mmol of lipids or red cell measurements?

6.1 Introduction

There is increasing evidence that plasma concentrations of a number of important micronutrient biomarkers are influenced by the presence of a systemic inflammatory response (Fell & Talwar 1998; Galloway, McMillan & Sattar 2000; Schweigert 2001). Such an effect has been reported in apparently healthy individuals (Gray et al. 2004; Gray et al. 2005; Oakes et al. 2008), patients with chronic disease (Talwar et al. 1997; McMillan et al. 2000; Almushatat et al. 2006; Leung et al. 2008; El Muhtaseb et al. 2009) and acute illness (Quasim et al. 2003; Quasim et al. 2005). In particular, plasma concentrations of vitamins are reduced as part of the systemic inflammatory response in apparently healthy individuals (Gray et al. 2005), chronic disease (Talwar et al. 1997; McMillan et al. 2000; McMillan et al. 2002; Almushatat et al. 2006) and acute illness (Goode et al. 1995; Nathens et al. 2002; Quasim et al. 2003) independent of status. Some workers have proposed either expressing plasma vitamin concentrations per lipid concentrations (Thurnham et al. 1986; 2008) or measurement of intracellular vitamin concentrations (Talwar et al. 2003b).

Vitamin E is the major chain breaking lipophilic antioxidant in cell membranes and plasma, where it protects polyunsaturated fatty acids against free radical mediated peroxidation. It is also essential, along with cholesterol, for the structural stability of membranes. The most biological active form of vitamin E is α-tocopherol which accounts for more than 83% of the vitamin E in tissues (Chow 1975). Vitamin E status is usually assessed by measurement of α-tocopherol in plasma although its correlation in tissues is not clearly established. Moreover,

plasma concentrations of vitamin E are strongly associated with their carrier lipids, principally cholesterol and triglycerides. To overcome this limitation it has been proposed the plasma α-tocopherol concentrations should be expressed in relation to plasma lipid concentrations, usually cholesterol (Thurnham et al. 1986; Thurnham et al. 2008; Doise et al. 2008).

Indeed, in healthy subjects undergoing elective surgery, there is a significant transient decrease in both α-tocopherol and cholesterol concentrations during the evolution of the systemic inflammatory response (Gray et al. 2005). However, when plasma α-tocopherol/mmol of cholesterol, as proposed by Thurnham and coworkers (1986), was applied there was no significant alteration in plasma α-tocopherol concentrations (Gray et al. 2005). However, we have observed that, in patients with systemic inflammatory response syndrome, when expressed per mmol of cholesterol, α-tocopherol concentrations were significantly higher than controls (Quasim et al. 2003). Therefore, it would appear that the simple expression of plasma α-tocopherol per mmol of cholesterol may overestimate vitamin E status in patients with systemic inflammatory response syndrome.

Since vitamin E is mainly present in cell membranes with plasma concentrations only representing a small fraction of total body vitamin E, it may be that the measurement of red cell α-tocopherol concentrations will prove to be a more reliable indicator of vitamin E status (Kitagawa, Nakagawa & Mino 1983). In animal studies, it has been reported that there is a good correlation between tissue and red cell α-tocopherol concentrations in the presence of variable plasma concentrations (Lehmann 1981). Also, red cell α-tocopherol concentrations have been used to assess vitamin E status in cystic fibrosis (Winklhofer-Roob et al. 1992), in cigarette smoking (Brown, Morrice & Duthie 1997), in type I diabetes (Jain, McVie & Smith 2000) and in atherosclerosis (Bonithon-Kopp et al. 1997). Results from these studies showed

significant correlations between red cell vitamin E status and markers of oxidative stress, or a dose response relationship in cases of supplementation.

Therefore, the aim of the present study was to examine the longitudinal inter-relationships between α-tocopherol in the plasma, in the plasma expressed per mmol of lipids and in the red cell of patients with systemic inflammatory response syndrome.

6.2 Materials and Methods

6.2.1 Controls, patients and study design

See paragraph 2.1.1

6.2.2 Collection and preparation of blood samples

The EDTA sample was centrifuged (500g, 4^{o}C, 10mins) and plasma was removed into a plastic tube for vitamin determination, the buffy coat was discarded and packed red cells were washed with saline (NaCl 0.9%). Red cells were centrifuged again and saline removed. To stabilise the vitamin E in the red cells 400µl of washed red cells were mixed with an equal volume of ascorbic acid solution (2%). For long term storage of samples at -70^{o} C, it was found necessary to stabilise the α-tocopherol in the red cell sample with ascorbic acid. Under these conditions the α-tocopherol was stable for at least 6 months.

6.2.3 Analytical Methods

6.2.3.1 Measurement of plasma and red cell vitamin E

See paragraph 2.1.2.7

6.2.3.2 Measurement of plasma and whole blood protein and lipid concentrations

See paragraph 2.1.2.2

6.2.4 Statistics

Data from normal subject and critically ill patient groups are presented as median and range. Comparisons between the control and critically groups were performed with the use of the

Mann-Whitney U test. Correlations between variables in the critically ill group were performed with the use of the Spearman's rank correlation (r_s). Data from different time points in the patient groups were tested for statistical significance with the use of the Wilcoxon's signed-rank test. Because of the number of statistical comparisons, a P value of <0.01 was considered to be significant. Analysis was performed with the use of SPSS software (version 15; SPSS Inc, Chicago, Illinois, U.S.A.).

6.3 Results

In total, sixty seven healthy controls and eighty two critically ill patients (medical n=38, surgical n=44) were studied (Table 6-1). The patients were not different in terms of age and sex compared with the controls. The patients had median APACHE II score of 21, predicted hospital mortality of 36% and SOFA score of 7. Patients had a median length of ICU stay of 6 days and median hospital stay of 23 days.

The 95% reference intervals in the normal subjects for plasma α-tocopherol, plasma α-tocopherol/mmol of cholesterol, plasma α-tocopherol/mmol of triglycerides and red cell α-tocopherol/g of haemoglobin were 14-45 umol/ l, 2.36-48.00 umol/ mmol, 2.17-6.23 umol/ mmol and 7.8-30.8 nmol/gHb respectively. Compared with controls, C-reactive protein concentrations were significantly higher and albumin concentrations were significantly lower in the critically ill patients group (both $p<0.001$). Plasma cholesterol concentrations were significantly lower, but triglycerides were similar ($p=0.218$) in the critically ill patients compared with the controls (all $p<0.001$). Plasma α-tocopherol was significantly lower in the critically ill patients compared with the controls (all $p<0.001$) with 41% of patients having concentrations below the reference interval. In contrast, when expressed per mmol of cholesterol, α-tocopherol concentrations were significantly higher in the critically ill patients compared with the controls group ($p<0.001$, 27% above the reference interval) and when expressed per mmol of triglycerides, α-tocopherol concentrations were significantly lower in the critically ill patients compared with the controls group ($p<0.001$). Red cell α-tocopherol/g of haemoglobin was similar ($p=0.852$) in the critically ill patients compared with control subjects.

The interrelationships between plasma and red cell concentrations of α-tocopherol, lipids and albumin in the control subjects are shown in Table 6-2. Plasma α-tocopherol was directly associated with cholesterol (r_s=0.35, p<0.01), triglycerides (r_s=0.40, p<0.01) and inversely with albumin (r_s=-0.46, p<0.01) but not red cell α-tocopherol/g of haemoglobin. Plasma α-tocopherol/mmol of cholesterol was directly associated with red cell α-tocopherol/g of haemoglobin (r_s=0.51, p<0.01).

The interrelationships between plasma and red cell concentrations of α-tocopherol, lipids and proteins in the critically-ill patients are shown in Table 6-3. Plasma α-tocopherol was directly associated with cholesterol (r_s=0.87, p<0.001), triglycerides (r_s=0.66, p<0.001) and albumin (r_s=0.60, p<0.001) but not red cell α-tocopherol/g of haemoglobin at the p<0.01 level. Plasma α-tocopherol/mmol of cholesterol was inversely associated with albumin (r_s=-0.41, p<0.01). Plasma α-tocopherol/mmol of triglycerides was directly associated with red cell α-tocopherol/g of haemoglobin (r_s=0.35, p<0.01).

Of the 82 patients who were admitted to the intensive care unit, 53 had longitudinal measurements of both plasma and red cell α-tocopherol concentrations (Table 6-4). Those patients with a follow-up sample had a higher APACHE II score than did those patients who did not (p<0.01). The patients who did not have a longitudinal measurement were either discharged from ICU (n=28) or dead in ICU (n=1). The time between admission and follow-up samples was a median of 4 days (range: 2–12 days). There were no significant differences in plasma or red cell α-tocopherol concentrations between the admission values of those with and without a follow-up sample.

Between the admission and follow-up measurements there was a decrease in albumin concentrations (p<0.01). There was also a significant increase in α-tocopherol/mmol of

cholesterol ($p<0.01$), but no difference in α-tocopherol/mmol of triglycerides ($p=0.117$) or red cell α-tocopherol concentrations/g of haemoglobin ($p=0.066$) at the $p<0.01$ level, between the admission and follow-up measurements.

6.4 Discussion

Plasma vitamin E (α-tocopherol) is most frequently used for assessing status although the relationship between plasma and tissue vitamin E concentrations has not been clearly established. In the plasma, α-tocopherol is recognised to circulate primarily bound to the lipoproteins in the lipid fraction, cholesterol and triglycerides (Rubinstein, Diez & Srinivasan 1969). It has been previously reported that where there are small changes in the plasma lipid fraction, plasma α-tocopherol concentrations are also altered (Bieri, Evarts & Thorp 1977). Therefore, plasma α-tocopherol concentration expressed per mmol of lipids (whether cholesterol or triglycerides) is considered to be a more reliable measurement. However, the basis for such a calculation in the presence of systemic inflammation, where there are marked changes in plasma lipid concentration that occur as part of the acute phase response (Carpentier & Scruel 2002), has not been established.

In the present study, compared with control subjects, the critically-ill patient group had lower plasma α-tocopherol concentrations and also when expressed per mmol of triglycerides. In contrast, α-tocopherol concentrations were higher when expressed per mmol of cholesterol despite these patients receiving no vitamin E supplementation prior or during their ICU stay. Moreover, neither plasma α-tocopherol nor plasma α-tocopherol/mmol of cholesterol were strongly correlated with red cell α-tocopherol concentrations in the critically-ill patients. Finally, median red cell α-tocopherol concentrations were the same in the healthy subjects and patients with critical illness and systemic inflammatory response syndrome. Taken together the results of the present study indicate that red cell α-tocopherol concentrations may be a more accurate marker of vitamin E status than plasma α-tocopherol in patients with a systemic inflammatory response however this would need further examination, e.g. examining the

effect of vitamin E supplementation on the plasma or red cell concentrations of depleted patients.

The low plasma α-tocopherol concentrations in critically ill patients with systemic inflammation are in agreement with previous reports (Goode et al. 1995; Borrelli et al. 1996; Kharb, Ghalaut & Ghalaut 1999; Nathens et al. 2002; Quasim et al. 2003). However, the basis of the elevated plasma α-tocopherol when expressed per mmol of lipids is not clear. From the present results, the basis appears to be due to the variable and disproportionately lower α-tocopherol (48%), cholesterol (61%) and triglyceride (15%) concentrations in the critically ill patients compared with controls. These variable changes in α-tocopherol, cholesterol and triglycerides are such that when α-tocopherol was expressed per mmol of cholesterol and mmol of triglycerides there were inconsistent values (31% higher and 43% lower, respectively). Given that, in the plasma, α-tocopherol is present in the greatest amount in the low density (LDL) and high density (HDL) lipoprotein fractions (McCormick, Cornwell & Brown 1960) and cholesterol concentrations are markedly reduced whilst very low density lipoprotein (VLDL) and triglycerides not significantly so, this may have contributed to the inconsistency of the results when expressed by mmol of these plasma lipids and thus to the unreliable estimation of α-tocopherol when expressed per mmol of these lipid fractions.

In vitro studies have shown that whilst α-tocopherol is transferred from all lipoprotein fractions to red cell membranes (and also presumably membranes of other cells), the relative ability to affect this transfer is 3 times greater with HDL and LDL compared with VLDL (Kayden & Bjornson 1972). It is recognised that, as part of the systemic inflammatory response, the concentration and composition of plasma lipid and lipoproteins is markely altered. In particular, plasma cholesterol concentrations are substantially reduced whereas plasma triglyceride concentrations are minimally reduced (Carpentier & Scruel 2002). In the

present study, critically-ill patients had a much reduced cholesterol/ triglyceride ratio compared with the controls largely due to the fall in plasma cholesterol concentration. LDL, in man, is derived from VLDL and the two lipoprotein classes differ in that VLDL is larger and contains more triglyceride. Therefore, in the presence of a systemic inflammatory response, it is likely that the transport of α-tocopherol is predominantly associated with triglycerides, i.e. VLDL rather the LDL, the concentration of which is relatively more reduced. This may have resulted in a reduced transfer of α-tocopherol from plasma to cell membranes and/or reduced turnover of plasma α-tocopherol relative to plasma cholesterol. As a result, in the presence of a systemic inflammatory response, plasma α-tocopherol concentration may fall less than cholesterol leading to an increase in lipid expressed α-tocopherol values in plasma. Clearly, the analysis of α-tocopherol in the above lipid fractions in the presence of a systemic inflammatory response is required to test this hypothesis.

In contrast, red cell α-tocopherol concentrations primarily reflect that in the red cell membrane (Chow 1975) and therefore, less influenced by the acute changes in plasma lipid fractions as part of the systemic inflammatory response. Indeed, Lehmann and coworkers (1988), in normal subjects, reported that red cell α-tocopherol was more sensitive to dietary intake than the corresponding plasma measurement. In addition, the susceptibility of red blood cells to haemolysis in the presence of hydrogen peroxide is more directly related to α-tocopherol concentration in red blood cells than in plasma. Also the results of the present study are similar to those of previous studies (Bieri, Tolliver & Catignani 1979; Bonithon-Kopp et al. 1997; Campoy et al. 2003). Taken together these observations indicate that red cell α-tocopherol is a more reliable measure of vitamin E status.

In summary, the results of the present study indicate that there is a discrepancy between vitamin E measurements in plasma, in plasma expressed per mmol of lipids and in red cells.

Although the value of expressing vitamin E concentrations per mmol of lipids is well established in population studies, the present study indicates that such calculation is unreliable in the presence of systemic inflammatory response syndrome and that vitamin E status should be assessed using red cell α-tocopherol measurements.

Table 6-1 Characteristics and vitamin E in critically-ill patients on admission to ICU

	Healthy subjects (n=67)	Critically–ill patients (n=82)	p-value[b]
Age (yr)	55 (29-79)	60 (18-81)	0.123
Sex (M/F)	36/ 31	52/ 30	0.233
Medical/ Surgical		38/ 44	
APACHE II		21 (3-38)	
Predicted mortality (%)		35.6 (1.2-92.6)	
SOFA score		7 (1-18)	
ICU Length of stay		6 (0.2-76.4)	
Hospital Length of stay		23 (0.4-508)	
Alive/ Dead		62/ 20	
Plasma			
C-reactive protein (mg/ l)	<6 (<6-<6)	110 (<6-565)	<0.001
Albumin (g/ l)	44 (38-50)	18 (9-45)	<0.001
Cholesterol (mmol/ l)	5.40 (3.10-7.80)	2.10 (0.40-6.40)	<0.001
Triglycerides (mmol/ l)	1.25 (0.50-4.05)	1.06 (0.30-5.05)	0.218
α-tocopherol (umol/ l)	29 (14-47)	15 (4-41)	<0.001
α-tocopherol/ cholesterol (μmol/ mmol)	5.2 (3.5-8.7)	6.8 (3.6-15.7)	<0.001
α-tocopherol/ triglycerides (μmol/ mmol)	22.3 (7.2-67.3)	12.8 (4.8-47.8)	<0.001
Red cell			
α-tocopherol (nmol/ gHb)	18.5 (12-28)[a]	18.6 (3.4-39.3)	0.852

Median (range)

[a]n=26, [b]Mann-Witney U test

Table 6-2 Spearman correlations between plasma and red cell vitamin E concentrations in the controls (n=67)

	Plasma α-tocopherol/ cholesterol	Plasma α-tocopherol/ triglycerides	Cholesterol	Triglycerides	Albumin	Red cell α-tocopherol/ Hb[a]
Plasma						
α-tocopherol	0.68***	0.00	0.35**	0.40**	-0.46**	0.32
α-tocopherol/ cholesterol		0.11	-0.39**	0.15	-0.50**	0.51**
α-tocopherol/ triglycerides			-0.09	-0.88***	-0.06	0.26
Cholesterol				0.26*	0.03	-0.07
Triglycerides					-0.03	-0.10

[a]n=26, *P<0.05, **P<0.01, ***P<0.001

Table 6-3 Spearman correlations between laboratory characteristics, plasma and red cell vitamin E concentrations in critically-ill patients on admission to ICU
(n=82)

	Plasma α-tocopherol/ cholesterol	Plasma α-tocopherol/ triglycerides	Plasma Cholesterol	Plasma Triglycerides	Plasma Albumin	Red cell α-tocopherol/ Hb
Plasma						
α-tocopherol	-0.12	0.17	0.87***	0.66***	0.60***	0.23*
α-tocopherol/ cholesterol		-0.15	-0.55***	-0.02	-0.41***	0.29*
α-tocopherol/ triglycerides			0.22	-0.57***	0.16	0.35**
Cholesterol				0.54***	0.72***	0.07
Triglycerides					0.37***	-0.04

*P<0.05, **P<0.01, ***P<0.001

Table 6-4 Characteristics and vitamin E concentrations in critically ill patients on admission and follow-up

	Critically ill patients (n=53)		
	Admission	Follow-up	p-value[b]
Age (yr)	61 (18-81)		
Sex (M/F)	37/ 16		
Patients (medical/ surgical)	25/ 28		
APACHE II	23 (7-38)		
Predicted mortality (%)	42.5 (4.3-92.6)		
SOFA score	7 (1-14)	7 (0-13)	0.212
ICU length of stay	10.3 (0.90-76.4)		
Hospital length of stay	27.5 (6-253)		
Alive/ Dead	34/ 19		
Plasma			
C-reactive protein (mg/ l)	96 (26-565)	136 (20-356)	0.270
Albumin (g/ l)	16 (9-45)	14 (9-29)	0.001
Cholesterol (mmol/ l)	1.70 (0.53-5.20)	1.80 (0.60-4.40)	0.565
Triglycerides (mmol/ l)	0.80 (0.30-3.40)	1.02 (0.20-3.20)	0.575
α-tocopherol (μmol/ l)	14 (5-41)	16 (6-36)	0.081
α-tocopherol/cholesterol (μmol/ mmol)	7.2 (3.6-15.7)	8.2 (3.4-12.9)	0.010
α-tocopherol/ triglycerides (μmol/ mmol)	13.6 (4.8-47.8)	15.0 (3.7-60.1)	0.117
Red cell			
α-tocopherol/ Hb (nmol/gHb)	19.04 (3.40-39.28)	21.86 (8.18-48.37)	0.066

Median (range)

[b]Wilcoxon signed-rank test

7 The relationship between lipid peroxidation and lipid soluble vitamin antioxidants in critically ill patients

7.1 Introduction

During the systemic inflammatory response there is activation of white blood cells which secrete oxygen free radicals leading to oxidation of cell membrane lipids. Polyunsaturated fatty acids are especially vulnerable to attack by free radicals and undergo lipid peroxidation. Indeed, products of this reaction such malondialdehyde (MDA) are routinely used as markers of free radical damage (Ogilvie et al. 1991; Grune & Berger 2007). Furthermore, MDA may exacerbate oxidative stress since it is toxic to the cardiovascular system (Gutteridge 1995) and also mutagenic having potentially genotoxic interactions with DNA and proteins (Del Rio, Stewart & Pellegrini 2005).

Vitamin E (α-tocopherol) and carotenoids have important antioxidant properties and are able to quench oxygen derived radicals and prevent lipid peroxidation (Bast et al. 1998; Galloway, McMillan & Sattar 2000). However, there is increasing evidence which suggests that the systemic inflammatory response associated with acute injury and infection may lower plasma antioxidant concentrations independent of tissue stores (Rahman et al. 1998; Galloway, McMillan & Sattar 2000; Schweigert 2001; Roth, Manhart &Wessner 2004). As part of this process, in acute inflammation there has been reported a transient decrease in plasma lipid soluble antioxidants such as α-tocopherol and carotenoids in healthy subjects (Galloway, McMillan & Sattar 2000; Kritchevsky et al. 2000; Gray et al. 2005) and medical patients (Curran et al. 2000; Chang et al. 2005). In chronic inflammation there are also significant decreases in plasma lipid soluble antioxidants such as α-tocopherol and carotenoids (Talwar et

al. 1997; McMillan et al. 2002), even when expressed per mmol of plasma lipids (Talwar et al. 1997; Leung et al. 2008).

This inflammation-associated reduction in plasma lipid soluble vitamin anti-oxidants also appears to be relevant in the critically-ill patient (Goode & Webster 1993). Quasim and colleagues (2003), in a small cross sectional study, reported that plasma concentrations of carotenoids (lutein, lycopene, α- and β-carotene) all appeared lower, compared with controls, even when expressed per mmol of cholesterol. Also, supplementation with vitamins E and C appears to be beneficial to the critically ill patient, in reducing oxidative stress and the risk of the development of multiple organ dysfunction syndrome (Nathens et al. 2002).

Clearly, if lipid soluble vitamin antioxidants were shown to be deficient in the critically-ill patient due to increased oxidative stress and were related to lipid peroxidation then they might provide an important therapeutic target in this difficult to treat group of patients. Alternatively, if lipid soluble vitamin antioxidants were shown to be related to vitamin A (retinol), which is very rarely deficient in Western populations (World Health Organisation 1995) because of its large body stores but falls transiently during the systemic inflammatory response (Gray et al. 2005), then the value of using lipid soluble vitamins to protect from oxidative stress might be questioned.

To date we are unaware of any studies that have examined the temporal plasma concentrations of MDA, retinol, α-tocopherol, and carotenoids in patients with critical illness. Therefore, the aim of the present study was to examine the longitudinal inter-relationships between circulation concentrations of malondialdehyde (total and free) and the lipid vitamin antioxidants retinol, α-tocopherol, lutein, lycopene, α- and β-carotene in patients with critical illness.

7.2 Materials and methods

7.2.1 Controls, patients and study design

See paragraph 2.1.1

7.2.2 Collection and preparation of blood samples

Venous blood samples (EDTA) were withdrawn on admission (day 1) and on follow –up, median 4 (range 2-12) days for the analysis of plasma total and free malondialdehyde, retinol, α-tocopherol, lutein, lycopene, α-carotene and β-carotene, C-reactive protein, albumin, cholesterol and triglycerides. The EDTA sample was centrifuged (500g, 4^{o}C, 10mins) and plasma was removed into a plastic tube for vitamin determination. All tubes were stored at -70^{o} C until analysis. All samples were assayed in a single batch for each analyte to minimise interbatch analytical variation.

7.2.3 Analytical Methods

7.2.3.1 Measurement of Malondialdehyde (MDA)

See paragraph 2.1.2.9

7.2.3.2 Measurement of lipid soluble antioxidants

See paragraph 2.1.2.7 and 2.1.2.8

7.2.3.3 Measurement of plasma proteins and lipids concentrations

See paragraph 2.1.2.2

7.2.4 Statistics

Comparisons between controls and critically ill patients were carried out using the Mann-Whitney U test. Data from critically ill patients groups are presented as median and range and Wilcoxon-signed rank test was used to test the longitudinal data. Correlations were carried out using the Spearman rank correlation (r_s). Analysis was performed using SPSS software (SPSS Inc., version 15, Chicago, Illinois, U.S.A.).

7.3 Results

In total, one hundred and twenty three critically ill (medical n=53, surgical n=70) patients and thirty eight controls were studied. The characteristics of the controls and the critically-ill patients on admission are shown in Table 7-1. The two groups were similar in terms of age and sex. The majority of patients were male and over the age of fifty. The majority of patients had a median APACHE II score greater than 20, with a predicted hospital mortality of 34%, and a median SOFA score of 7. One hundred patients survived and twenty three died in the ICU. The patient group had significantly higher C-reactive protein concentrations ($p<0.001$) and significantly lower concentrations of total protein, albumin and cholesterol (all $p<0.001$) compared with the control group. Free MDA and free MDA fraction were significantly higher in the critically ill patients, compared with the controls ($p<0.05$ and $p=0.001$, respectively) while more patients than controls had total MDA concentrations above 1.0 umol/l ($p<0.05$; Table 7-2).

Given that cholesterol concentrations in the critically ill patients were below the normal range and that carotenoids are lipophilic, associated with lipoproteins in plasma, and that cholesterol concentrations were significantly correlated with α-tocopherol ($r_s=0.84$; $p<0.001$), lutein ($r_s=0.56$, $p<0.001$), lycopene ($r_s=0.60$, $p<0.001$), α-carotene ($r_s=0.40$, $p<0.001$) and β-carotene ($r_s=0.45$, $p<0.001$) their ratio to cholesterol concentrations were determined (Thurnham et al. 1986). Plasma concentrations of α-carotene were below the limit of detection in 30 (24%) of the critically-ill patients and therefore were not expressed per mmol of cholesterol. Compared with the controls, α-tocopherol, lutein, lycopene, α- and β-carotene concentrations were significantly lower in the critically-ill patients (all $p<0.001$). In contrast, when expressed per mmol of cholesterol, α-tocopherol concentrations were higher ($p<0.001$) while lutein,

lycopene and β-carotene concentrations remained significantly lower in the critically-ill patients (all $p<0.001$).

The interrelations between lipid soluble vitamins and MDA concentrations in the control population are shown in Table 7-3. Total MDA was directly associated with total MDA/g total protein ($r_s=0.97$, $p<0.001$), free MDA ($r_s=0.81$, $p<0.001$) and inversely associated with free MDA fraction ($r_s=-0.63$, $p<0.001$). Total MDA/g total protein was directly associated with free MDA ($r_s=0.77$, $p<0.001$) and inversely associated with free MDA fraction ($r_s=-0.63$, $p<0.001$). Plasma lutein was directly associated with plasma α-carotene ($r_s=0.50$, $p<0.01$). Concentrations of α-carotene were directly associated with concentrations of β-carotene ($r_s=0.49$, $p<0.001$).

The interrelations between lipid soluble vitamins and MDA concentrations in the patient population are shown in Tables 7-4 and 7-5. Total MDA was directly associated with total MDA /g total protein ($r_s=0.81$, $p<0.001$), free MDA ($r_s=0.30$, $p<0.01$), plasma retinol ($r_s=0.40$, $p<0.001$) and plasma α-tocopherol ($r_s=0.46$, $p<0.001$).Total MDA/g total protein was directly associated with free MDA ($r_s=0.30$, $p<0.01$). Free MDA was directly associated with free MDA fraction ($r_s=0.82$, $p<0.001$) and inversely associated with α-carotene ($r_s=-0.30$, $p<0.01$). Free MDA fraction was inversely associated with plasma α-tocopherol ($r_s=-0.30$, $p<0.01$), plasma lutein ($r_s=-0.30$, $p<0.01$), plasma lycopene ($r_s=-0.34$, $p<0.01$) and plasma α-carotene ($r_s=-0.29$, $p<0.01$). Plasma retinol was directly associated with plasma α-tocopherol ($r_s=0.63$, $p<0.001$), plasma lutein ($r_s=0.59$, $p<0.001$), plasma lycopene ($r_s=0.34$, $p<0.001$), plasma α-carotene ($r_s=0.45$, $p<0.001$) and plasma β-carotene ($r_s=0.47$, $p<0.001$). Plasma α-tocopherol was directly associated with plasma lutein ($r_s=0.60$, $p<0.001$), plasma lycopene ($r_s=0.58$, $p<0.001$), plasma α-carotene ($r_s=0.39$, $p<0.001$) and plasma β-carotene ($r_s=0.52$, $p<0.001$). Plasma lutein was directly associated with plasma lycopene ($r_s=0.76$, $p<0.001$), plasma α-

carotene (r_s=0.53, p<0.001) and plasma β-carotene (r_s=0.63, p<0.001). Plasma lycopene was directly associated with plasma α-carotene (r_s=0.54, p<0.001) and plasma β-carotene (r_s=0.62, p<0.001). Plasma α-carotene was directly associated with plasma β-carotene (r_s=0.69, p<0.001).

Of the 123 patients that were admitted in the ICU, 59 had follow-up measurements (Table 7-6). On follow-up, the patients had significantly lower albumin (p=0.001) concentrations.

The interrelations between the changes in lipid soluble antioxidants and MDA concentrations between admission to ICU and follow-up in critically ill patients (n=59) are shown in Tables 7-7 and 7-8. The change in total MDA was directly associated with the change in total MDA/ g total protein (r_s=0.89, p<0.001). The change in total MDA/g total protein was directly associated with the change in free MDA (r_s=0.44, p<0.01). The change in free MDA was directly associated with the change in free MDA fraction (r_s=0.69, p<0.001). The change in free MDA fraction was inversely associated with the change in plasma lutein (r_s=-0.42, p<0.01).

The change in plasma retinol was directly associated with the changes in plasma α-tocopherol (r_s=0.63, p<0.001), plasma lutein (r_s=0.54, p<0.001), plasma lycopene (r_s=0.40, p<0.01), plasma α-carotene (r_s=0.35, p<0.01) and plasma β-carotene (r_s=0.40, p<0.001). The change in plasma α-tocopherol was directly associated with the changes in plasma lutein (r_s=0.68, p<0.001), plasma lycopene (r_s=0.53, p<0.001), plasma α-carotene (r_s=0.53, p<0.001) and plasma β-carotene (r_s=0.47, p<0.001). The change in .plasma lutein was directly associated with the changes in plasma lycopene (r_s=0.70, p<0.001), plasma α-carotene (r_s=0.55, p<0.001) and plasma β-carotene (r_s=0.49, p<0.001). The change in plasma lycopene was directly associated with the changes in plasma α-carotene (r_s=0.67, p<0.001) and plasma β-carotene

(r_s=0.55, p<0.001). The change in plasma α-carotene was directly associated with the change in plasma β-carotene (r_s=0.60, p<0.001).

7.4 Discusssion

Increased production of reactive oxygen species (ROS) associated with the inflammatory response is normally buffered by cellular antioxidants. In humans, the carotenoids have an important role as chain-breaking antioxidants that can retard ROS proliferation (Halliwell 1994). The mechanism by which carotenoids protect against ROS-mediated damage depends largely on physical quenching (Sies & Stahl 1995). Their interaction with ROS results in fragmentation and loss of the carotenoid molecule (Tsuchiya et al. 1992; Boehm, Tinkler & Trusctott 1995).

In the present study, on admission, the ICU patients had significantly higher total MDA/g total protein and free MDA fraction, and lower carotenoid concentrations compared with the control group. The basis of such an increase in MDA to total protein and free MDA fraction in critically ill patients is not clear. An elevated free MDA fraction, in the presence of normal total MDA concentrations, is likely to be due to decreased plasma protein binding of MDA since plasma protein concentrations were low, in particular albumin. Furthermore, increased free MDA fraction may also have the potential to directly damage cellular proteins and DNA (Fleming et al. 1982; Cross et al. 1987; Hruszkewycz 1988).

Previous studies have explored the clinical significance of measuring free MDA (Lee & Csallany 1987; Carbonneau et al. 1991; Draper et al. 1993; Cighetti et al. 2005; Hong et al. 2000; Grotto et al. 2007). Indeed, Lee & Csallany (1987) showed that free MDA was 15 times higher but bound MDA was less than 2 times higher bound MDA in tissues of vitamin E deficient animals compared with vitamin E supplemented animals. Carbonneau and co-workers (1991) showed that there is a significant increase in plasma free MDA in cancer and hemodialysis patients compared with healthy controls (by 4.9 and 4.1 times respectively,

$p<0.001$) where there was no difference in total MDA measurements between these three groups. Cighetti and co-workers (2005) showed that critically ill patients had 4 times higher concentrations of free MDA compared with controls ($p<0.001$). Hong and co-workers (2000) showed that free MDA was not related to either bound or total MDA and that bound MDA plus free MDA were equal to total MDA in healthy subjects. In the present study, free and total MDA concentrations were not significantly different between the critically ill patients and healthy controls. A closer look to the data however showed that 32% of the patients had free MDA concentrations above the upper reference interval while none of the controls had free MDA concentrations above the reference interval ($p<0.001$). On the other had only 16% of the patients had total MDA concentrations above the reference interval while only 5% of the healthy controls had total MDA concentrations above the reference interval. Furthermore, it was of interest that the critically ill patients had significantly higher free MDA fraction results compared with the controls ($p=0.001$), and that there were no correlations between the change in albumin and C-reactive protein concentrations, and the change in free MDA fraction between admission and follow-up. In addition, free MDA fraction was only weakly related to albumin and cholesterol on admission to the ICU. Therefore, from these results an increased free MDA fraction possibly reflects increased free radical damage rather than the effect of the systemic inflammatory response.

Given that plasma α-tocopherol and carotenoids are lipophilic and associated with lipoproteins in plasma, their ratio to cholesterol concentrations was determined (Thurnham et al. 1986). However, it was of interest that, in the critically ill patients, the carotenoid concentrations, even when expressed per mmol of cholesterol, were low compared with the control group. In contrast, α-tocopherol concentrations, when expressed per mmol of cholesterol, were higher

compared with the control group. Therefore, in the critically ill patient, simply expressing per mmol of cholesterol is unlikely to reflect vitamin E and carotenoid status.

The basis of the low concentrations of carotenoids after expressing them per mmol of cholesterol, in the present and previous studies of critically ill patients (Schweigert 2001; Quasim et al. 2003), is not clear. It may be that many patients admitted to ICU are deficient in carotenoids and that carotenoids are utilized rapidly following injury. Indeed, there is some evidence that patients admitted to the ICU have reduced antioxidant capacity (Ogilvie et al. 1991; Goode et al. 1995; Borrelli et al. 1996; Cowley et al. 1996; Alonso de Vega et al. 2000; Alonso de Vega et al. 2002; Tsai et al. 2000). In addition, it may be that there is some consumption that takes place in addition to redistribution. It was also of interest that there was an inverse association between free MDA fraction and α-tocopherol and the majority of the carotenoids measured in the critically ill patients. Thus, one interpretation of such data could be that these lipid soluble antioxidants are consumed during the lipid peroxidation process. However, the majority of patients had plasma retinol concentrations below the reference interval on admission to ICU and were directly and significantly related to α-tocopherol and the carotenoids. Moreover, this relationship was maintained on follow-up. Given that the hepatic stores of retinol are large and that deficiency is rare in Western populations (World Health Organisation 1995) this would suggest that the low concentrations of α-tocopherol and carotenoids, in addition to being consumed, maybe lowered by other factors.

One such factor recognised to lower plasma lipid soluble antioxidant concentrations is the presence of a systemic inflammatory response. Gray and coworkers (2005) showed that in patients undergoing elective surgery, there was a transient decrease in plasma α-tocopherol and carotenoid concentrations during the systemic inflammatory response, as evidenced by increasing concentrations of C-reactive protein and reducing concentrations of albumin.

Indeed, in the present study, both albumin and C-reactive protein were consistently associated with these lipid soluble antioxidant concentrations. Therefore, the reduction of lipid soluble antioxidant concentrations, in particular carotenoids, is likely to be multifactorial and not solely dependent on consumption during lipid peroxidation.

In summary, the results of the present study show that, on admission to ICU, total MDA tot total protein, free MDA and free MDA fraction were increased and carotenoid concentrations were low in patients with critical illness. However, although increased free MDA fraction is likely to reflect primarily increased lipid peroxidation, low plasma carotenoid concentrations are unlikely to reflect primarily consumption.

Table 7-1 Characteristics and measurements of MDA and lipid soluble vitamins in controls and critically ill patients on admission to ICU

	Reference Intervals	Healthy controls (n=38)	Critically ill patients (n=123)	p-value[a]
Age (yr)		55 (43-73)	60 (20-100)	0.156
Sex (M/F)		21/ 17	81/ 42	0.238
Patients (medical/ surgical)			53/ 70	
APACHE II			21 (3-38)	
Predicted hospital mortality (%)			33.7 (0.2-92.6)	
SOFA score			7 (0-14)	
Total protein (g/ l)	60-80	73 (66-81)	46 (23-83)	<0.001
C-reactive protein (mg/ l)	<6	<6 (<6-<6)	108 (<6-565)	<0.001
Albumin (g/ l)	32-45	44 (38-50)	17 (9-47)	<0.001
Cholesterol (mmol/ l)	3.5-5.5	5.45 (3.85-7.30)	2.19 (0.40-6.40)	<0.001
Triglycerides (mmol/l)	<2.3	1.25 (0.50-4.05)	0.95 (0.30-5.05)	0.395
Total MDA (μmol/l)	0.30-1.00	0.70 (0.36-1.10)	0.66 (0.22-14.27)	0.657
Total MDA/ total protein (μmol/ g)		0.009 (0.00-0.02)	0.016 (0.01-0.53)	<0.001
Free MDA (μmol/ l)	0.04-0.10	0.08 (0.04-0.10)	0.09 (0.02-5.96)	0.030
Free MDA fraction		0.11 (0.06-0.15)	0.14 (0.03-1.04)	0.001
Retinol (μmol/l)	1.0-2.8	2.1 (1.3-3.6)	0.7 (0.02-4.2)	<0.001
α-tocopherol (μmol/ l)	14-39	30 (14-42)	14 (4-41)	<0.001
α-tocopherol/ chol (μmol/ mmol)		5.3 (3.5-8.4)	6.7 (2.1-15.7)	<0.001
Lutein (μg/ l)	82-202	130 (58-329)	22 (<5-246)	<0.001
Lutein/ chol (μg/ mmol)		23.4 (9.7-62.7)	12.6 (1.1-169)	<0.001
Lycopene (μg/l)	100-300	199 (64-505)	29 (<5-318)	<0.001
Lycopene/ chol (μg/ mmol)		39.3 (13.3-93.8)	17 (0.6-346)	<0.001
α-carotene (μg/ l)	14-60	17 (10-75)	9 (<5-173)	<0.001
β-carotene (μg/ l)	92-312	162 (57-708)	21 (<5-522)	<0.001
β-carotene/ chol (μg/ mmol)		30.9 (9.9-137.5)	10 (0.1-239)	<0.001

Median (range), [a]Mann-Whitney U test

Table 7-2 Measurement categories of carotenoids and MDA concentrations in controls and critically ill patients on admission to ICU

	Healthy controls (n= 38)	Critically ill patients (n= 123)	p-value[a]
Total protein (<60/ >60g/l)	0/ 38	96/ 27	<0.001
C-reactive protein (<10/ >10mg/l)	38/ 0	18/ 104	<0.001
Albumin (<35/ >35g/l)	0/ 38	114/ 9	<0.001
Cholesterol (<3.5/ >3.5mmol/l)	0/ 38	91/ 26	<0.001
Triglycerides (<2.3/ >2.3mmol/l)	29/ 2	103/ 12	0.505
Total MDA(<1.0/ >1.0μmol/l)	36/ 2	81/ 20	0.037
Free MDA (0.1</ >0.1μmol/l)	38/ 0	61/ 39	<0.001
Retinol (<1.0/ >1.0 μmol/l)	0/ 38	80/ 43	<0.001
α-tocopherol (<14/ >14 μmol/l)	1/ 37	63/ 60	<0.001
Lutein (<82/ >82 μg/l)	6/ 32	112/ 11	<0.001
Lycopene (<100/>100 μg/l)	3/ 35	99/ 24	<0.001
α-carotene (<14/ >14 μg/l)	11/ 27	97/ 25	<0.001
β-carotene (<92/ >92 μg/l)	4/ 34	104/ 19	<0.001

[a]Chi-square test

Table 7-3 Spearman correlations between lipid soluble vitamins and MDA concentrations in the control population (n=38)

	Total MDA/ total protein	Free MDA	Free MDA fraction	Retinol	α-tocopherol	Lutein	Lycopene	α-carotene	β-carotene
Total MDA	0.97***	0.81***	-0.63***	0.09	0.09	0.03	0.11	-0.18	-0.25
Total MDA/ total protein		0.77***	-0.63***	0.10	0.15	0.06	0.13	-0.12	-0.17
Free MDA			-0.11	0.03	0.11	0.04	0.05	-0.10	-0.22
Free MDA fraction				-0.09	-0.08	0.13	-0.06	0.30	0.17
Retinol					-0.01	0.13	-0.06	-0.10	-0.39*
α-tocopherol						0.23	0.06	0.16	0.34*
Lutein							0.36*	0.50**	0.22
Lycopene								0.25	0.12
α-carotene									0.49**

*p<0.05, **p<0.01, ***p<0.001

Table 7-4 Spearman correlations between laboratory characteristics, lipid soluble antioxidants and MDA concentrations in critically ill patients on admission to ICU
(n=123)

	Total protein	CRP	Albumin	Cholesterol	Triglycerides
CRP	-0.14				
Albumin	0.71***	-0.35***			
Cholesterol	0.62***	-0.39***	0.69***		
Triglycerides	0.33***	-0.05	0.30**	0.50***	
Total MDA	0.31**	-0.26**	0.37***	0.53***	0.38***
Total MDA/ total protein	-0.25*	-0.21*	-0.01	0.19	0.24*
Free MDA	0.05	0.07	-0.13	-0.10	0.04
Free MDA fraction	-0.20	0.22*	-0.30**	-0.35***	-0.19
Retinol	0.47***	-0.50***	0.58***	0.65***	0.34***
α-tocopherol	0.59***	-0.28**	0.59***	0.84***	0.62***
Lutein	0.43***	-0.35***	0.60***	0.56***	0.09
Lycopene	0.43***	-0.45***	0.57***	0.60***	0.15
α-carotene	0.29**	-0.25**	0.33***	0.40***	-0.00
β-carotene	0.33***	-0.24**	0.37***	0.45***	-0.00

*p<0.05, **p<0.01, ***p<0.001

Table 7-5 Spearman correlations between laboratory characteristics, lipid soluble antioxidants and MDA concentrations in critically ill patients on admission to ICU
(n=123)

	Total MDA/ total protein	Free MDA	Free MDA fraction	Retinol	α-tocopherol	Lutein	Lycopene	α-carotene	β-carotene
Total MDA	0.81***	0.30**	-0.24*	0.40***	0.46***	0.24*	0.22*	0.05	0.17
Total MDA/ Total protein		0.30**	-0.17	0.17	0.14	-0.01	-0.02	-0.12	-0.03
Free MDA			0.82***	-0.05	-0.08	-0.22*	-0.25*	-0.30**	-0.17
Free MDA fraction				-0.24*	-0.30**	-0.30**	-0.34**	-0.29**	-0.22*
Retinol					0.63***	0.59***	0.34***	0.45***	0.47***
α-tocopherol						0.60***	0.58***	0.39***	0.52***
Lutein							0.76***	0.53***	0.63***
Lycopene								0.54***	0.62***
α-carotene									0.69***

*p<0.05, **p<0.01, ***p<0.001

Table 7-6 Characteristics, lipid soluble vitamin and MDA concentrations in critically ill patients on admission and follow-up

	Critically ill patients (n=59)		
	Admission	Follow up	p-value[a]
Age (yr)	61 (20-86)		
Sex (M/F)	42/ 17		
BMI	26 (16-54)		
Patients (medical/ surgical)	25/ 34		
APACHE II	23 (7-38)		
Predicted mortality (%)	42.2 (4.3-92.6)		
SOFA score	7 (1-14)	7 (1-16)	0.106
Total protein (g/ l)	41 (23-83)	42 (26-62)	0.018
C-reactive protein (mg/ l)	100 (<6-565)	132 (20-356)	0.390
Albumin (g/ l)	15 (9-45)	14 (9-29)	<0.001
Cholesterol (mmol/ l)	1.70 (0.53-5.20)	1.80 (0.60-4.40)	0.812
Triglycerides (mmol/ l)	0.80 (0.30-4.50)	1.00 (0.20-3.20)	0.804
Total Malondialdehyde (μmol/ l)	0.65 (0.22-14.27)	0.54 (0.32-20.40)	0.769
Total Malondialdehyde/ total protein (μmol/ g)	0.02 (0.01-0.53)	0.01 (0.01-0.57)	0.420
Free Malondialdehyde (μmol/ l)	0.12 (0.04-5.96)	0.13 (0.03-9.41)	0.194
Free Malondialdehyde fraction	0.12 (0.04-5.96)	0.13 (0.03-9.41)	0.509
Lutein (μg/ l)	22 (2-246)	19 (4-107)	0.030
Lycopene (μg/ l)	27 (1-291)	27 (2-176)	0.182
α-carotene (μg/ l)	9 (1-173)	9 (1-143)	0.125
β-carotene (μg/ l)	18 (<1-522)	27 (<1-248)	0.037

Median (range)

[a]Wilcoxon signed rank test

Table 7-7 Spearman correlations between the changes, between admission to ICU and follow-up, in laboratory characteristics, lipid soluble antioxidants and MDA concentrations in critically ill patients (n=59)

	Total protein	CRP	Albumin	Cholesterol	Triglycerides
CRP	0.02				
Albumin	0.62***	-0.11			
Cholesterol	0.53***	-0.41**	0.57***		
Triglycerides	0.50***	-0.20	0.67***	0.64***	
Total MDA	0.32*	0.09	0.45**	0.30	0.57***
Total MDA/ total protein	-0.01	0.03	0.26	0.09	0.34*
Free MDA	-0.14	0.09	0.11	-0.13	0.14
Free MDA fraction	-0.35*	0.03	-0.19	-0.28	-0.21
Retinol	0.36**	-0.64***	0.47***	0.59***	0.42**
α-tocopherol	0.58***	-0.27*	0.39**	0.81***	0.59***
Lutein	0.60***	-0.33*	0.57***	0.79***	0.58***
Lycopene	0.38**	-0.26	0.39**	0.62***	0.30*
α-carotene	0.44**	-0.15	0.27*	0.58***	0.19
β-carotene	0.37**	-0.17	0.17	0.43**	0.20

*p<0.05, **p<0.01, ***p<0.001

Table 7-8 Spearman correlations between the changes, between admission to ICU and follow-up, in laboratory characteristics, lipid soluble antioxidants and MDA concentrations in critically ill patients (n=59)

	Total MDA/ total protein	Free MDA	Free MDA fraction	Retinol	α-tocopherol	Lutein	Lycopene	α-carotene	β-carotene
Total MDA	0.89***	0.34*	-0.31*	0.27	0.29	0.26	0.32*	0.27	0.06
Total MDA/ total protein		0.44**	-0.16	0.17	0.05	0.04	0.16	0.09	-0.06
Free MDA			0.69***	0.01	-0.10	-0.28	-0.07	-0.12	0.03
Free MDA fraction				-0.15	-0.26	-0.42**	-0.19	-0.35*	-0.06
Retinol					0.63***	0.54***	0.40**	0.35**	0.40***
α-tocopherol						0.68***	0.53***	0.53***	0.47***
Lutein							0.70***	0.55***	0.49***
Lycopene								0.67***	0.55***
α-carotene									0.60***

*p<0.05, **p<0.01, ***p<0.001

8 The effect of B1, B2, B6 and C vitamin supplementation on plasma and intracellular vitamin concentrations in patients with critical illness

8.1 Introduction

It has been recognised for some time that during the systemic inflammatory response, plasma vitamin concentrations may not be responsive to supplementation. Indeed, in 46 critically ill patients, Huang and co-workers (2002) showed that vitamin B6 supplementation (15 mg) was unable to increase plasma pyridoxal 5'-phosphate and pyridoxal concentrations and both markers decreased by approximately 20%. In agreement it has been reported that, on supplementation (150mg), PLP concentrations were increased in red cells but not in plasma in critically ill patients (Talwar et al. 2003b).

Vitamin C behaviour seems to follow similar patterns during the systemic inflammatory response. 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 (Rumelin et al. 2002). In the same manner, supplementation of intravenous 1,000 mg vitamin C for 3 days was unable to prevent the fall of plasma concentrations to undetectable levels by day 12 in alcoholic critically ill patients (Mishra et al. 2005). However, in critically ill surgical patients, very high amounts of a combined parenteral supplement of a-tocopherol (1000 IU/d) and ascorbic acid (1000 mg/d) eight times per day was able to increase plasma concentrations of these vitamins (Nathens et al. 2002). White cell vitamin C has been found to decrease during the systemic inflammatory response by approximately 40% in patients undergoing orthopaedic surgery (Louw et al. 1992)

however to date there have been no studies examining the effect of ascorbic acid supplementation on white cell ascorbic acid concentrations in patients with critical illness.

As plasma B and C vitamin concentrations seem to be less responsive to supplementation and also have been shown to fall transiently during the systemic inflammatory response trying to guide supplementation by interpretation of these values could be of questionable value. In the previous chapters, we have shown that red and white cell B and C vitamin concentrations are more stable and less affected by the systemic inflammatory response syndrome, thus the question that arises is whether they reflect B and C vitamin supplementation and thus be alternative markers of vitamin status and be used to guide supplementation in patients with critical illness.

The aim of the present study was to examine the effect of B and C vitamin supplementation on plasma and intracellular B and C vitamin concentrations in patients with critical illness.

8.2 Materials and Methods

8.2.1 Patients and study design

See paragraph 2.1.1

8.2.2 Collection and preparation of blood samples

An EDTA tube containing approximately 6 mL of whole blood was taken for each patient on admission and follow-up. The EDTA sample was centrifuged (500g, 4^{o}C, 10mins) and 500 uL of plasma was removed into another plastic tube containing, metaphosphoric acid 6%, for plasma vitamin C determination. After removing the buffy coat, the packed red cells were stored for red cell vitamin determination. All tubes were stored at -70^{o} C until analysis. All samples were assayed in a single batch for each of the analytes to minimise interbatch analytical variation.

8.2.2.1 White cell preparation

See paragraph 2.1.2.1

Following the final brief centrifugation (10,000 rpm, 3mins), the supernatant was removed and the resulting pellet was dispersed in 125μL of deionised water and stabilized with 125uL of metaphosphoric acid 6% for the vitamin C analysis. The cell suspension was stored at -80°C. Before analysis, the cell suspension was sonicated for 10 min to lyse the cell membranes.

8.2.3 Analytical Methods

8.2.3.1 Measurements of vitamins B1, B2 and B6

See paragraph 2.1.2.3, 2.1.2.4 and 2.1.2.5

8.2.3.2 Measurement of vitamin C

See paragraph 2.1.2.6

8.2.4 Statistics

Data from critically ill patient groups are presented as median and range. Comparisons between the supplemented and not supplemented critically groups were performed with the use of the Mann-Whitney U test. Data from different time points in the patient groups were tested for statistical significance with the use of the Wilcoxon's signed-rank test. Correlations were carried out using the Spearman rank correlation (r_s). Because of the number of statistical comparisons, a P value of <0.01 was considered to be significant. Analysis was performed with the use of SPSS software (version 15; SPSS Inc, Chicago, Illinois, U.S.A.).

8.3 Results

In total, one hundred and twenty six patients were studied (Table 8.1 and 8.2). Patients were initially grouped by whether or not they had recorded supplementation of B and C vitamins prior to ICU admission. On admission, one hundred patients had no recorded supplementation and twenty six had received supplements of B and C vitamins prior to ICU admission. Eleven out of the 26 patients were on chronic B vitamin supplementation in the community prior to ICU admission (B Co strong, contains: vitamin B1 5mg, vitamin B2 2mg and vitamin B6 2mg). The remaining 15 out of 26 patients had a record of receiving a median amount of Pabrinex doses equal to 5 (range 1-95) prior their admission in ICU. The two groups did not differ in terms of age, sex, patient type, APACHE II and SOFA scores, ventilation days and ICU and hospital mortality. The patients with recorded supplementation prior to admission in ICU, stayed in ICU significantly longer than the patients that did not ($p<0.01$). In terms of vitamin B1, whole blood TDP was significantly higher on admission in patients that had recorded supplementation prior to ICU admission compared to the patients that did not ($p<0.001$). In terms of vitamin B2, red cell FAD ($p=0.001$), red cell FMN ($p=0.001$), plasma riboflavin ($p<0.001$), red cell riboflavin ($p<0.001$) and white cell riboflavin ($p=0.001$), were significantly higher on admission in patients that had recorded supplementations prior to ICU admission compared to the patients that did not. In terms of vitamin B6, red cell PLP ($p<0.001$), white cell PLP ($p<0.01$), plasma PL ($p<0.001$), red cell PL ($p<0.001$) and white cell PL ($p<0.001$) were significantly higher on admission in patients that had recorded supplementations prior to admission compared to the patients that did not. In terms of vitamin C, white cell ascorbic acid was significantly higher on admission in patients that had recorded supplementations prior to admission compared to the patients that did not ($p<0.01$).

From the one hundred patients that had no recorded ICU supplementation prior to ICU admission, nineteen patients continued having no recorded supplementation until their follow-up sample was taken (median 3 days, range 2-8; Table 8.3 and 8.4). On follow up, there were no significant differences in plasma, red cell and white cell vitamin B1, B2, B6 and C concentrations.

From the one hundred patients that had no recorded ICU supplementation prior to ICU admission, twenty two patients had record of supplementation in ICU (median 3 Pabrinex doses, range 1-12) until their follow-up sample was taken (median 4 days, range 2-5; Table 8.5 and 8.6). In terms of vitamin B1, on follow up, whole blood TDP was significantly higher compared with admission ($p=0.001$). In terms of vitamin B2, on follow up, white cell FMN and white cell riboflavin were significantly higher compared with admission (both $p<0.01$). In terms of vitamin B6 and vitamin C there were no significant differences between admission and follow up.

The number of Pabrinex doses the patients received during their ICU stay, between admission and follow up sample, were significantly related with the follow-up concentrations of plasma riboflavin ($r_s=0.54$, $p=0.004$), red cell FAD ($r_s=0.65$, $p<0.001$), red cell riboflavin ($r_s=0.51$, $p=0.002$), red cell PLP ($r_s=0.67$, $p<0.001$), plasma PL ($r_s=0.50$, $p=0.003$), red cell PL ($r_s=0.54$, $p=0.001$) and white cell PL ($r_s=0.52$, $p=0.006$) but were not related with the follow up concentrations of whole blood TDP ($r_s=0.38$, $p=0.020$), plasma FAD ($r_s=-0.05$, $p=0.772$), white cell FAD ($r_s=-0.08$, $p=0.684$), plasma FMN ($r_s=0.27$, $p=0.448$), red cell FMN ($r_s=0.46$, $p=0.013$), white cell FMN ($r_s=-0.23$, $p=0.306$), white cell riboflavin ($r_s=0.33$, $p=0.100$), plasma PLP ($r_s=0.08$, $p=0.652$), white cell PLP ($r_s=0.22$, $p=0.279$), plasma vitamin C ($r_s=0.44$, $p=0.01$) and white cell vitamin C ($r_s=0.53$, $p=0.023$).

The number of Pabrinex doses the patients received during their ICU stay, between admission and follow up sample, were significantly related with the change in white cell ascorbic acid concentrations (r_s=0.71, p=0.001) between admission and follow-up sample but not with the changes in whole blood TDP (r_s=0.11, p=0.525), plasma FAD (r_s=0.01, p=0.935), red cell FAD (r_s=039, p=0.018), white cell FAD (r_s=0.19, p=0.347), plasma FMN (r_s=-0.78, p=0.225), red cell FMN (r_s=0.16, p=0.400), white cell FMN (r_s=-0.06, p=0.815), plasma riboflavin (r_s=0.15, p=0.474), red cell riboflavin (r_s=0.27, p=0.122), white cell riboflavin (r_s=0.33, p=0.116), plasma PLP (r_s=-0.19, p=0.262), red cell PLP (r_s=0.16, p=0.346), white cell PLP (r_s=0.10, p=0.632), plasma PL (r_s=0.14, p=0.431), red cell PL (r_s=-0.02, p=0.918), white cell PL (r_s=0.22, p=0.304) and plasma ascorbic acid (r_s=0.28, p=0.147) between admission and follow up.

Tables 8.7 and 8.8 show the comparison between the characteristics and the change from baseline in plasma, red cell and white cell B and C vitamin concentrations between the patients that were admitted in ICU without prior recorded supplementation and either had records of ICU supplementation (n=22) or not (n=19). The two groups were not different in terms of age, sex, type, APACHE II and SOFA scores, ventilation days, ICU and hospital length of stay and ICU and hospital mortality. In terms of vitamin B1, the increase in whole blood TDP was significantly higher in the group that had recorded supplementation compared with the group that did not (p<0.001). In terms of vitamin B2, white cell FMN decreased on follow-up in the non-supplemented group and increased in the supplemented group (p<0.01). Also the increase in white cell riboflavin was significantly higher in the group that had recorded supplementation compared with the group that did not (p<0.001). In terms of vitamin B6, red cell PLP and red cell PL significantly decreased on follow-up in the non-supplemented group and significantly increased in the supplemented group (p=0.001 and

$p<0.01$, respectively). The increase in plasma PL and white cell PL was significantly higher in the group that had recorded supplementation compared with the group that did not (both $p<0.01$).

There were only nine patients on admission with plasma FAD concentrations below the reference interval that had no recorded supplementation during their ICU stay and nineteen patients that on admission had plasma FAD concentrations below the reference interval and had recorded supplementation during their ICU stay (Table 8.9). Statistical comparisons were not possible with the small numbers of the non-supplemented group. Nevertheless, on follow-up, apart from plasma FAD concentrations that remained below the reference interval, the rest of the vitamin B2 vitamers remained within the reference interval in the patients that were admitted with low plasma FAD concentrations and received no supplementation during their ICU stay. On follow –up, only red cell FAD and red cell FMN concentrations significantly increased in the patients that were admitted with low plasma FAD concentrations and received supplementation during their ICU stay (both $p<0.01$). Plasma FAD concentrations remained below the reference interval and plasma riboflavin concentrations remained above the reference interval, while the rest of the vitamers remained within the reference interval in this group of patients on follow-up.

There was only one patient with red cell FAD concentrations below the reference interval on admission to ICU and this patient did not have follow up data.

There were only three patients on admission with plasma FAD concentrations above the reference interval that had no recorded supplementation during their ICU stay and seven patients that on admission had plasma FAD concentrations below the reference interval and had recorded supplementation during their ICU stay (Table 8.10). Statistical comparisons

were not possible with the small numbers of both the groups. Nevertheless, on follow-up, apart from plasma FAD concentrations that remained above the reference interval, the rest of the vitamin B2 vitamers remained within the reference interval in the patients that were admitted with high plasma FAD concentrations whether they had recorded or not supplementation during their ICU stay.

There were only eleven patients on admission with plasma PLP concentrations below the reference interval that had no recorded supplementation during their ICU stay and eleven patients that on admission had plasma PLP concentrations below the reference interval and had recorded supplementation during their ICU stay (Table 8.11). There were no significant differences in all the B6 vitamers between admission and follow-up in the group that did not have recorded supplementation during ICU stay. On follow-up, plasma PLP and red cell PLP concentrations remained below the reference interval and the rest of the vitamin B6 vitamers remained within the reference interval in the patients that were admitted with low plasma PLP concentrations and received no supplementation during their ICU stay. On follow –up, only red cell PLP and plasma PL concentrations significantly increased in the patients that were admitted with low plasma PLP concentrations and received supplementation during their ICU stay (both $p<0.01$). Plasma PLP concentrations showed a trend of increase from below the reference interval on admission to within the reference interval on follow up in the supplemented group ($p=0.010$). Red cell PLP and plasma PL concentrations increased significantly from below the reference interval on admission to above the reference interval on follow up in the supplemented group (both $p<0.01$).

In total fifty seven patients had low red cell PLP concentrations on admission to ICU. There were thirteen patients with low red cell PLP concentrations on admission to ICU that had records of supplementation in ICU and thirteen that did not. There was no significant

difference in plasma, red cell and white cell PLP concentrations in patients that did not receive supplementation in ICU. Plasma PLP, red cell PLP and plasma PL concentrations significantly increased between admission and follow-up in patients that received supplementation with the medians of red cell PLP and plasma PL increasing above the reference interval on follow up.

There were only four patients on admission with plasma PLP concentrations above the reference interval that had no recorded supplementation during their ICU stay and two patients that on admission had plasma PLP concentrations below the reference interval and had recorded supplementation during their ICU stay (Table 8.12). Statistical comparisons were not possible with the small numbers of both the groups. On follow-up, all the vitamin B6 vitamers were within the reference interval in the patients that were admitted with high plasma PLP concentrations and did not have recorded supplementation during their ICU stay. On follow-up, apart from plasma PLP that returned within the reference interval, all the vitamin B6 vitamers were above the reference interval in the patients that were admitted with high plasma PLP concentrations and had recorded supplementation during their ICU stay.

There were only eleven patients on admission with plasma ascorbic acid concentrations below the reference interval that had no recorded supplementation during their ICU stay and nineteen patients that on admission had plasma ascorbic acid concentrations below the reference interval and had recorded supplementation during their ICU stay (Table 8.13). Statistical comparisons were not possible with the small patient numbers of the non-supplemented group. Nevertheless, on follow-up, apart from plasma ascorbic acid concentrations that remained below the reference interval, white cell ascorbic acid concentrations remained within the reference interval in the patients that were admitted with low plasma ascorbic acid concentrations whether they had recorded supplementation during their ICU stay or not.

There were ten patients on admission to ICU with white cell ascorbic acid concentrations below the reference interval. Three of these patients had follow up data. Two patients did not receive supplementation in ICU and one did. For both groups (supplemented and unsupplemented) both medians of plasma and white cell ascorbic acid concentrations remained below the reference interval.

There was only one patient on admission with plasma ascorbic acid concentrations above the reference interval that had no recorded supplementation during their ICU stay and four patients that on admission had plasma ascorbic acid concentrations above the reference interval and had recorded supplementation during their ICU stay (Table 8.14). Statistical comparisons were not possible with the small patient numbers in the two groups. The patient that received no supplementation during ICU stay, had plasma ascorbic acid concentrations below the reference interval (<10 umol/L) on follow up, while his white cell concentrations remained close to the upper reference interval on both admission and follow-up. Also on follow up, there was no increase in the median of plasma ascorbic acid concentrations in the group that had recorded ICU supplementation while the median of white cell concentrations moved from within the reference range on admission to above the reference range in the patients that had high plasma ascorbic acid concentrations on admission to ICU and had recorded supplementation on follow up.

8.4 Discussion

The results of the present study show a discrepancy in the behaviour of plasma and intracellular B2, B6 and C vitamin concentrations during supplementation in patients with critical illness. Indeed, on admission to ICU, almost 1/3 of the patients had plasma vitamin B2, B6 and C concentrations below the reference interval. This could be interpreted as indicating deficiency however it has been shown that plasma B and C vitamin concentrations can decrease transiently due to the effect of the systemic inflammatory response (Louw et al. 1992; Gray et al. 2003; Mishra et al. 2005) and that supplementation is not reflected in plasma concentrations in these patients (Huang et al. 2002; Rumelin et al. 2002; Talwar et al. 2003b; Mishra et al. 2005; Quasim et al. 2005). The longitudinal data presented in the current study are in agreement with these studies as plasma FAD, PLP and ascorbic acid concentrations did not increase with supplementation and remained below the reference interval on follow-up.

In contrast, on admission to ICU, red cell FAD, red cell PLP and white cell ascorbic acid concentrations were significantly higher in the patients with records of prior supplementation compared with the patients without. In addition, the medians of these intracellular vitamers were all significantly higher for the group with records of supplementation prior to ICU admission compared with the group without, while they were within the reference intervals for red cell FAD, above the reference interval for white cell ascorbic acid and below the reference interval for red cell PLP in the group that had records of supplementation prior to ICU. Moreover, in patients with plasma vitamin B2 and B6 concentrations below the reference interval and that did not have records of previous supplementation, red cell FAD and red cell PLP concentrations significantly increased after supplementation with their medians being above the reference interval on follow-up. These results are in agreement with previous studies (Talwar et al. 2003b; Quasim et al. 2004; Huang et al. 2005).

Hustad et al. (2002) have proposed that both plasma riboflavin and red cell FAD could be valuable markers of vitamin B2 status compared with plasma FAD. Indeed in the present study, both plasma riboflavin and red cell FAD concentrations were strongly related on follow up, to the number of supplementation doses, with red cell FAD concentrations having the strongest relationship. The median of plasma riboflavin was above the reference interval in the group that had recorded supplementation prior to ICU admission while it was within the reference interval in the group that did not. This may indicate that plasma riboflavin could be a good marker of vitamin B2 status in patients with critical illness. However, in the present study, plasma riboflavin did not significantly increase with supplementation on follow up while red cell FAD concentrations did.

Similar, findings were observed with plasma and red cell PL concentrations with their medians being above the reference interval in the group that had recorded supplementation prior to ICU admission. This may indicate that plasma or red cell PL could be good markers of vitamin B6 status in patients with critical illness. Indeed in this study, both plasma and red cell PL concentrations were strongly related to number of supplementation doses on follow up. However, red cell PL did not reflect supplementation on follow up while both red cell PLP and plasma PL concentrations significantly increased with supplementation, in patients that were admitted in ICU with low plasma PLP concentrations. In addition, PL is not the active vitamer of vitamin B6, thus its use in clinical practice for interpretation of vitamin B6 status is uncertain.

In the present study, both white cell riboflavin and white cell PL were sensitive to supplementation. Indeed, changes in white cell riboflavin and white cell PL concentrations between admission and follow up were significantly different between the groups that did not have record of supplementation prior to ICU admission and received, or not, supplementation

in ICU. This possibly reflects that, within the short period between admission and follow up samples, white cells may more sensitively reflect changes in vitamin concentrations compared with either plasma or red cells. Unfortunately, the number of longitudinal white cell riboflavin and white cell PL measurements was low in patients with low plasma FAD and low plasma PLP concentrations on admission, respectively, with recorded supplementation in ICU, and also reference intervals were not available from a control population in order to interpret the white cell riboflavin and white cell PL results. This limits the interpretation of the value of white cell riboflavin and white cell PL as markers of vitamin B2 and B6 status, respectively, in patients with critical illness.

In the present study, compared with plasma ascorbic acid, white cell ascorbic acid concentrations seemed to be a better marker of vitamin C status in patients with critical illness. Indeed, white cell ascorbic acid concentrations were above the reference interval in the group with evidence of prior ICU supplementation and their change between admission and follow up was related to the total number of supplementation doses received in ICU. Previous studies have shown that, there is a good correlation between plasma and white cell vitamin C concentrations in healthy subjects (Bates 1977; Jacob, Skala & Omaye 1987; Omaye et al. 1987) and also that, white cell ascorbic acid concentrations reflect cellular stores and vitamin C status (Omaye et al. 1987).

There was no relationship between the number of Pabrinex doses and any of the change in vitamin B2 and B6 vitamers between admission and follow up. This possibly means that the effect of supplementation may need longer to be evident at the intracellular compartment than the number of days between the admission and our follow up sample and possibly the relationship between the number of doses with the follow up measurements reflect a longer period of supplementation that many of these patients had starting prior to their ICU

admission. Moreover, in the subgroups with records of ICU supplementation and plasma vitamin concentrations above the reference interval on admission, the medians of red cell PLP, plasma PL and red cell PL concentrations did not increase further, possibly suggesting that either saturation or increased utilisation and excretion or both took place between the admission and the follow up sample. Such a metabolic effect could not be examined in the present study as there were no measurements of the relevant metabolic products in either urine or blood. A recent previous study though has shown that there are increased pyridoxic acid concentrations in plasma and urine in patients with critical illness (Cheng et al. 2006). Similar results were observed for the intracellular concentrations of FAD, FMN and riboflavin and also for white cell ascorbic acid concentrations in the subgroups with high plasma FAD and ascorbic acid concentrations on admission, respectively, that had records of supplementation in ICU.

Low concentrations of both plasma and red cell PLP concentrations were identified in approximately half of the patients in this study, suggesting that true vitamin B6 deficiency may be more frequent in the critically ill patients and that supplementation may be essential. Vitamin B6 has been shown to be essential for immune function and recent research has been conducted to establish the effect and efficacy of vitamin B6 supplementation in patients with critical illness (Huang et al. 2005; Cheng et al. 2006).

Taken together, these findings suggest that cellular concentrations of vitamins B2, B6 and C may be more accurate markers of their respective vitamin status and may be expected to be a more true reflection of the presence of supplementation than their respective plasma concentrations. Also, from these data, critical illness could be related to true vitamin B6 and C deficiency and supplementation could be important for some patients admitted in ICU.

Table 8-1 Characteristics and measurements of proteins in critically ill patients on admission to ICU that had either had or not recorded supplementation prior to ICU admission

	Reference intervals	No supplementation Admission (n=100)	Supplementation Admission (n=26)	p-value*
Age (yr)		60 (18-100)	56 (33-81)	0.824
Sex (M/F)		62/ 37	19/ 7	0.323
Patients (medical/ surgical)		42/ 57	14/ 12	0.299
ICU death (yes/ no)		19/ 80	5/ 21	0.996
APACHE II		20 (3-34)	23 (11-38)	0.107
Predicted hospital mortality (%)		31.5 (0.2-86.3)	46 (9.9-92.6)	0.025
SOFA score		7 (0-18)	8 (2-14)	0.125
Ventilation (days)		3 (0-60)	5 (0-75)	0.057
ICU length of stay (days)		2.9 (0.2-59.5)	8.4 (0.8-76.4)	0.006
Hospital length of stay (days)		19 (0.4-253)	31.3 (4-508)	0.072
Hospital death (yes/ no)		24/ 75	8/ 18	0.499
C-reactive protein (mg/ l)	<6	109 (<6-434)	106 (<6-565)	0.961
Albumin (g/ l)	32-45	18 (9-47)	14 (9-45)	0.210

Median (range)

*Mann-Whitney U-test

Table 8-2 Measurements of water and lipid soluble vitamins in critically ill patients on admission to ICU that had either had or not recorded supplementation prior to ICU admission

	Reference intervals	No supplementation Admission (n=100)	Supplementation Admission (n=26)	p-value*
Whole blood TDP (ng/ gHb)	275-675	690 (196-2552)	1703 (544-3650)	<0.001
Plasma FAD (nmol/ l)	51-160	43.6 (10.4-1179)	56.3 (10.3-1916)	0.310
Red cell FAD (nmol/ gHb)	1.0-3.4	1.44 (0.97-2.58)	1.77 (1.23-2.30)	0.001
White cell FAD (pmol/10^6 cells)	NA	(n=59) 12.77 (7.89-90.15)	(n=18) 13.98 (6.58-18.25)	0.057
Plasma FMN (nmol/ l)	3.3-14.1**	(n=18) 16.95 (2.80-34.2)	(n=8) 15.05 (6.10-75.60)	0.644
Red cell FMN (nmol/ gHb)	0.04-0.44**	(n=86) 0.09 (0.05-0.48)	(n=22) 0.13 (0.07-0.28)	0.001
White cell FMN (pmol/ 10^6 cells)	NA	(n=51) 0.62 (0.27-1.73)	(n=15) 0.74 (0.37-1.51)	0.242
Plasma riboflavin (nmol/ l)	4-34**	(n=82) 26 (3-374)	(n=21) 107 (7-748)	<0.001
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	(n=97) 0.03 (0.00-0.23)	(n=26) 0.07 (0.00-0.22)	<0.001
White cell riboflavin (pmol/ 10^6 cells)	NA	(n=55) 0.54 (0.23-3.27)	(n=16) 0.77 (0.28-1.32)	0.001
Plasma PLP (nmol/ l)	17-135	19 (<2-333)	30 (3-306)	0.015
Red cell PLP (pmol/ gHb)	250-680	242 (32-27441)	2530 (111-25583)	<0.001
White cell PLP (pmol/ 10^6 cells)	NA	(n=57) 2.12 (0.37-8.24)	(n=16) 2.96 (1.58-6.16)	0.005
Plasma PL (nmol/ l)	5-26	(n=96) 9 (2-1112)	(n=25) 90 (2-1346)	<0.001
Red cell PL (pmol/ gHb)	25-195	(n=36) 109 (12.24-8680)	(n=17) 1432 (15-28868)	<0.001
White cell PL (pmol/ 10^6 cells)	NA	(n=55) 0.43 (0.7-7.78)	(n=15) 4.02 (0.44-10.29)	<0.001
Plasma ascorbic acid (μmol/ l)	15-40	(n=69) <10 (<10-58)	(n=15) 11 (<10-136)	0.019
White cell ascorbic acid (μmol/ 10^9 cells)	1.5-2.5***	(n=33) 1.81 (0.46-3.30)	(n=9) 3.02 (1.47-8.77)	0.002

Median (range), *Mann-Whitney U-test, **Reference intervals from controls, ***Reference intervals Lee et al. (1982)

Table 8-3 Characteristics and measurements of proteins between admission and follow up in critically ill patients that did not have recorded supplementation prior or within ICU

		Critically ill patients (n=19) No supplementation		
	Reference intervals	Admission	Follow-up	p-value*
Age (yr)		67 (26-81)		
Sex (M/F)		14/ 5		
Patients (medical/ surgical)		6/ 13		
ICU death (yes/ no)		8/ 11		
APACHE II		20 (7-34)		
Predicted hospital mortality (%)		44.4 (4.8-86.3)		
SOFA score		7 (1-11)		
Ventilation (days)		12 (0-60)		
ICU length of stay (days)		10.7 (0.9-59.5)		
Hospital length of stay (days)		29 (11.1-253)		
Hospital death (yes/ no)		8/ 11		
C-reactive protein (mg/ l)	<6	137 (<6-259)	149 (27-251)	0.845
Albumin (g/ l)	32-45	19 (9-42)	14 (9-28)	0.016

Median (range)
*Wilcoxon signed rank test

Table 8-4 Measurements of water and lipid soluble vitamins between admission and follow up in critically ill patients that did not have recorded supplementation prior or within ICU

		Critically ill patients (n=19) No supplementation		
	Reference intervals	Admission	Follow-up	p-value*
Whole blood TDP (ng/ gHb)	275-675	800 (196-1379)	689 (207-1538)	0.904
Plasma FAD (nmol/ l)	51-160	53 (25-1179)	56 (19-1225)	0.983
Red cell FAD (nmol/ gHb)	1.0-3.4	1.31 (1.04-2.21)	1.33 (0.96-2.19)	1.000
White cell FAD (pmol/10^6 cells) (n=14)	NA	13.14 (11.22-15.81)	13.02 (10.47-16.25)	0.177
Plasma FMN (nmol/ l) (n=6)	3.3-14.1**	10 (4-34)	6 (4-23)	0.046
Red cell FMN (nmol/ gHb)	0.04-0.44**	0.10 (0.05-0.17)	0.10 (0.05-0.17)	0.904
White cell FMN (pmol/ 10^6 cells) (n=14)	NA	0.63 (0.43-0.82)	0.58 (0.37-0.90)	0.300
Plasma riboflavin (nmol/ l) (n=13)	4-34**	20 (7-170)	17 (3-300)	0.753
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	0.03 (0.01-0.09)	0.03 (0.00-0.17)	0.841
White cell riboflavin (pmol/ 10^6 cells) (n=14)	NA	0.57 (0.33-0.83)	0.61 (0.38-0.81)	0.925
Plasma PLP (nmol/ l)	17-135	17 (2-296)	10 (2-357)	0.133
Red cell PLP (pmol/ gHb)	250-680	216 (149-721)	224 (78-514)	0.198
White cell PLP (pmol/ 10^6 cells) (n=14)	NA	1.96 (1.39-3.56)	1.93 (1.38-2.14)	0.510
Plasma PL (nmol/ l) (n=16)	5-26**	5 (2-83)	5 (2-30)	0.048
Red cell PL (pmol/ gHb) (n=15)	25-195**	50 (17-130)	54 (12-301)	0.211
White cell PL (pmol/ 10^6 cells) (n=14)	NA	0.54 (0.26-1.15)	0.66 (0.17-1.45)	0.026
Plasma ascorbic acid (μmol/ l) (n=12)	15-40	<10 (<10-50)	<10 (<10-18)	0.154
White cell ascorbic acid (μmol/ 10^9 cells) (n=6)	1.5-2.5***	1.80 (0.60-2.45)	1.78 (0.67-3.12)	0.249

Median (range)

*Wilcoxon signed rank test, **Reference intervals from controls, ***Reference intervals Lee et al. (1982)

Table 8-5 Characteristics and measurements of proteins between admission and follow up in critically ill patients that had recorded supplementation only within ICU

		Critically ill patients (n=22) Supplementation		
	Reference intervals	Admission	Follow-up	p-value *
Age (yr)		56 (18-86)		
Sex (M/F)		14/ 8		
Patients (medical/ surgical)		10/ 12		
ICU death (yes/ no)		7/ 15		
APACHE II		23 (9-33)		
Predicted hospital mortality (%)		38.6 (4.3-80.4)		
SOFA score		7 (1-14)		
Ventilation (days)		8 (0-49)		
ICU length of stay (days)		8.3 (1.9-48.1)		
Hospital length of stay (days)		26 (6-119)		
Hospital death (yes/ no)		9/ 13		
C-reactive protein (mg/ l)	<6	58 (3-257)	144 (28-303)	0.055
Albumin (g/ l)	32-45	16 (9-33)	15 (9-29)	0.055

Median (range)
*Wilcoxon signed rank test

Table 8-6 Measurements of water and lipid soluble vitamins between admission and follow up in critically ill patients that had recorded supplementation only within ICU

		Critically ill patients (n=22) Supplementation		
	Reference intervals	Admission	Follow-up	p-value*
Whole blood TDP (ng/ gHb)	275-675	685 (288-2287)	1646 (333-5491)	0.001
Plasma FAD (nmol/ l)	51-160	37 (14-827)	43 (12-1698)	0.794
Red cell FAD (nmol/ gHb) (n=21)	1.0-3.4	1.46 (1.13-2.56)	1.68 (1.24-2.63)	0.019
White cell FAD (pmol/10^6 cells) (n=16)	NA	12.63 (7.89-16.89)	13.64 (5.85-29.25)	0.163
Plasma FMN (nmol/ l) (n=1)	3.3-14.1**	NA	NA	
Red cell FMN (nmol/ gHb) (n=16)	0.04-0.44**	0.09 (0.06-0.33)	0.11 (0.06-0.25)	0.034
White cell FMN (pmol/ 10^6 cells) (n=11)	NA	0.53 (0.27-0.84)	0.67 (0.34-0.94)	0.004
Plasma riboflavin (nmol/ l) (n=13)	4-34**	24 (3-338)	44 (15-577)	0.221
Red cell riboflavin (nmol/ gHb) (n=19)	0.01-0.13**	0.02 (0.01-0.21)	0.03 (0.01-0.28)	0.033
White cell riboflavin (pmol/ 10^6 cells) (n=14)	NA	0.49 (0.23-1.36)	0.67 (0.46-2.07)	0.003
Plasma PLP (nmol/ l)	17-135	21 (<2-126)	34 (9-175)	0.158
Red cell PLP (pmol/ gHb) (n=21)	250-680	241 (131-10788)	1000 (132-5881)	0.016
White cell PLP (pmol/ 10^6 cells) (n=14)	NA	2.62 (0.89-4.06)	3.39 (1.94-7.24)	0.041
Plasma PL (nmol/ l) (n=18)	5-26**	9 (<2-912)	33 (7-1057)	0.058
Red cell PL (pmol/ gHb) (n=15)	25-195**	39.74 (18.07-38223)	333 (83.33-8680)	0.088
White cell PL (pmol/ 10^6 cells) (n=14)	NA	0.43 (0.18-7.78)	3.02 (0.43-10.32)	0.016
Plasma ascorbic acid (μmol/ l) (n=18)	15-40	<10 (<10-69)	<10 (<10-69)	0.609
White cell ascorbic acid (μmol/ 10^9 cells) (n=10)	1.5-2.5***	1.92 (0.46-2.93)	2.35 (1.02-5.80)	0.074

Median (range)
*Wilcoxon signed rank test, **Reference intervals from controls, ***Reference intervals Lee et al. (1982)

Table 8-7 Characteristics and changes in proteins between admission and follow up in critically ill patients that did not have recorded supplementation prior to ICU and did or did not have recorded supplementation within ICU

	Critically ill patients		
	No supplementation (n=19) Changes	Supplementation (n=22) Changes	p-value*
Age (yr)	67 (26-81)	56 (18-86)	0.440
Sex (M/F)	14/ 5	14/ 8	0.496
Patients (medical/ surgical)	6/ 13	10/ 12	0.370
ICU death (yes/ no)	8/ 11	7/ 15	0.501
APACHE II	20 (7-34)	23 (9-33)	0.896
Predicted hospital mortality (%)	44.4 (4.8-86.3)	38.6 (4.3-80.4)	0.676
SOFA score	7 (1-11)	7 (1-14)	0.460
Ventilation (days)	12 (0-60)	8 (0-49)	0.885
ICU length of stay (days)	10.7 (0.9-59.5)	8.3 (1.9-48.1)	0.906
Hospital length of stay (days)	29 (11.1-253)	26 (6-119)	0.556
Hospital death (yes/ no)	8/ 11	9/ 13	0.939
C-reactive protein (mg/ l)	-5 (-96 - +249)	62 (-184 - +276)	0.242
Albumin (g/ l)	-1 (-15 - +6)	-3 (-15 - +18)	0.927

Change in median (change in range)
*Mann-Whitney U-test

Table 8-8 Changes water and lipid soluble vitamins between admission and follow up in critically ill patients that did not have recorded supplementation prior to ICU and did or did not have recorded supplementation within ICU

	Critically ill patients		
	No supplementation (n=19)	Supplementation (n=22)	p-value*
Whole blood TDP (ng/ gHb)	9 (-519 - +628)	885 (-1034 - +4600)	<0.001
Plasma FAD (nmol/ l)	2 (-200 - +845)	6 (-230 - +1377)	0.850
Red cell FAD (nmol/ gHb)	-0.02 (-0.12 - +0.29)	0.10 (-0.36 - +1.50)	0.060
White cell FAD (pmol/10^6 cells)	(n=14) -0.24 (-4.32 - +3.91)	(n=16) 0.85 (-3.28 - +12.50)	0.022
Plasma FMN (nmol/ l)	(n=6) -4 (-27 - +1)	(n=1) NA	NA
Red cell FMN (nmol/ gHb)	 -0.00 (-0.02 - +0.02)	(n=16) 0.02 (-0.08 - +0.11)	0.017
White cell FMN (pmol/ 10^6 cells)	(n=14) -0.02 (-0.23- +0.18)	(n=11) 0.12 (-0.01- +0.24)	0.003
Plasma riboflavin (nmol/ l)	(n=13) 1 (-17 - +130)	(n=13) 12 (-165 - +571)	0.293
Red cell riboflavin (nmol/ gHb)	(n=19) -0.00 (-0.03 - +0.07)	(n=19) 0.01 (-0.09 - +0.24)	0.042
White cell riboflavin (pmol/ 10^6 cells)	(n=14) 0.001 (-0.19 - +0.16)	(n=14) 0.24 (-0.16 - +0.72)	<0.001
Plasma PLP (nmol/ l)	-2 (-147 - +62)	4.3 (-68 - +132)	0.150
Red cell PLP (pmol/ gHb)	-53 (-274 - +352)	749 (-7038 - +5690)	0.001
White cell PLP (pmol/ 10^6 cells)	(n=14) 0.01 (-1.70 - +0.56)	(n=14) 1.26 (-1.53 - +4.98)	0.054
Plasma PL (nmol/ l)	(n=16) 2 (-3 - +12)	(n=18) 15 (-857 - +1048)	0.009
Red cell PL (pmol/ gHb)	(n=15) -2.89 (-13.27 - +263)	(n=15) 292 (-34757 - +8653)	0.007
White cell PL (pmol/ 10^6 cells)	(n=14) 0.01 (-1.70 - +0.56)	(n=14) 1.26 (-1.53 - +4.98)	0.004
Plasma ascorbic acid (μmol/ l)	(n=12) -0.55 (-47 - +2.10)	(n=18) 0.50 (-22 - +67)	0.155
White cell ascorbic acid (μmol/ 10^9 cells)	(n=6) 0.09 (-0.46 - +0.66)	(n=10) 0.49 (-0.83 - +3.85)	0.278

Change in median (change in range) *Mann-Whitney U-test

Table 8-9 Plasma, red cell and white cell vitamin B2 concentrations in patients that had plasma FAD concentrations below the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with low plasma FAD concentrations on admission					
	Reference intervals	Admission (n=9)	No supplementation Follow-up (n=9)	p-value*	Admission (n=19)	Supplementation Follow-up (n=19)	p-value*
Plasma FAD (nmol/ l)	51-160	30 (24-46)	33 (23-882)	0.327	23 (10-44)	36 (12-72)	0.020
Red cell FAD (nmol/ gHb)	1.0-3.4	1.3 (1.13-2.21)	1.27 (1.08-2.19)	0.110	(n=18) 1.54 (1.13-2.56)	(n=18) 1.83 (1.40-2.63)	0.003
White cell FAD (pmol/10^6 cells)	NA	(n=5) 15 (12-16)	(n=5) 12 (10-16)		(n=13) 13 (8-17)	(n=13) 12 (6-29)	0.221
Plasma FMN (nmol/ l)	3.3-14.1**	(n=4) 6.6 (3.6-28.1)	(n=4) 4.8 (4.1-23.1)		(n=2) 12.4 (2.8-21.9)	(n=2) 12.0 (4.6-19.3)	0.655
Red cell FMN (nmol/ gHb)	0.04-0.44**	0.08 (0.06-0.19)	0.08 (0.05-0.19)		(n=16) 0.10 (0.06-0.33)	(n=16) 0.14 (0.08-0.25)	0.008
White cell FMN (pmol/ 10^6 cells)	NA	(n=5) 0.68 (0.49-0.78)	(n=5) 0.59 (0.38-0.90)		(n=11) 0.43 (0.27-0.99)	(n=11) 0.51 (0.28-0.88)	0.155
Plasma riboflavin (nmol/ l)	4-34**	(n=7) 15 (7-34)	(n=7) 11 (3-40)		(n=16) 43 (3-572)	(n=16) 86 (15-617)	0.163
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	0.02 (0.01-0.08)	0.02 (0.01-0.07)		(n=17) 0.03 (0.01-0.21)	(n=17) 0.06 (0.01-0.25)	0.076
White cell riboflavin (pmol/ 10^6 cells)	NA	(n=5) 0.55 (0.47-0.71)	(n=5) 0.53 (0.38-0.69)		(n=11) 0.53 (0.23-1.36)	(n=11) 0.67 (0.52-2.07)	0.013

Median (range), *Wilcoxon signed rank test, ** Reference intervals from controls

Table 8-10 Plasma, red cell and white cell vitamin B2 concentrations in patients that had plasma FAD concentrations above the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with high plasma FAD concentrations on admission					
	Reference intervals	Admission (n=3)	No supplementation Follow-up (n=3)	p-value*	Admission (n=7)	Supplementation Follow-up (n=7)	p-value*
Plasma FAD (nmol/ l)	51-160	546 (322-1179)	414 (347-1225)		530 (170-1916)	908 (74-5479)	
Red cell FAD (nmol/ gHb)	1.0-3.4	1.85 (1.58-2.04)	2.15 (1.74-2.15)		1.86 (1.33-2.17)	2.02 (1.24-2.55)	
White cell FAD (pmol/10^6 cells)	NA	12.97 (11.22-13.08)	13.17 (12.86-15.13)		(n=6) 14.87 (12.30-17.29)	(n=6) 13.56 (11.58-15.82)	
Plasma FMN (nmol/ l)	3.3-14.1**	NA	NA		NA	NA	
Red cell FMN (nmol/ gHb)	0.04-0.44**	0.15 (0.10-0.17)	0.16 (0.11-0.17)		(n=4) 0.13 (0.08-0.16)	(n=4) 0.15 (0.08-0.18)	
White cell FMN (pmol/ 10^6 cells)	NA	0.52 (0.52-0.82)	0.58 (0.56-0.76)		(n=3) 0.81 (0.59-0.82)	(n=3) 0.64 (0.56-0.94)	
Plasma riboflavin (nmol/ l)	4-34**	NA	NA		NA	NA	
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	0.07 (0.07-0.09)	0.08 (0.06-0.17)		(n=6) 0.12 (0.04-0.19)	(n=6) 0.10 (0.02-0.62)	
White cell riboflavin (pmol/ 10^6 cells)	NA	0.81 (0.54-0.82)	0.78 (0.58-0.81)		(n=6) 0.84 (0.34-1.28)	(n=6) 0.85 (0.46-1.00)	

Median (range), *Wilcoxon signed rank test, ** Reference intervals from controls

Table 8-11 Plasma, red cell and white cell vitamin B6 concentrations in patients that had plasma PLP concentrations below the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with low plasma PLP concentrations on admission					
	Reference intervals	No supplementation Admission (n=11)	No supplementation Follow-up (n=11)	p-value*	Supplementation Admission (n=11)	Supplementation Follow-up (n=11)	p-value*
Plasma PLP (nmol/ l)	17-135	8 (2-17)	7 (2-12)	0.059	9 (<2-16)	22 (9-63)	0.010
Red cell PLP (pmol/ gHb)	250-680	201 (111-625)	205 (51-399)	0.091	209 (131-1014)	1558 (185-22001)	0.008
White cell PLP (pmol/ 10^6 cells)	NA	(n=8) 1.79 (1.39-2.34)	(n=8) 1.90 (1.38-2.09)	0.779	(n=6) 2.32 (0.89-3.47)	(n=6) 3.14 (1.94-5.18)	0.249
Plasma PL (nmol/ l)	5-26**	(n=10) 5 (<2-10)	(n=10) 5 (2-12)	0.123	(n=10) 4 (<2-19)	(n=10) 44 (3-1057)	0.007
Red cell PL (pmol/ gHb)	25-195**	(n=8) 25 (17-130)	(n=8) 46 (15-127)	0.093	(n=9) 39 (18-242)	(n=9) 824 (54-28868)	0.011
White cell PL (pmol/ 10^6 cells)	NA	(n=8) 0.43 (0.26-1.15)	(n=8) 0.67 (0.17-1.45)	0.069	(n=6) 0.41 (0.18-1.22)	(n=6) 2.51 (0.42-7.17)	0.075

Median (range), *Wilcoxon signed rank test, **Reference intervals from controls

Table 8-12 Plasma, red cell and white cell vitamin B6 concentrations in patients that had plasma PLP concentrations above the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with high plasma PLP concentrations on admission					
	Reference intervals	No supplementation Admission (n=4)	No supplementation Follow-up (n=4)	p-value*	Supplementation Admission (n=2)	Supplementation Follow-up (n=2)	p-value*
Plasma PLP (nmol/ l)	17-135	162 (141-296)	24 (7-357)		252 (198-306)	73 (72-74)	
Red cell PLP (pmol/ gHb)	250-680	321 (205-13717)	301 (207-514)		18748 (11912-25583)	4250 (4233-4268)	
White cell PLP (pmol/ 10^6 cells)	NA	2.08 (1.74-3.98)	1.89 (1.81-2.14)		NA		
Plasma PL (nmol/ l)	5-26**	11 (3-865)	6 (<2-30)		NA		
Red cell PL (pmol/ gHb)	25-195**	(n=3) 65 (50-12640)	(n=3) 71 (55-76)		30122 (6765-53480)	2232 (1747-2716)	
White cell PL (pmol/ 10^6 cells)	NA	0.64 (0.54-8.06)	0.64 (0.48-1.48)		NA		

Median (range), *Wilcoxon signed rank test, **Reference intervals from controls

Table 8-13 Plasma and white cell vitamin C concentrations in patients that had plasma ascorbic acid concentrations below the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with low plasma vitamin C concentrations on admission				
	Reference intervals	No supplementation Admission (n=11)	No supplementation Follow-up (n=11)	p-value*	Supplementation Follow-up (n=19)	p-value*
Plasma ascorbic acid (µmol/ l)	15-40	<10 (<10-14)	<10 (<10-10)	0.332	<10 (<10-69)	0.017
White cell ascorbic acid (µmol/ 10^9 cells)	1.5-2.5**	(n=27) 1.6 (0.46-8.77)	(n=5) 1.45 (0.67-3.12)	0.345	(n=10) 2.20 (1.02-5.80)	0.646

Median (range), *Wilcoxon signed rank test, **Reference intervals from Lee et al. (1982)

Table 8-14 Plasma and white cell vitamin C concentrations in patients that had plasma ascorbic acid concentrations below the reference interval on admission to ICU and had or not recorded supplementation in ICU

		Critically ill patients with low white cell vitamin C concentrations on admission				
	Reference intervals	Admission (n=10)	No supplementation Follow-up (n=2)	p-value*	Supplementation Follow-up (n=1)	p-value*
Plasma ascorbic acid (µmol/ l)	15-40	<10 (<10-17)	<10 (<10-<10)	0.317	NA	NA
White cell ascorbic acid (µmol/ 10^9 cells)	1.5-2.5**	0.87 (0.46-1.47)	0.76 (0.67-0.84)	0.180	NA	NA

Median (range), *Wilcoxon signed rank test, **Reference intervals from Lee et al. (1982)

9 The relationship between vitamin B2, B6, C, A, E and carotenoids'concentrations, lipid peroxidation and hospital mortality in patients with critical illness

9.1 Introduction

Nutritional support, in the form of parenteral and enteral nutrition, is a cornerstone of care for patients with critical illness. The concept of feeding such patients is to maintain energy balance and micronutrient status (Kreymann et al. 2006). In particular, micronutrients have been proposed to prevent cellular damage by reactive oxygen species, for example lipid peroxidations and malondialdehyde (MDA) formation, which have been linked to the development of multiple organ dysfunction syndrome and poor prognosis in patients with critical illness (Hill & Hill 1998; Mishra et al. 2005).

Antioxidant status has been reported to be suboptimal in critical illness (Cowley et al. 1996; Alonso de Vega et al. 2000; Mishra et al. 2005; Doise et al. 2008). Therefore, there is continuing interest in optimising micronutrient status for critically ill patients admitted to ICU (Heyland et al. 2005).

Vitamin supplementation should be guided by the assessment of vitamin status. However, reliable measurement of vitamin status in the critically ill is problematical. In health, plasma concentrations of most vitamins have been shown to be associated with status (Fell & Talwar 1998). However, in the critically ill patient, plasma concentrations are often perturbed by the presence of systemic inflammatory response syndrome. Indeed, there is evidence that plasma concentrations of water-soluble B (Hustad et al. 2002; Gray et al. 2004; Quasim et al. 2005) and C vitamins (Louw et al. 1992), and lipid-soluble retinol, α-tocopherol, and carotenoids

(Quasim et al. 2004; Gray et al. 2005) do not reflect status in the presence of a systemic inflammatory response. Therefore, although low plasma concentrations are often used as the rationale for vitamin supplementation in the critically ill the basis for such supplementation is not well founded.

Recently, there has been increasing evidence that intracellular vitamin concentrations in blood may be a more reliable measure of status in the patient with critical illness and systemic inflammatory response syndrome. Red cell B2 and B6 have been reported to be more reliable indicators of status compared with plasma concentrations (Gray et al. 2004; Quasim et al. 2005). Also, red cell vitamin E and white cell vitamin C have also been reported to be more reliable indicators of status compared with plasma concentrations (Kitagawa, Nakagawa & Mino 1983; Omaye et al.1987; Lehmann et al. 1988; Louw et al. 1992).

Therefore, the aim of the present study was to examine the relationships between MDA, water and lipid soluble vitamins and survival in patients with critical illness.

9.2 Materials and Methods

9.2.1 Patients and study design

See paragraph 2.1.1

9.2.2 Collection and preparation of blood samples

An EDTA tube containing approximately 6 mL of whole blood was taken for each patient on admission and follow-up. The EDTA sample was centrifuged (500g, 4^{o}C, 10mins) and 500 uL of plasma was removed into another plastic tube containing, metaphosphoric acid 6%, for plasma vitamin C determination. After removing the buffy coat, the packed red cells were stored for red cell vitamin determination. All tubes were stored at -70^{o} C until analysis. All samples were assayed in a single batch for each of the analytes to minimise interbatch analytical variation.

9.2.2.1 White cell preparation

See paragraph 2.1.2.1

Following the final brief centrifugation (10,000 rpm, 3mins), the supernatant was removed and the resulting pellet was dispersed in 125μL of deionised water and stabilized with 125uL of metaphosphoric acid 6% for the vitamin C analysis. The cell suspension was stored at -80°C. Before analysis, the cell suspension was sonicated for 10 min to lyse the cell membranes.

9.2.3 Analytical Methods

9.2.3.1 Measurements of vitamins B1, B2 and B6

See paragraph 2.1.2.3, 2.1.2.4 and 2.1.2.5

9.2.3.2 Measurement of vitamin C

See paragraph 2.1.2.6

9.2.3.3 Measurement of plasma and red cell vitamin E and plasma carotenoids

See paragraph 2.1.2.7 and 2.1.2.8

9.2.3.4 Measurement of total and free malondialdehyde (MDA)

See paragraph 2.1.2.9

9.2.3.5 Measurement of whole blood and plasma proteins concentrations

See paragraph 2.1.2.2

9.2.4 Statistics

Data from critically ill patient groups are presented as median and range. Comparisons between critically groups were performed with the use of the Mann-Whitney U test. Correlations between variables in the critically ill group were performed with the use of the Spearman's rank correlation (r_s) and a P value of <0.01 was considered to be significant due to the number of observations. Outcome data were analysed by binary logistic regression analysis. Analysis was performed with the use of SPSS software (version 15; SPSS Inc, Chicago, Illinois, U.S.A.).

9.3 Results

In total, one hundred and twenty six critically ill patients (medical n=56, surgical n=70) were studied. The comparisons between the patients at the lowest APACHE II score quartile (n=33) and the patients at the highest APACHE II score quartile (n=34) are shown in Table 9-1. The patients in the lowest APACHE II score quartile were younger ($p<0.001$), mostly surgical ($p<0.01$), had lower hospital predicted mortality ($p<0.001$) and SOFA score ($p<0.001$) and stayed in the ICU for less days ($p<0.01$) compared with the patients in the highest quartile. Albumin, cholesterol and triglyceride concentrations were significantly lower in the group of patients at the highest APACHE II score quartile compared with the group at the lowest quartile ($p<0.01$, $p<0.001$ and $p<0.01$, respectively). Free MDA fraction was significantly higher in the group of patients at the highest APACHE II score quartile compared with the group at the lowest quartile ($p<0.01$). There were no significant differences in water soluble vitamin concentrations between the two groups (Table 9-2). Plasma α-tocopherol was significantly lower in the group at the highest APACHE II score quartile compared with the group at the lowest quartile (Table 9-3, $p<0.01$).

Ninety four patients survived and thirty two died in hospital after their ICU admission (Table 9-4). Non-survivors were older and had significantly higher APACHE II score ($p<0.01$), predicted hospital mortality ($p<0.001$), ventilation days ($p<0.001$) and ICU length of stay ($p<0.001$) compared with the survivors. Survivors had significantly higher hospital length of stay compared with non-survivors ($p<0.01$).

On admission, compared with survivors, free MDA and free MDA fraction were higher in the non-survivors ($p<0.01$ and $p<0.001$, respectively). In contrast, plasma concentrations of albumin ($p<0.01$), retinol ($p<0.05$), α-tocopherol ($p<0.05$), lutein ($p<0.01$) and lycopene

($p<0.01$) were significantly lower in the non-survivor critically ill patient group on admission to ICU (Tables 9-4, 9-5 and 9-6).

The relationship between clinical pathological characteristics, vitamin status and hospital death in patients with critical illness on admission to ICU is shown in Table 9-7. On multivariate logistic regression analysis of the individual significant parameters only albumin ($p<0.05$) and free MDA fraction ($p<0.05$) were independently associated with hospital death. When albumin was removed from the model, free MDA fraction (OR=55.20, 95% CI=3.88-785, p=0.003) and lycopene (OR=0.98, 95% CI=0.97-1.00, p=0.033) were independently associated with hospital death.

The interrelationships between outcome scores and concentrations of plasma proteins, lipids, free MDA fraction, plasma α-tocopherol and carotenoids in the critically-ill patients on admission to ICU are shown in Table 9-8. APACHE II score was directly associated with age ($r_s=0.44$, $p<0.001$), SOFA score ($r_s=0.50$, $p<0.001$) and free MDA fraction ($r_s=0.34$, $p<0.01$) and inversely associated with albumin ($r_s=-0.29$, $p<0.01$), cholesterol ($r_s=-0.38$, $p<0.001$), triglycerides ($r_s=-0.27$, $p<0.01$), plasma α-tocopherol ($r_s=-0.32$, $p<0.001$) and lycopene ($r_s=-0.30$, $p<0.01$). Albumin was directly associated with cholesterol ($r_s=0.68$, $p<0.001$), triglycerides ($r_s=0.28$, $p<0.01$), retinol ($r_s=0.58$, $p<0.001$), α-tocopherol ($r_s=0.58$, $p<0.001$), lutein ($r_s=0.60$, $p<0.001$) and lycopene ($r_s=0.57$, $p<0.001$) and β-carotene ($r_s=0.37$, $p<0.001$), and inversely associated with APACHE II ($r_s=-0.29$, $p<0.01$), SOFA score ($r_s=-0.39$, $p<0.001$) and free MDA fraction ($r_s=-0.29$, $p<0.01$). Free MDA fraction was directly associated with SOFA score ($r_s=0.41$, $p<0.001$) inversely associated with cholesterol ($r_s=-0.34$, $p<0.01$), plasma α-tocopherol ($r_s=-0.29$, $p<0.01$), lutein ($r_s=-0.30$, $p<0.01$) and lycopene ($r_s=-0.34$, $p<0.01$).

9.4 Discussion

In the present study, from the relationship between lipid peroxidation, water and lipid soluble vitamins and outcome in critically ill patients, it was shown that lipid peroxidation, as evidenced by elevated free MDA fraction, was most significantly associated with death in hospital. This study included all the vitamers measured in all different blood compartments and tried to examine whether low plasma or intracellular vitamin concentrations are prognostic of outcome.

In keeping with previous studies (Goode et al. 1995; Cowley et al. 1996), the results of this study show that plasma α-tocopherol concentrations on admission were related to the severity of illness, as measured by APACHE II score, in the critically ill patients. However, taking into consideration that cholesterol and triglyceride concentrations also significantly decreased between the two quartiles and that plasma α-tocopherol concentrations were not significantly different between survivors and non-survivors, the clinical relevance of the association between plasma α-tocopherol concentrations and severity of illness is uncertain. From these data, there was no relationship between plasma or intra-cellular vitamin concentrations and APACHE II scores in patients with critical illness.

In the present study, serum albumin was found to be an independent predictor of hospital outcome in patients with critical illness, independent of severity of illness as assessed by APACHE II score. Indeed, serum albumin has been found to be the best single indicator of concurrent sepsis and anergy and predictor of mortality in seriously ill hospitalized patients (Harvey et al., 1981). An initial albumin of <2.2 g/dL has been associated with a greater than 75% chance of having concurrent anergy and sepsis and dying during hospitalization (Harvey et al., 1981). Surgery has been associated with a fall in serum albumin concentrations, which

persisted despite nutritional support (Harvey et al., 1981). A decrease in serum albumin from concentrations greater than 46 g/L to less than 21 g/L was associated with an exponential increase in mortality rates from less than 1% to 29% and in morbidity rates from 10% to 65%, particularly sepsis and major infections, in surgical patients followed up for 30 days postoperatively (Gibbs et al., 1999).

To date, the present study is the first to report the independent prognostic value of free MDA fraction in patients with critical illness. It was of interest that, despite their recognised antioxidant role, the lipid soluble vitamins although associated with free MDA fraction, had no independent prognostic value. One interpretation of these results would be that it is the degree of lipid peroxidation, rather than the depletion of lipid soluble vitamins, that determines outcome in patients with critical illness. However, for most patients the concentrations of retinol and the carotenoids in the plasma were well below the reference intervals or were undetected and therefore may account for the lack of effect on outcome. This is further complicated by albumin being directly associated with the lipid soluble vitamins in the plasma, in particular retinol, α-tocopherol, lutein and lycopene, suggesting redistribution of these lipid soluble antioxidants. Indeed, consistent with previous studies (Goldwasser & Felman, 1997; Vincent et al., 2003), albumin was shown to have prognostic value independent of APACHE II and when albumin was removed from the multivariate model, lycopene became an independent predictor of hospital death. Taken together these results would suggest that low concentrations of lipid soluble vitamins may be partly due to redistribution in the same way as albumin concentrations fall as part of the systemic inflammatory response syndrome. However, it remains to be established whether such low plasma concentrations are mainly due to increased consumption or redistribution or both.

Similarly, the present study showed that there was no significant difference in C-reactive protein concentrations between survivors and non-survivors. For this reason, we decided not to include C-reactive protein in the univariate-multivariate model. These results are consistent with the results of Pettila and co-workers (2002) although changes in C-reactive protein concentrations have been shown to be predictive of outcome independently of severity of illness (Lobo et al. 2003; Ho et al. 2008).

The basis of the present relationship between an elevated free MDA fraction and poor outcome is not clear. However, it has previously been reported that elevated MDA has been associated with multiple organ dysfunction (Mishra et al. 2005). Indeed, in the present study free MDA fraction was directly associated with APACHE II and SOFA scores on admission to ICU. Therefore, it may be that free MDA fraction as well as being an important prognostic factor, may also provide a useful therapeutic target in patients with critical illness.

In summary, the results of the present study have shown that free malondialdehyde fraction is an independent prosnostic factor for survival in the hospital after admission in the intensive care unit and could be used as a therapeutic target in patients with critical illness.

Table 9-1 Characteristics and measurements of proteins and malondialdehyde between critically ill patients at the lowest and those at the highest quartiles of APACHE II score on admission to ICU

	Reference intervals	APACHE II Lowest quartile (n=33)	APACHE II Highest quartile (n=34)	p-value*
Age (yr)		43 (18-74)	63 (39-81)	<0.001
Sex (M/F)		20/ 13	23/ 11	0.551
Patients (medical/ surgical)		10/ 23	22/ 12	0.005
APACHE II score		12 (3-15)	31 (26-38)	<0.001
Predicted hospital mortality (%)		10.1 (0.2-27.4)	74.3 (4.4-92.6)	<0.001
SOFA score		4 (0-14)	8 (4-14)	<0.001
Ventilation (days)		2 (0-49)	6 (0-35)	0.017
ICU death (yes/ no)		3/ 30	11/ 23	0.020
ICU length of stay (days)		2.3 (0.2-48.1)	7.0 (0.9-34.6)	0.003
Hospital death (yes/ no)		4/ 29	13/ 21	0.015
Hospital length of stay (days)		14 (0.4-77)	24.5 (1.8-240)	0.259
C-reactive protein (mg/ l)	<6	48 (<6-434)	138 (3-565)	0.030
Albumin (g/ l)	35-75	22 (10-47)	15 (9-33)	0.005
Total protein (g/ l)	60-80	48 (32-75)	44 (25-73)	0.236
Cholesterol (mmol/ l)	3.5-5.5	(n=30) 2.7 (0.9-6.4)	 1.6 (0.4-5.0)	<0.001
Triglycerides (mmol/ l)	<2.3	(n=30) 1.2 (0.3-4.7)	(n=33) 0.8 (0.3-2.4)	0.005
Total Malondialdehyde (umol/ l)	0.30-1.00	(n=27) 0.77 (0.24-1.65)	(n=29) 0.65 (0.22-2.34)	0.142
Total Malondialdehyde/ Total protein (μmol/ g)		(n=27) 0.017 (0.01-0.03)	(n=29) 0.014 (0.01-0.09)	0.329
Free Malondialdehyde (μmol/ l)	0.04-0.10	(n=26) 0.07 (0.02-0.38)	(n=29) 0.12 (0.02-0.62)	0.017
Free Malondialdehyde fraction		(n=26) 0.10 (0.04-0.37)	(n=29) 0.19 (0.03-0.58)	0.002

Median (range), *Mann-Whitney U test

Table 9-2 Measurements of water soluble vitamins between critically ill patients at the lowest and those at the highest quartiles of APACHE II score on admission to ICU

	Reference intervals	APACHE II Lowest quartile (n=33)	APACHE II Highest quartile (n=34)	p-value*
Whole blood TDP (ng/ gHb)	275-675	697 (257-2574)	863 (300-3650)	0.087
Plasma FAD (nmol/ l)	51-160	46 (18-290)	(n=33) 41 (10-1916)	0.773
Red cell FAD (nmol/ gHb)	1.0-3.4	(n=32) 1.46 (1.02-2.10)	1.65 (1.06-2.56)	0.186
White cell FAD (pmol/10^6 cells)	NA	(n=19) 12.69 (8.40-90.15)	(n=20) 13.03 (9.24-17.29)	0.482
Plasma FMN (nmol/ l)	3.3-14.1**	(n=11) 17 (5-29)	(n=4) 30 (22-76)	0.013
Red cell FMN (nmol/ gHb)	0.04-0.44**	(n=28) 0.08 (0.05-0.48)	(n=33) 0.11 (0.05-0.33)	0.256
White cell FMN (pmol/ 10^6 cells)	NA	(n=16) 0.67 (0.38-1.73)	(n=20) 0.60 (0.37-0.94)	0.504
Plasma riboflavin (nmol/ l)	4-34**	(n=32) 29 (6-279)	(n=23) 34 (4-748)	0.384
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	(n=32) 0.02 (0.01-0.22)	(n=33) 0.04 (0.00-0.21)	0.208
White cell riboflavin (pmol/ 10^6 cells)	NA	(n=16) 0.60 (0.33-1.04)	(n=20) 0.58 (0.36-1.36)	0.236
Plasma PLP (nmol/ l)	17-135	21 (<9-198)	29 (<9-333)	0.306
Red cell PLP (pmol/ gHb)	250-680	(n=32) 248 (105-25583)	321 (32-27441)	0.140
White cell PLP (pmol/ 10^6 cells)	NA	(n=17) 2.07 (1.30-6.16)	(n=20) 2.01 (0.37-5.18)	0.345
Plasma PL (nmol/ l)	5-26	9 (<9-301)	(n=33) 10 (<9-1346)	0.114
Red cell PL (pmol/ gHb)	25-195	(n=29) 50.0 (6.4-53480)	(n=31) 64.9 (12.0-38223)	0.300
White cell PL (pmol/ 10^6 cells)	NA	(n=17) 0.43 (0.17-10.29)	(n=20) 0.58 (0.07-9.79)	0.223
Plasma ascorbic acid (μmol/ l)	15-40	(n=23) <9 (<9-136)	(n=21) <9 (<9-102)	0.315
White cell ascorbic acid (μmol/ 10^9 cells)	1.5-2.5***	(n=11) 1.79 (0.74-2.57)	(n=11) 1.70 (0.60-8.77)	0.718

Median (range), * Mann-Whitney U test, ** Reference intervals from controls, ***Lee et al. (1982)

Table 9-3 Measurements of lipid soluble vitamins between critically ill patients at the lowest and those at the highest quartiles of APACHE II score on admission to ICU

	Reference intervals	APACHE II Lowest quartile (n=33)	APACHE II Highest quartile (n=34)	p-value*
Plasma retinol (μmol/l)	1.0-2.8	0.75 (0.09-3.10)	0.61 (0.02-2.50)	0.269
Plasma α-tocopherol (μmol/ l)	14-39	17 (6-36)	12 (4-23)	0.004
Red cell α-tocopherol (nmol/gHb)	12-28**	(n=16) 16.9 (11.3-25.3)	(n=23) 18.5 (12.4-29.8)	0.123
Lutein (μg/ l)	82-202	(n=32) 30 (<9-106)	19 (<9-142)	0.036
Lycopene (μg/ l)	100-300	(n=32) 50 (<9-318)	18 (<9-291)	0.016
α-carotene (μg/ l)	14-60	(n=32) 9 (<9-44)	(n=33) 9 (<9-29)	0.157
β-carotene (μg/ l)	92-312	(n=32) 15 (<9-518)	15 (<9-201)	0.126

Median (range), *Mann-Whitney U test

Table 9-4 Characteristics and measurements of proteins and malondialdehyde between survivor and non-survivor critically ill patients on admission to ICU

	Reference intervals	Survivors (n=94)	Non-survivors (n=32)	p-value*
Age (yr)		56 (22-100)	67 (18-81)	0.014
Sex (M/F)		61/ 33	21/ 11	0.940
Patients (medical/ surgical)		42/ 52	14/ 18	0.927
APACHE II		19 (3-38)	24 (10-34)	0.003
Predicted hospital mortality (%)		25.4 (0.2-92.6)	53.3 (11.7-86.3)	<0.001
SOFA score		6 (0-18)	8 (1-14)	0.027
Ventilation (days)		2 (0-35)	12 (0-75)	<0.001
ICU length of stay (days)		3 (1-36)	11 (1-77)	<0.001
Hospital length of stay (days)		24 (1-508)	12 (2-123)	0.002
C-reactive protein (mg/ l)	<6	97 (<6-434)	119 (2-565)	0.577
Albumin (g/ l)	32-45	19 (9-47)	14 (9-33)	0.002
Total protein (g/ l)	60-80	47 (23-83)	43 (26-73)	0.364
Cholesterol (mmol/ l)	3.5-5.5	2.20 (0.53-6.40)	1.90 (0.40-5.20)	0.072
Triglycerides (mmol/ l)	<2.3	1.10 (0.30-5.05)	0.80 (0.30-2.80)	0.041
Total Malondialdehyde (umol/ l)	0.30-1.00	0.69 (0.24-2.34) (n=75)	0.59 (0.22-14.27) (n=29)	0.090
Total Malondialdehyde/ Total protein (μmol/ g)		0.02 (0.01-0.09) (n=75)	0.01 (0.01-0.53) 0.02 (n=29)	0.121
Free Malondialdehyde (μmol/ l)	0.04-0.10	0.08 (0.02-1.37) (n=74)	0.12 (0.04-5.96) (n=29)	0.007
Free Malondialdehyde fraction		0.13 (0.03-1.00) (n=74)	0.21 (0.05-1.04) (n=29)	<0.001

Median (range), *Mann-Whitney U-test

Table 9-5 Measurements of water soluble vitamins between survivor and non-survivor critically ill patients on admission to ICU

	Reference intervals	Survivors (n=94)	Non-survivors (n=32)	p-value*
Whole blood TDP (ng/ gHb)	275-675	778 (257-3650) (n=93)	728 (196-3524)	0.157
Plasma FAD (nmol/ l)	51-160	44 (14-1916) (n=92)	56 (10-1179)	0.263
Red cell FAD (nmol/ gHb)	1.0-3.4	1.49 (0.97-2.58)	1.46 (1.04-2.30)	0.788
White cell FAD (pmol/10^6 cells)		13 (7-90) (n=55)	13 (8-18) (n=22)	0.558
Plasma FMN (nmol/ l)	3.3-14.1**	17 (3-76) (n=23)	19 (12-31) (n=3)	0.446
Red cell FMN (nmol/ gHb)	0.04-0.44**	0.09 (0.05-0.48) (n=80)	0.09 (0.05-0.19) (n=29)	0.773
White cell FMN (pmol/ 10^6 cells)		0.68 (0.37-1.73) (n=46)	0.54 (0.27-1.51) (n=20)	0.132
Plasma riboflavin (nmol/ l)	4-34**	30 (3-458) (n=85)	35 (4-748) (n=19)	0.480
Red cell riboflavin (nmol/ gHb)	0.01-0.13**	0.03 (0.00-0.23)	0.04 (0.01-0.17) (n=31)	0.676
White cell riboflavin (pmol/ 10^6 cells)		0.61 (0.31-3.27) (n=49)	0.59 (0.23-1.32) (n=22)	0.728
Plasma PLP (nmol/ l)	17-135	22 (<10-333)	16 (<10-296)	0.064
Red cell PLP (pmol/ gHb)	250-680	50 (<10-53480) (n=93)	60 (12-13499)	0.995
White cell PLP (pmol/ 10^6 cells)		2.31 (1.02-8.24) (n=51)	2.08 (0.37-6.15) (n=22)	0.186
Plasma PL (nmol/ l)	5-26**	<10 (<10-1346) (n=90)	<10 (<10-286)	0.189
Red cell PL (pmol/ gHb)	25-195**	50 (<10-53480) (n=83)	60 (12-13499) (n=28)	0.664
White cell PL (pmol/ 10^6 cells)		0.49 (0.17-10.29) (n=49)	0.54 (0.07-6.15) (n=21)	0.833
Plasma ascorbic acid (μmol/ l)	15-40	5 (1-136) (n=64)	4 (1-102) (n=20)	0.441
White cell ascorbic acid (μmol/ 10^9 cells)	1.5-2.5***	1.92 (0.96-8.77) (n=28)	1.83 (0.46-3.06) (n=14)	0.571

Table 9-6 Measurements of water and lipid soluble vitamins between survivor and non-survivor critically ill patients on admission to ICU

Plasma retinol (μmol/l)	1.0-2.8	0.80 (0.09-3.10)	0.40 (0.02-4.20)	0.019
Plasma α-tocopherol (μmol/ l)	14-39	15 (5-41)	12 (4-34)	0.020
Red cell α-tocopherol (nmol/gHb)	12-28	18.35 (3.40-31.49) (n=58)	19.14 (11.30-39.28) (n=24)	0.210
Lutein (μg/ l)	82-202	28 (<5-168) (n=92)	14 (<5-246) (n=31)	0.003
Lycopene (μg/ l)	100-300	42 (<5-318) (n=92)	19 (<5-103) (n=31)	0.009
α-carotene (μg/ l)	14-60	9 (<5-173) (n=91)	9 (<5-44) (n=31)	0.626
β-carotene (μg/ l)	92-312	25 (<5-402) (n=92)	16 (<5-522) (n=31)	0.060

Median (range) * Mann-Whitney U test, ** Reference intervals from controls, ***Lee et al. (1982)

Table 9-7 The relationship between clinical characteristics, vitamin status and hospital death in patients with critical illness on admission to ICU
Univariate and multivariate binary logistic regression analysis

	Univariate analysis	p-value	Multivariate analysis	p-value
	Odds ratio (95% CI)		Odds ratio (95% CI)	
Age	1.03 (1.00-1.06)	0.041*		0.896
APACHE II	1.09 (1.03-1.16)	0.004*	1.07 (1.00-1.15)	0.069
SOFA score	1.11 (0.99-1.24)	0.071*		0.995
Albumin	0.92 (0.86-0.98)	0.006*	0.92 (0.85-0.99)	0.025
Cholesterol	0.72 (0.51-1.01)	0.059*		0.660
Triglycerides	0.49 (0.25-0.97)	0.040*		0.637
Free MDA	4.10 (0.44-38.42)	0.217		
Free MDA fraction	41.35 (3.23-529.34)	0.004*	12.59 (1.13-140.18)	0.039
Retinol	0.55 (0.28-1.08)	0.084*		0.400
Plasma α-tocopherol	0.93 (0.87-0.99)	0.022*		0.603
Plasma lutein	0.98 (0.97-1.00)	0.073*		0.874
Plasma lycopene	0.98 (0.97-1.00)	0.011*		0.311
Plasma β-carotene	1.00 (1.00-1.01)	0.933		

*Variables included in the multivariate model.

Table 9-8 Spearman correlations between outcome scores and concentrations of plasma proteins, lipids, free MDA fraction, plasma α-tocopherol and carotenoids in the critically-ill patients on admission to ICU (n=126)

	APACHE II	SOFA	Albumin	Cholesterol	Trigly-cerides	Free MDA fraction	Retinol	α-tocopherol	Lutein	Lycopene	β-carotene
Age	0.44***	0.11	-0.21*	-0.15	0.23*	0.16	-0.03	-0.09	-0.08	-0.25**	-0.06
APACHE II		0.50***	-0.29**	-0.38***	-0.27**	0.34**	-0.14	-0.32***	-0.22*	-0.30**	-0.15
SOFA			-0.39***	-0.49***	-0.17	0.41***	-0.41***	-0.43***	-0.39***	-0.45***	-0.36***
Albumin				0.68***	0.28**	-0.29**	0.58***	0.58***	0.60***	0.57***	0.37***
Cholesterol					0.48***	-0.34**	0.65***	0.84***	0.56***	0.60***	0.45***
Triglycerides						-0.17	0.30**	0.60***	0.09	0.15	-0.01
Free MDA fraction							-0.24*	-0.29**	-0.30**	-0.34**	-0.22*
Retinol								0.63***	0.59***	0.59***	0.45***
α-tocopherol									0.60***	0.58***	0.52***
Lutein										0.76***	0.63***
Lycopene											0.62***

*P<0.05, **P<0.01, ***P<0.001

10 Discussion

The aims of the thesis were to examine whether intracellular vitamin concentrations, as measured in blood cells, are a more reliable measure of status in patients with critical illness, and also, to examine the relationship between plasma and intracellular vitamin concentrations and hospital mortality in patients with critical illness. These were addressed by a prospective longitudinal study that examined the relationship between plasma and intracellular concentrations of a number of vitamins (both water and lipid soluble) and outcome in patients with critical illness.

The major findings of the study were:

1. Intracellular vitamin measurements may be more reliable in assessing vitamin status and in guiding supplementation in patients with systemic inflammatory response. Assessment of vitamin status by measuring plasma concentrations is discouraged in patients with evidence of systemic inflammatory response syndrome.

2. True vitamin B6 deficiency may be more frequent in the critically ill patient and thus vitamin B6 supplementation may be essential.

3. Neither plasma nor intracellular vitamin B2 nor B6 nor C concentrations were related to severity of illness and hospital mortality in patients with critical illness.

10.1 Accurate and reliable assessment of vitamin status in patients with critical illness

Clinical assessment of the general nutritional status of the critically ill patients can be addressed by the use of different tools such as reported recent weight changes, dietary assessment, clinical signs of vitamins deficiency, functional and anthropometric measurements. In the clinical setting, there is a discrepancy between nutritional screening and nutritional assessment with the first including tools for identification of malnourished patients while the second aims to help with the understanding of the present nutritional status and the calculation of current nutritional needs and requirements. Biochemical vitamin measurements are one of the methods traditionally used to assess patient vitamin status in the clinical setting and one of the key pieces of the vitamin status puzzle.

10.1.1 Assessment of plasma vitamin concentrations

A good marker of vitamin status should be able to reflect deficiency and supplementation. On admission to ICU, almost 1/3 of the critically ill patients had plasma vitamin B2, B6 and C concentrations below the reference interval and remained below the reference interval on follow-up. This could be interpreted as indicating deficiency. However it has been shown that plasma B and C vitamin concentrations can decrease transiently due to the effect of the systemic inflammatory response (Louw et al. 1992; Gray et al. 2003; Mishra et al. 2005) and that supplementation is not reflected in plasma concentrations in these patients (Huang et al. 2002; Rumelin et al. 2002; Talwar et al. 2003b; Mishra et al. 2005; Quasim et al. 2005) and the data of the current study are in agreement with these results as plasma concentrations of these vitamins did not increase with supplementation on follow up.

Compared with control subjects, the critically ill patients had lower plasma α-tocopherol concentrations even when expressed per mmol of triglycerides. In contrast, α-tocopherol concentrations were higher when expressed per mmol of cholesterol (although these patients did not receive vitamin E supplementation). Moreover, neither plasma α-tocopherol nor plasma α-tocopherol expressed per mmol of plasma lipids were strongly correlated with red cell α-tocopherol concentrations in the critically ill patients.

Plasma carotenoid concentrations, even when expressed per mmol of cholesterol, remained low compared with the control group. One interpretation of such data could be that these lipid soluble antioxidant vitamins may be consumed during the lipid peroxidation process. However, the majority of patients had plasma retinol concentrations below the reference interval on admission to ICU and these were directly and significantly related to alpha-tocopherol and the carotenoids. Moreover, this relationship was maintained on follow-up. Given that the hepatic stores of retinol are large and that deficiency is rare in Western populations (World Health Organisation 1995) this would suggest that the low concentrations of alpha-tocopherol and carotenoids, in addition to being consumed, maybe lowered by other factors. Indeed, in the present study, there was no or only a weak relationship between the different carotenoids and between the carotenoids and retinol in control subjects. In contrast, in patients with critical illness, the correlations between the different carotenoids became significant and particularly stong illustrating the effect of the systemic inflammatory response and redistribution in these carotenoid concentrations.

10.1.2 Assessment of red cell vitamin concentrations

In contrast, on admission to ICU, red cell FAD and red cell PLP concentrations were significantly higher in the patients with records of prior supplementation compared with the

patients without. Moreover, in patients with plasma vitamin B2 and B6 concentrations below the reference interval, who did not have records of previous supplementation, red cell FAD and red cell PLP concentrations significantly increased after supplementation, in agreement with previous studies (Talwar et al. 2003b; Quasim et al. 2004; Huang et al. 2005).

The critically ill patients of the present study did not receive vitamin E supplementation and their median red cell vitamin E concentrations were found to be similar to healthy subjects.

10.1.3 Assessment of white cell vitamin concentrations

White cell B2 and B6 vitamin concentrations seemed to also be able to reflect supplementation in the present study, with both white cell riboflavin and white cell PL being sensitive to supplementation. Indeed, changes in white cell riboflavin and white cell PL concentrations between admission and follow up were significantly different between the groups that received (group 1), or not (group 2), supplementation in ICU (and did not have record of supplementation prior to ICU admission). This possibly reflects that, within the short period between admission and follow up samples, white cells may more sensitively reflect vitamin B2 and vitamin B6 supplementation compared with either plasma or red cells. Unfortunately, the number of longitudinal white cell riboflavin and white cell PL measurements was low in patients with low plasma FAD and low plasma PLP concentrations on admission, respectively, with recorded supplementation in ICU, and also reference intervals were not available from a control population in order to interpret the white cell riboflavin and white cell PL results. This limits the interpretation of the value of white cell riboflavin and white cell PL as markers of vitamin B2 and B6 status, respectively, in patients with critical illness.

Also, in the present study, compared with plasma ascorbic acid, white cell ascorbic acid concentrations seemed to be a better marker of vitamin C status in patients with critical illness.

On admission to ICU, white cell ascorbic acid concentrations were significantly higher in the patients with records of prior supplementation compared with the patients without. Indeed, white cell ascorbic acid concentrations were above the reference interval in the group with evidence of prior ICU supplementation and their change between admission and follow up was related to the total number of supplementation doses received in ICU. Previous studies have shown that, there is a good correlation between plasma and white cell vitamin C concentrations in healthy subjects (Bates 1977; Jacob, Skala & Omaye 1987; Omaye et al. 1987) and also that, white cell ascorbic acid concentrations reflect cellular stores and vitamin C status (Omaye et al. 1987).

Taken together, these findings suggest that intra-cellular concentrations of vitamins B2, B6, C and E may be more accurate markers of their respective vitamin status in patients with critical illness.

10.2 The importance of supplementation in patients with critical illness

Low concentrations of both plasma and red cell PLP concentrations were identified in approximately half of the patients in this study, although a number of these patients had records of supplementation prior to ICU admission, suggesting that true vitamin B6 deficiency may be more frequent in the critically ill patients and that supplementation may be essential. Vitamin B6 has been shown to be essential for immune function and recent research has been conducted to establish the effect and efficacy of vitamin B6 supplementation in patients with critical illness (Huang et al. 2005; Cheng et al. 2006). Similarly, approximately 1 in 4 patients admitted in ICU had low white cell ascorbic acid concentrations, also suggesting that some of these patients may have true vitamin C deficiency on their admission. In contrast, there was only one patient with red cell FAD concentrations just below the reference interval on admission to ICU, suggesting that vitamin B2 deficiency may not be so common in patients with critical illness and that vitamin B2 supplementation may be unnecessary in these patients.

On admission to ICU, intracellular B2, B6 and C vitamin concentrations and more specifically, red cell FAD, red cell PLP and white cell ascorbic acid concentrations were significantly higher in the patients with records of prior supplementation compared with the patients without. In addition, the medians of these intracellular vitamers were all significantly higher for the group with records of supplementation prior to ICU admission compared with the group without, while they were within the reference intervals for red cell FAD, above the reference interval for white cell ascorbic acid and below the reference interval for red cell PLP in the group that had records of supplementation prior to ICU. Moreover, in patients with plasma vitamin B2 and B6 concentrations below the reference interval and that did not have records of previous supplementation, red cell FAD and red cell PLP concentrations

significantly increased after supplementation with their medians being above the reference interval on follow-up. These results are in agreement with previous studies (Talwar et al. 2003b; Quasim et al. 2004; Huang et al. 2005).

There was no relationship between the number of Pabrinex doses and the change in any of the changes in vitamin B2 and B6 vitamers between admission and follow up. This possibly means that the effect of supplementation may need longer to be evident at the intracellular compartment than the number of days between the admission and our follow up sample and possibly that the relationship between the number of doses with the follow up measurements reflect a longer period of supplementation that many of these patients had starting prior to their ICU admission. Moreover, in the subgroups with records of ICU supplementation and plasma vitamin concentrations above the reference interval on admission, the medians of red cell PLP, plasma PL and red cell PL concentrations did not increase further, possibly suggesting that either saturation or increased utilisation and excretion or both took place between the admission and the follow up sample. Such a metabolic effect could not be examined in the present study as there were no measurements of the relevant metabolic products in either urine or blood. A recent previous study though has shown that there are increased pyridoxic acid concentrations in plasma and urine in patients with critical illness (Cheng et al. 2006). Similar results were observed for the intracellular concentrations of FAD, FMN and riboflavin and also for white cell ascorbic acid concentrations in the subgroups with high plasma FAD and ascorbic acid concentrations on admission, respectively, that had records of supplementation in ICU.

Taken together, these findings suggest that cellular concentrations of vitamins B2, B6 and C may be expected to be a more true reflection of the presence of supplementation than their respective plasma concentrations. Also, from these data, critical illness seems to be associated

with vitamin B6 and possibly vitamin C deficiency without these though to be important for patient severity of illness, as measured by APACHE II score, or survival. Still, correction of vitamin deficiency could be important for some of the patients admitted in ICU and intracellular measurements of these vitamins, from the results of this study, could identify patient vitamin status and guide supplementation in ICU.

10.3 The relationship between intracellular vitamin concentrations, the magnitude of injury and outcome in patients with critical illness

The relationship between admission plasma and intracellular blood concentrations of vitamins B2, B6, C and E and outcome in patients with critical illness was examined. Neither plasma nor intracellular concentrations of B2, B6, C and E were significantly and independently associated with hospital mortality ($p>0.10$). These results would suggest that these vitamins may not play a major role in determining outcome in patients with critical illness.

A higher APACHE II score was associated with lower plasma α-tocopherol concentrations in patients with critical illness at the highest APACHE II quartile compared with the patients at the lowest APACHE II quartile. Taken though into consideration, that at the same time plasma lipids (cholesterol and triglyceride) concentrations were also significantly lower in patients at the highest APACHE II quartile compared with the patients at the lowest and that plasma α-tocopherol concentrations were not related to red cell α-tocopherol concentrations in patients with critical illness or plasma α-tocopherol concentrations in healthy controls, and that plasma α-tocopherol concentrations have been shown to transiently fall in the presence of a systemic inflammatory response (Louw et al. 1992; Gray et al. 2005), the clinical relevance of such an observation is questionable. Neither plasma carotenoids nor plasma nor intracellular concentrations of B2, B6 and C vitamins nor red cell E vitamin were significantly related with severity of illness in the patients of the present study as assessed by APACHE II score.

Free MDA fraction was also found to be an independent prognostic factor of outcome prognosis in patients with critical illness. This relationship and clinical significance of this

finding is not clear especially when neither total MDA nor free MDA were significantly related to hospital mortality in patients with critical illness.

From the data of the present study, it would appear that outcome depends more on the severity of the insult, which lead to the state of critical illness, and its effect on metabolism rather than the presence of low plasma or intra-cellular vitamin concentrations.

10.4 Limitations of the study

In the present study, biochemical measurements in three different blood compartments were used to assess vitamin status in patients with critical illness. Other methods for assessing general nutritional status exist such as anthropometric measurements (changes in weight, BMI, muscle mass, skinfold thickness) and previous diet history (24h hour recall, 7 day food diary, food frequency questionnaires) however most of the above methods of assessing nutritional status are often of limited value in the critically ill patient, bringing challenge to the assessment of vitamin status (Manning & Shenkin 1995; Winkler & Malone 2004). The critically ill patients are a heterogenous group with different medical backgrounds, and are usually unable to provide a dietary history due to sedation/confusion, weight measurements may be erroneous after fluid resuscitation and/ or oedema, and anthropometric measurements are not easily attainable, may be erroneous due to oedema, have been shown to be less sensitive to acute changes of status (Harvey et al. 1981; Winkler & Malone 2004). In addition, clinical symptoms occur at the final stages of vitamin deficiency thus their value as markers of vitamin deficiency and predictors of outcome in patients with critical illness comes possibly too late for the clinician and the patient.

One limitation of the present work was that a number of the critically ill patients had received intravenously B and C vitamin supplementation either before their admission to ICU and/ or during their ICU stay. Clearly, since these patients received vitamin supplementation this may have had a confounding effect on some of the results obtained. For example, it meant that few patients had evidence of intracellular B vitamin deficiency on admission limiting the conclusions of the study on whether low intracellular B vitamin concentrations may be related to poor outcome.

Examining the effect of supplementation in the specific study was challenging as some of our patients had records of supplementation prior to ICU admission. Also, the upper range of some of the vitamins in the non-supplemented group prior to ICU admission were very high suggesting that some of these patients had recently received B and C vitamin supplementation prior to their admission to ICU but to our knowledge there were no records of this in the medical files.

Also, in the present study, there were no control values for white cell vitamin concentrations. In the first 6 months of this study, I had identified approximately 100 anonymous patient blood samples from the Haematology department in which I extracted the white cells and analysed white cell B2 and B6 vitamin concentrations in order to calculate a non-critically ill population reference range. On the completion of this, I was advised that these results could not be used for such a purpose as the majority of the patients admitted in the hospital would be under systemic inflammatory response and thus the vitamin concentrations could be biased.

Another limitation was the smaller number of plasma and white cell vitamin C samples. There was a problem in stabilising the blood samples for vitamin C analysis soon enough as stabilisation has to take place within the first 12 hours of the sample extracted. A number of patients were admitted in the ICU directly from theatre in the evening hours resulting in a big number of samples being unsuitable for vitamin C analysis. In addition, some patients had very low white cell numbers resulting in insufficient white cell pellets during extraction which further compromised the number of white cell vitamin C samples. Thus, these small numbers may have compromised the significance of plasma and white cell vitamin C concentrations in terms of outcome.

A problem with numbers of observations also occurred during the analysis of some of the vitamers especially FMN. The chromatogram produced had split peaks which did not allow the calculation of the vitamer concentration in the sample. That lead to the difference in numbers between the vitamers in the same matrix.

There was no vitamin E or carotenoid supplementation in the critically ill patients admitted in the intensive care unit. Thus the effect of supplementation on red cell vitamin E or plasma carotenoid concentrations could not be examined.

Also it remains to be determined, in patients with critical illness, whether red or white cell concentrations of B2, B6, C and E reflect those in other tissues before a definitive statement can be made as to their value in measuring whole body status. However, given the clinical and ethical problems of removing tissue samples from the critically-ill this issue may remain unresolved for some time.

10.5 Future studies

Future research is required both to confirm these results and also to further investigate the clinical value of the intracellular blood vitamin measurements in patient management.

10.5.1 Studies to confirm the usefulness of intracellular vitamin measurements

The relationship between intracellular vitamin status and tissue stores could be investigated by analysing tissue (liver and muscle being the major stores) obtained from these patients. Removing tissue from critically ill patients has major ethical implications. One way this could be achieved without compromising the health of the patient and without further invasive procedures would be to collect liver biopsy specimens at the time of laparotomy for intra-abdominal sepsis.

Establishing reference intervals for vitamins B2 and B6 in white cells would be required in order to examine whether white cell vitamin concentrations are more reliable than red cell vitamin concentrations.

10.5.2 Intervention studies

Further examination of the effect of supplementation on white cell vitamin C concentrations in patients with critical illness is required with a bigger number of longitudinal samples. Such a task would require longer term patient recruitment, sample collection and white cell extraction than the duration of this study.

10.5.3 Studies that examine the effect of vitamin supplementation on mortality

Studies examining the importance of normal intracellular vitamin concentrations, in terms of outcome for the critically ill patient, could further examine the differences in outcome between patients who receive supplementation and those that they do not. For example, a subgroup of patient samples from the Accident and Emergency Unit could be identified at the Haematology Department immediately after the patients are admitted and consented in the ICU and analysed for vitamin concentrations. It is expected that these samples would be free of IV or oral hospital supplementation treatments. Also, future studies could include results from different centres where vitamin supplementation protocols differ.

More specifically, future studies could examine the effect of vitamin E and carotenoid supplementation on red cell vitamin E and plasma carotenoids in patients with critical illness. Interestingly, in the present study, plasma lycopene was independently associated with hospital mortality when albumin was removed from the multivariate analysis model. Taking into consideration, the established role of poor antioxidant status and adverse outcome in patients with critical illness (Borrelli et al. 1996; Tsai et al. 2000; Alonso de Vega et al. 2002; Nathens et al. 2002; Crimi et al. 2004), a study examining the role of carotenoid supplementation in improving hospital mortality in patients with critical illness would be of substantial interest.

10.6 Final comments

This work, to my knowledge, is the most detailed systematic examination of how best to assess vitamin status and its role in outcome in large cohort of patients with critical illness. These results have led to changes in the methodology and practice of vitamin analysis in the Scottish Trace Elements and Micronutrients Reference Laboratory, Glasgow Royal Infirmary. In particular, analysis of vitamins B2 and B6 for assessing their status is now carried out in red cells rather than plasma in the acutely ill patient in Glasgow Royal Infirmary. In these patients such measurements may be more informative and clinically relevant. Furthermore, such measurements may be more reliable in guiding supplementation in patients with systemic inflammatory response. Assessment of vitamin status by measuring plasma concentrations is discouraged in patients with evidence of systemic inflammatory response syndrome.

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12 Appendices

12.1 Appendix 1 Data collection proforma

Intensive Care Proforma

	Admission (1)	(2)	(3)	Discharge
Dates - Time				
Tube coding for routine				
Tube coding				
APACHE II				
Predicted mortality (%)				
MOF (systems)				
E/P Nutrition (sort/rate)				
Vitamin supplements				
Oral anticoagulants (warfarin/enoxaparin)				
Weight (kg)			Height (m)	
Smoker (yes/no)		Cigar/day		
Alcohol intake/Drugs				
Diagnosis for ICU admission				
Did the patient die? (Y/N) Date				
Pabrinex or oral B vitamin supplementation (Number/timing)	Prior to admission (1)	Prior to sample (2)	Prior to sample (3)	
CRP				
Albumin				
WBC				

12.2 Appendix 2 Consent form for participation in the study

Patient Identification Number for this study:

19th June 2006

CONSENT FORM

The relationship between the systemic inflammatory response, micronutrient status and outcome in patients with critical illness

(Version 2A)

Name of Researcher: Dr John Kinsella

I confirm that I have read and understand the information sheet dated 5th June 2006 (version 2A) for the above study and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my medical care or legal rights being affected.

I give my permission for my GP to be informed about my participation in the above study.

I understand that the blood samples taken from me for routine purposes will be kept and analysed for research purposes which include vitamin and trace element concentrations in different blood compartments. I understand that these samples will not be returned to me.

I understand that the sections of any of my medical notes may be looked at by responsible individuals from the research team where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

_____	_____	_____
Name of patient	Date	Signature
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Name of welfare guardian or Next of kin	Date	Signature

Nearest relative: Yes / No If yes, relationship to patient:_____

Welfare guardian: Yes / No

_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient, 1 for researcher, 1 to be kept with hospital notes

12.3 Appendix 3 Information leaflet for patients and relatives

19th June 2006

PATIENT INFORMATION SHEET

The relationship between the systemic inflammatory response, micronutrient status and outcome inpatients with critical illness

(Version 2A)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled 'Medical Research and You.' This leaflet gives more information about medical research and looks at some questions you may want to ask. If you would like to have a copy we will provide one.

Thank you for reading this.

What is the Purpose of the Study?
We wish to investigate the changes that occur in the concentrations of vitamins and other micronutrients in the blood during and after critical illness. This will require the further analysis of some of the blood samples that were routinely taken during your illness and a further blood sample after you have left intensive care.
We will also ask you some questions about your state of health and your diet. From this information we intend to establish the best method of assessing the vitamin and trace element nutritional status in critically ill patients.

Why have I been chosen?

All patients admitted to Glasgow Royal Infirmary ICU are being considered for admission into this study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

Your treatment will not be altered by taking part to the study.

Phase 1 - When in the intensive care unit we would like to run some extra tests on your blood samples that have already been taken from you as part of routine. We will not ask you for anything else other than your consent to measure the vitamin and trace element concentrations in different compartments of your blood. This assessment is not done routinely and it will help us better assess the health of other patients admitted in the intensive care unit.

Phase 2 - If you agree to that we will also ask you to give us one more blood sample at clinic, and an interview about your health 3 – 6 months after your discharge from the intensive care unit. At this time we would also like you to provide us with a 7 day food diary that you will complete before coming to see us.

What do I have to do?

You don't have to do anything while in hospital (phase 1). If you agree to take part in phase 2 (at clinic) you only have to complete a 7 day food diary before visiting the surgical clinic. Please try not to change your usual eating habits when completing the diary. You should also follow your doctor's instructions and take your medication as prescribed.

Will any of my travel expenses be covered?

Travel expenses for routine hospital visits will not be covered. However, if you have to come to the hospital just for the purposes of the study your travel expenses will be covered in full.

What are the side effects of taking part?

There are no side effects of taking part in this study. It is purely an observational study does not include drug or nutritional intervention. In addition, if at any point you become concerned in any way please contact Dr John Kinsella, Consultant in Critical Care, tel. 0141 211 4625.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks of taking part to this study.

What are the possible benefits of taking part?
As your treatment will be unchanged, there is no intended clinical benefit from taking part in the study. However, the information that we collect may help us to improve the treatment of future patients admitted to the intensive care unit.

What happens when the study stops?
Your treatment and follow up will continue as normal.

What if something goes wrong?
We have seen that the study will be absolutely safe for the patient as being observational in nature. If you are harmed by taking part in this study, there are no special compensation arrangements. Standard NHS indemnity does, however, apply in any case of adverse event. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Will my taking part in this study be kept confidential?
Yes. All information, which is collected about you during the course of research, will be kept strictly confidential. All the information required for the study will be kept strictly within the hospital departments. We have also set up a coding system so that your name will be replaced by a unique number and all of your information and results will be matched to this unique number and not your name.
If you agree, we will write to your GP and inform them of your participation in the study.

What will happen to the results of the research study?

The results of the study will be in large part to PhD thesis studies and may be published in scientific journals. Moreover, some of the results may also be presented in scientific meetings, e.g. conferences. We expect to publish our first results in 2007. If you wish you can obtain a copy of the published material from the University Department of Anaesthesia.
It is important to understand that at no publication or presentation of the results your name and/or personal details about your health will be identified.

Who is organizing and funding the research?
The funding of this research is from collaborative group of clinicians and scientists in the Glasgow Royal Infirmary.

Who has reviewed the study?
The study has been reviewed and approved by the Local Research Ethics Committee of the Royal Infirmary of Glasgow and the MREC for Scotland, Committee A.

Contact for Further Information
If you have any further questions or wish to obtain more information, please do not hesitate to contact the chief investigator –
Dr John Kinsella
Consultant
University Department of Anaesthesia
Royal Infirmary
Tel - 0141 211 4625

12.4 Appendix 4 Information leaflet for General Practitioner

Patient Name: ……

19th June 2006

GENERAL PRACTIONER INFORMATION SHEET

The relationship between the systemic inflammatory response, micronutrient status and outcome inpatients with critical illness

(Version 2)

We would like to inform you that your patient has consented to take part to the study mentioned above while in Intensive Care Unit in Glasgow Royal Infirmary.

This is an observational study that aims to investigate the changes in micronutrient status that occur during and after critical illness. This will require the further analysis of some of the samples that were routinely taken during your patient's illness and a further blood sample after the patient has left intensive care.

There are no side effects or benefits of taking part in this study. It is purely an observational study does not include drug or nutritional intervention.

The study has been reviewed and approved by the Local Research Ethics Committee of the Royal Infirmary of Glasgow and the MREC for Scotland, Committee A.

Contact for Further Information

If you have any further questions or wish to obtain more information, please do not hesitate to contact Dr John Kinsella, tel. 0141 211 4625.

12.5 Appendix 5 Development of a white cell extraction method

First of all I had to stabilize the technique of separating a good proportion (> 40%) of white cells from small quantities (2 mL) of fresh whole blood. To do that I used anonymous blood samples from haematology. I started with the technique that T.P. Chang had developed for his summer projected but found that severe clotting, and platelets and haemoglobin concentrations were in my final preparations. Reading papers that described white cell separation, I found out that using the water bath at 37^{o}C and centrifuging my tubes at room temperature were creating these clots (Hinks, Colmsee & Delves 1982). Instead, I used cold temperatures to avoid clotting. Together with Dr Dinesh Talwar, we established a method of bringing our blood to the right temperature (2-3^{o}C) without freezing it and we show that this solved the problem of clotting and removed all the haemoglobin that was contaminating our white cells. Moreover, I centrifuge the tubes at 4-5^{o}C. Instead of using NH_4Cl, as described in the previous method, I read that there would be less clumping and better separation of mononuclear cells from platelets if our lysing solution contained EDTA, as EDTA prevents platelet binding to leukocytes (Milne, Ralston & Wallwork 1985). Ammonium chloride has the disadvantage that associated changes in pH can damage and destroy the white cells, thus producing low yields of a final leucocyte preparation that may not be representative tissue (Hinks, Colmsee & Delves 1982). Our red blood cells' lysing solution (described in the methods in the following pages) has a pH of 7.4 so white cell losses have been minimized. The last problem was how to remove the platelets. The method of T.P. Chang was describing three centrifugations of the tubes at 1000 rpm. Centrifugation of the samples for the third time at 500 rpm rather than 1000 rmp was able to remove all platelets as they are lighter and remain in the supernatant so they are removed easily. The method provided a white cell extraction yield of 63% (n=76, 50-93%) containing mainly neutrophils (>90%).

Because of the clumping problems I performed protein determination of my white cell samples together with a white cell count, using the Bradford reagent and measuring absorbance after 15 minutes incubation at 595 nm (method described in the following pages). The rational behind this is that the machine that does the cell count can only measure intact cells. So cells that might have burst will not be detected. However, a protein determination would give us more robust results as it is not affected by cell integrity. Our cell counts correlated extremely well with the protein determination results (see figure 8-1).

White cell extraction method description

For the white cell extraction conical plastic tubes (15 ml) were used. All reagents and centrifugation were kept at a temperature of approximately 4oC. This temperature prevented the white cells from forming clots and aided in the removal of haemoglobin. Two hundred μL of whole blood were transferred into Eppendorff tubes (1.9 mL) to be used for comparison of white cells in the count machine. Two mL of whole blood were put in a conical plastic tube and 9 mL of diluted sterile red blood cell lysing solution was added. The tubes were then gently mixed by a roller for 2 minutes and stored in the freezer for five minutes. Mixing by the roller and freezing for the same timings was repeated for once more, then the tubes were mixed again for 2 minutes and kept in the fridge for 15 minutes. The tubes were centrifuged at 1,000 rpm for 10 minutes at 4-5oC. The supernatant was carefully removed so as not to disturb the white cell pellet. Ten mL of cold PBS solution (Invitrogen, Paisley, UK) were added to the pellet of each tube and the latter was resuspended by mixing. The tubes were again centrifuged, supernatant was removed and the pellet was resuspended for once more as described above. The tubes were finally centrifuged at 500 rpm for 10 minutes at 4-5oC, the supernatant was removed and 1 mL of PBS solution was added to the final pellet. The pellet was well resuspended in the solution and the final mixture was transferred to Eppendorff tubes

(1.9 mL). The samples were taken to the blood count machine (KX – 21N, Sysmex, UK Ltd) and both the whole blood samples and white cell samples were assessed. The white cell samples were then centrifuged at 10,000 rpm for 3 minutes, the supernatant was removed and the pellet was resuspended by adding 500 μL of cold water.

Protein Determination

Standard 3.1 mL Assay Protocol

Dilute 100 mg BSA to 10 mL of H_2O (10 mg/mL) – stock solution.

Take 1 mL of stock solution to 9 mL of H_2O (1 mg/mL) – tube A.

Take 1 mL of tube A to fresh tube and add 1 mL of H_2O (0.5 mg/mL) – tube B.

Take 1 mL of tube B to fresh tube and add 1 mL of H_2O (0.25 mg/mL) – tube C.

Take 1 mL of tube C to fresh tube and add 1 mL of H_2O (0.125 mg/mL) – tube D.

Take 100 μL of each tube to new tubes and add 3 mL Bradford Reagent.

To make blank tube: take 100 μL of H_2O and add 3 mL Bradford Reagent – tube E.

Vortex gently but thoroughly all tubes and let them incubate at room temperature for 20 minutes.

Transfer the samples into cuvets and measure the absorbance at 595 nm.

Micro 2 mL Assay Protocol

Dilute 100 mg BSA to 10 mL of H_2O (10 mg/mL) – stock solution.

Take 1 mL of stock solution to 9 mL of H_2O (1 mg/mL) – tube O.

Take 100 μL of tube O to 9.9 mL of H_2O (10 μg/mL) – tube A.

Take 2 mL of tube A to fresh tube and add 2 mL of H_2O (5 μg/mL) – tube B.

Take 2 mL of tube B to fresh tube and add 2 mL of H_2O (2.5 μg/mL) – tube C.

Take 2 mL of tube C to fresh tube and add 2 mL of H_2O (1.25 μg/mL) – tube D.

Take 1 mL of each tube to new tubes and add 1 mL Bradford Reagent.

To make blank tube: take 1 mL of H_2O and add 1 mL Bradford Reagent – tube E.

Vortex gently but thoroughly all tubes and let them incubate at room temperature for 20 minutes.

Transfer the samples into cuvets and measure the absorbance at 595 nm.

Unknown sample protein determination

To the frozen samples add 275 μL of H_2O and mix well to redistribute the pallet.

Put in the freezer to -50^oC for 15 minutes.

Thaw the samples and mix them well.

Sonicate them for 20 minutes.

Mix them well with the vortex mixer.

Take 25 μL into fresh glass tube and add 75 μL of H_2O. Add 3 mL of Bradford Reagent.

Take 25 μL into fresh glass tube and add 975 μL of H_2O. Add 1 mL of Bradford Reagent.

Reagents

1. Bradford solution

2. Sterile Red Blood Cell Lysing Solution

Sterile RBC Lysing Solution has a reagent formula = 155 mM NH4Cl, 10 mM NaHCO3, 0.1 mM EDTA; pH = 7.4. The solution is used cold at 2-8 C and has a shelf life of six months when made and kept sterile. For the white cell extraction a dilution with water 1:10 was prepared and used.

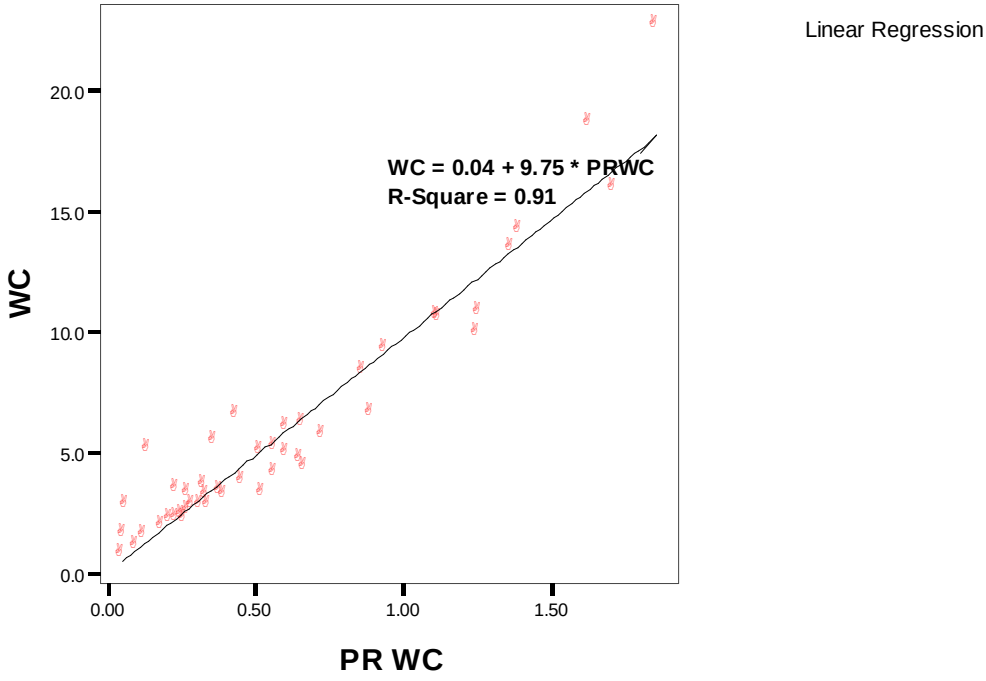

Figure 12-1 The relationship between white cell counts (WC) and white cell protein (PR WC) in anonymous hospital samples (n=76)

12.6 Appendix 6 Data coding

Pre-ICU Pabrinex doses: Doses of recorded vitamin B and C supplementation each patient received prior to their admission to the intensive care unit. The number 1000 is a code and refers to B vitamin supplementation prescribed to the patient on a regular basis in the community.

Pre + ICU Pabrinex doses: The total recorded vitamin B and C supplementation doses the patient received by the time the follow-up sample was taken.

12.7Appendix 7 Chapter 3 data

Appendix 7. Characteristics and biochemical measurements of controls (Chapter 3)

Control No.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma FAD (nmol/l)	Plasma FMN (nmol/l)	Plasma riboflavin (nmol/l)	Plasma FAD/ Riboflavin	Red cell FAD (pmol/g Hb)	Red cell FMN (pmol/g Hb)	Red cell riboflavin (pmol/g Hb)	Red cell FAD/ Riboflavin
1.00	45.00	0.00	6.00	82.00	6.60	9.00	9.11	0.70	--	0.03	24.14
2.00	47.00	1.00	6.00	117.00	7.20	8.00	14.63	0.90	0.05	0.02	50.00
3.00	51.00	1.00	6.00	92.00	4.40	12.00	7.67	1.00	0.11	0.02	45.45
4.00	39.00	0.00	6.00	67.00	3.90	4.00	16.75	1.10	0.13	0.08	13.58
5.00	53.00	1.00	6.00	88.00	4.90	7.00	12.57	1.10	0.15	0.03	37.93
6.00	47.00	1.00	6.00	68.00	5.70	7.00	9.71	1.20	0.13	0.02	54.55
7.00	54.00	1.00	6.00	145.00	7.20	10.00	14.50	1.20	0.05	--	--
8.00	50.00	1.00	6.00	150.00	8.40	34.00	4.41	1.20	0.05	--	--
9.00	71.00	0.00	6.00	63.00	--	5.00	12.60	1.30	0.07	0.01	108.33
10.00	58.00	1.00	6.00	89.00	5.10	4.00	22.25	1.30	0.05	0.01	92.86
11.00	48.00	0.00	6.00	94.00	7.20	17.00	5.53	1.30	0.04	--	--
12.00	68.00	1.00	6.00	124.00	9.30	23.00	5.39	1.30	0.07	--	--
13.00	73.00	1.00	6.00	130.00	6.00	20.00	6.50	1.30	0.04	0.02	76.47
14.00	61.00	1.00	6.00	69.00	8.30	5.00	13.80	1.40	0.15	0.03	42.42
15.00	45.00	0.00	6.00	102.00	4.80	15.00	6.80	1.40	0.07	0.01	100.00
16.00	44.00	0.00	6.00	107.00	7.50	10.00	10.70	1.40	0.08	0.02	77.78
17.00	73.00	1.00	6.00	126.00	10.00	28.00	4.50	1.40	0.07	--	--
18.00	60.00	1.00	6.00	127.00	5.40	12.00	10.58	1.40	0.07	0.11	13.21
19.00	68.00	0.00	6.00	60.00	13.80	5.00	12.00	1.50	--	0.03	44.12
20.00	58.00	0.00	6.00	69.00	--	7.00	9.86	1.50	0.09	--	--
21.00	42.00	0.00	6.00	75.00	--	12.00	6.25	1.50	0.12	0.02	78.95
22.00	60.00	0.00	6.00	88.00	5.40	8.00	11.00	1.50	0.06	--	--
23.00	65.00	1.00	6.00	100.00	6.60	21.00	4.76	1.50	0.06	--	--
24.00	48.00	0.00	6.00	103.00	--	11.00	9.36	1.50	0.12	0.05	30.61

Control No.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma FAD (nmol/l)	Plasma FMN (nmol/l)	Plasma riboflavin (nmol/l)	Plasma FAD/ Riboflavin	Red cell FAD (pmol/g Hb)	Red cell FMN (pmol/g Hb)	Red cell riboflavin (pmol/g Hb)	Red cell FAD/ Riboflavin
25.00	41.00	0.00	6.00	73.00	3.90	4.00	18.25	1.60	0.17	0.03	51.61
26.00	53.00	1.00	6.00	87.00	4.00	7.00	12.43	1.60	--	0.02	106.67
27.00	66.00	1.00	6.00	87.00	--	10.00	8.70	1.60	0.13	0.03	64.00
28.00	42.00	0.00	6.00	94.00	10.50	15.00	6.27	1.60	0.09	0.05	32.65
29.00	42.00	0.00	6.00	96.00	4.50	7.00	13.71	1.60	0.08	0.01	123.08
30.00	56.00	0.00	6.00	101.00	6.90	9.00	11.22	1.60	0.06	0.01	123.08
31.00	55.00	0.00	6.00	107.00	--	14.00	7.64	1.60	0.07	--	--
32.00	61.00	0.00	6.00	109.00	3.90	7.00	15.57	1.60	0.05	--	--
33.00	41.00	1.00	6.00	122.00	8.40	7.00	17.43	1.60	0.13	0.09	17.58
34.00	38.00	0.00	6.00	148.00	9.90	23.00	6.43	1.60	0.12	0.03	59.26
35.00	70.00	0.00	6.00	57.00	6.00	8.00	7.13	1.70	0.05	0.02	100.00
36.00	64.00	1.00	6.00	69.00	6.30	13.00	5.31	1.70	0.09	0.02	89.47
37.00	64.00	0.00	6.00	92.00	4.40	7.00	13.14	1.70	0.16	0.02	100.00
38.00	47.00	1.00	6.00	94.00	5.00	11.00	8.55	1.70	0.07	--	--
39.00	59.00	1.00	6.00	98.00	5.40	16.00	6.13	1.70	0.21	0.03	68.00
40.00	40.00	0.00	6.00	117.00	7.50	23.00	5.09	1.70	0.08	0.01	121.43
41.00	61.00	1.00	6.00	138.00	5.40	6.00	23.00	1.70	0.05	--	--
42.00	72.00	1.00	6.00	170.00	12.60	27.00	6.30	1.70	0.09	0.01	130.77
43.00	62.00	0.00	6.00	73.00	5.70	13.00	5.62	1.80	0.11	0.01	180.00
44.00	39.00	0.00	6.00	77.00	5.70	5.00	15.40	1.80	0.09	0.02	78.26
45.00	43.00	1.00	6.00	77.00	3.30	5.00	15.40	1.80	0.11	--	--
46.00	72.00	1.00	6.00	81.00	4.20	7.00	11.57	1.80	0.12	0.01	150.00
47.00	71.00	1.00	6.00	87.00	5.10	9.00	9.67	1.80	0.15	0.02	100.00
48.00	45.00	0.00	6.00	92.00	3.80	8.00	11.50	1.80	0.06	0.06	28.13
49.00	49.00	1.00	6.00	93.00	7.20	13.00	7.15	1.80	0.08	--	--
50.00	48.00	1.00	6.00	100.00	5.90	13.00	7.69	1.80	0.05	0.01	180.00
51.00	48.00	0.00	6.00	100.00	6.30	19.00	5.26	1.80	0.12	0.04	45.00
52.00	46.00	1.00	6.00	102.00	4.80	4.00	25.50	1.80	0.14	0.02	105.88
53.00	39.00	0.00	6.00	103.00	3.60	10.00	10.30	1.80	0.07	0.02	100.00
54.00	70.00	0.00	6.00	116.00	6.60	6.00	19.33	1.80	0.15	0.06	32.73
55.00	39.00	0.00	6.00	118.00	5.40	4.00	29.50	1.80	--	0.01	150.00
56.00	47.00	1.00	6.00	120.00	8.40	19.00	6.32	1.80	0.09	--	--
57.00	51.00	0.00	6.00	121.00	6.30	10.00	12.10	1.80	0.13	0.02	105.88
58.00	41.00	1.00	6.00	140.00	6.90	23.00	6.09	1.80	0.08	0.02	81.82

Control No.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma FAD (nmol/l)	Plasma FMN (nmol/l)	Plasma riboflavin (nmol/l)	Plasma FAD/ Riboflavin	Red cell FAD (pmol/g Hb)	Red cell FMN (pmol/g Hb)	Red cell riboflavin (pmol/g Hb)	Red cell FAD/ Riboflavin
59.00	38.00	0.00	6.00	71.00	6.60	19.00	3.74	1.90	--	0.01	158.33
60.00	57.00	0.00	6.00	77.00	5.70	9.00	8.56	1.90	0.05	0.01	135.71
61.00	35.00	0.00	6.00	83.00	5.10	8.00	10.38	1.90	0.05	0.02	126.67
62.00	67.00	1.00	6.00	93.00	5.10	10.00	9.30	1.90	0.20	0.06	29.69
63.00	72.00	1.00	6.00	95.00	5.90	11.00	8.64	1.90	0.27	0.08	23.46
64.00	45.00	1.00	6.00	140.00	7.50	8.00	17.50	1.90	0.09	--	--
65.00	54.00	1.00	6.00	77.00	6.00	7.00	11.00	2.00	0.11	--	--
66.00	58.00	1.00	6.00	77.00	4.80	6.00	12.83	2.00	0.15	0.03	64.52
67.00	58.00	0.00	6.00	85.00	6.90	9.00	9.44	2.00	0.14	--	--
68.00	57.00	0.00	6.00	115.00	7.20	8.00	14.38	2.00	0.07	0.03	60.61
69.00	38.00	0.00	6.00	116.00	5.40	9.00	12.89	2.00	0.06	0.02	125.00
70.00	47.00	1.00	6.00	117.00	7.20	10.00	11.70	2.00	0.08	0.02	133.33
71.00	53.00	0.00	6.00	143.00	5.40	8.00	17.88	2.00	0.08	0.04	51.28
72.00	47.00	0.00	6.00	94.00	7.80	9.00	10.44	2.10	0.13	0.03	67.74
73.00	51.00	1.00	6.00	106.00	6.70	19.00	5.58	2.10	0.12	0.02	116.67
74.00	63.00	1.00	6.00	112.00	6.00	19.00	5.89	2.10	0.22	0.02	105.00
75.00	51.00	0.00	6.00	71.00	3.30	4.00	17.75	2.20	0.17	0.04	57.89
76.00	63.00	1.00	6.00	77.00	9.60	20.00	3.85	2.20	0.10	0.02	146.67
77.00	52.00	0.00	6.00	88.00	--	13.00	6.77	2.20	0.16	0.10	21.57
78.00	69.00	0.00	6.00	89.00	4.80	8.00	11.13	2.20	0.11	0.02	146.67
79.00	49.00	0.00	6.00	98.00	--	15.00	6.53	2.20	0.11	0.02	100.00
80.00	57.00	0.00	6.00	101.00	6.70	12.00	8.42	2.20	0.14	0.02	91.67
81.00	53.00	0.00	6.00	118.00	7.00	10.00	11.80	2.20	0.09	0.03	75.86
82.00	67.00	1.00	6.00	119.00	7.40	12.00	9.92	2.20	0.09	0.02	91.67
83.00	53.00	1.00	6.00	132.00	11.10	22.00	6.00	2.20	0.14	0.03	70.97
84.00	57.00	0.00	6.00	137.00	14.10	28.00	4.89	2.20	0.31	0.04	61.11
85.00	57.00	0.00	6.00	108.00	6.00	28.00	3.86	2.30	0.44	0.13	17.42
86.00	48.00	0.00	6.00	122.00	7.80	15.00	8.13	2.30	0.06	0.03	67.65
87.00	59.00	0.00	6.00	78.00	4.80	12.00	6.50	2.40	0.23	0.03	72.73
88.00	66.00	0.00	6.00	79.00	3.90	4.00	19.75	2.40	0.06	--	--
89.00	43.00	0.00	6.00	80.00	8.10	25.00	3.20	2.40	0.11	0.01	171.43
90.00	60.00	0.00	6.00	105.00	5.70	18.00	5.83	2.40	0.25	0.05	50.00
91.00	70.00	0.00	6.00	137.00	10.80	31.00	4.42	2.40	0.30	0.07	32.43
92.00	62.00	0.00	6.00	70.00	3.70	8.00	8.75	2.60	0.10	0.02	136.84

Control No.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma FAD (nmol/l)	Plasma FMN (nmol/l)	Plasma riboflavin (nmol/l)	Plasma FAD/ Riboflavin	Red cell FAD (pmol/g Hb)	Red cell FMN (pmol/g Hb)	Red cell riboflavin (pmol/g Hb)	Red cell FAD/ Riboflavin
93.00	37.00	0.00	6.00	110.00	6.10	19.00	5.79	2.60	0.08	0.02	108.33
94.00	45.00	0.00	6.00	115.00	4.80	5.00	23.00	2.60	0.19	0.03	100.00
95.00	50.00	0.00	6.00	100.00	6.60	8.00	12.50	2.70	0.07	0.01	225.00
96.00	62.00	1.00	6.00	116.00	7.70	19.00	6.11	2.70	0.14	0.03	79.41
97.00	58.00	1.00	6.00	140.00	5.00	19.00	7.37	2.70	0.05	0.01	225.00
98.00	69.00	1.00	6.00	94.00	5.50	6.00	15.67	2.80	0.07	0.01	280.00
99.00	51.00	0.00	6.00	96.00	6.00	25.00	3.84	2.80	0.22	0.03	100.00
100.00	49.00	1.00	6.00	119.00	--	19.00	6.26	2.80	0.18	0.03	96.55
101.00	55.00	1.00	6.00	123.00	8.10	14.00	8.79	2.80	0.13	0.02	147.37
102.00	66.00	1.00	6.00	95.00	5.90	9.00	10.56	2.90	0.10	0.01	241.67
103.00	57.00	0.00	6.00	139.00	--	17.00	8.18	2.90	0.11	0.03	93.55
104.00	53.00	0.00	6.00	86.00	4.80	13.00	6.62	3.00	0.20	0.03	111.11
105.00	70.00	0.00	6.00	96.00	7.20	8.00	12.00	3.00	0.21	0.03	88.24
106.00	55.00	0.00	6.00	102.00	5.70	19.00	5.37	3.00	0.24	0.03	111.11
107.00	44.00	1.00	6.00	108.00	6.70	4.00	27.00	3.00	0.10	0.03	111.11
108.00	44.00	0.00	6.00	120.00	6.90	14.00	8.57	3.00	0.21	0.04	81.08
109.00	54.00	0.00	6.00	87.00	3.60	4.00	21.75	3.10	0.26	0.02	182.35
110.00	47.00	0.00	6.00	106.00	6.90	12.00	8.83	3.10	0.24	0.02	129.17
111.00	45.00	1.00	6.00	116.00	7.50	16.00	7.25	3.10	0.08	--	--
112.00	66.00	0.00	6.00	121.00	9.90	12.00	10.08	3.10	0.20	0.04	81.58
113.00	63.00	0.00	6.00	82.00	4.20	10.00	8.20	3.30	0.26	0.03	126.92
114.00	71.00	0.00	6.00	105.00	11.80	14.00	7.50	3.30	0.08	0.02	220.00
115.00	56.00	1.00	6.00	121.00	6.80	16.00	7.56	3.30	0.15	0.03	122.22
116.00	48.00	1.00	6.00	124.00	7.10	13.00	9.54	3.30	0.11	0.01	275.00
117.00	70.00	1.00	6.00	130.00	9.90	12.00	10.83	3.70	0.13	0.02	154.17
118.00	38.00	0.00	6.00	159.00	9.30	21.00	7.57	3.70	0.15	0.01	264.29
119.00	45.00	1.00	6.00	150.00	9.10	23.00	6.52	3.80	0.14	0.03	131.03

Appendix 7. Characteristics and biochemical measurements of patients (Chapter 3)

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex doses	ICU Death	ICU stay (days)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
12.00	61.00	0.00	29.00	76.60	0.00	0.00	0.00	0.00	7.40	7.00	7.00	2.00
13.00	43.00	1.00	12.00	11.70	1.00	0.00	2.00	0.00	48.10	5.00	9.00	3.00
14.00	71.00	1.00	18.00	28.90	1.00	0.00	0.00	1.00	12.90	8.00	9.00	2.00
15.00	53.00	1.00	19.00	32.20	0.00	0.00	1.00	0.00	4.80	8.00	2.00	5.00
16.00	61.00	0.00	23.00	28.50	0.00	0.00	--	0.00	1.00	6.00	--	--
17.00	18.00	0.00	12.00	20.20	0.00	0.00	3.00	1.00	6.20	2.00	0.00	4.00
18.00	67.00	0.00	18.00	44.40	1.00	0.00	0.00	1.00	59.50	10.00	10.00	4.00
19.00	50.00	0.00	21.00	38.90	0.00	0.00	3.00	1.00	46.30	10.00	9.00	4.00
21.00	61.00	0.00	34.00	76.80	1.00	0.00	--	0.00	2.00	8.00	--	--
22.00	52.00	0.00	13.00	9.90	0.00	95.00	101.00	0.00	3.70	2.00	3.00	4.00
23.00	71.00	0.00	23.00	42.50	1.00	9.00	10.00	1.00	20.90	8.00	4.00	7.00
24.00	77.00	1.00	21.00	35.00	0.00	0.00	--	0.00	2.00	4.00	--	--
27.00	76.00	1.00	23.00	46.00	0.00	1.00	3.00	0.00	21.70	5.00	4.00	3.00
29.00	70.00	0.00	16.00	20.20	1.00	4.00	10.00	0.00	5.10	4.00	4.00	4.00
30.00	73.00	1.00	25.00	53.30	1.00	0.00	2.00	1.00	15.50	11.00	7.00	3.00
31.00	62.00	0.00	19.00	27.10	1.00	0.00	--	0.00	0.50	11.00	--	--
32.00	55.00	1.00	15.00	21.00	1.00	0.00	--	0.00	2.20	6.00	--	--
33.00	80.00	1.00	31.00	84.20	1.00	0.00	0.00	1.00	12.00	7.00	8.00	5.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	0.00	29.40	6.00	7.00	4.00
35.00	20.00	0.00	18.00	39.70	1.00	0.00	1.00	1.00	44.00	10.00	7.00	3.00
36.00	43.00	1.00	38.00	92.60	0.00	5.00	10.00	0.00	25.50	13.00	10.00	4.00
37.00	60.00	0.00	27.00	63.10	0.00	0.00	0.00	0.00	21.80	10.00	9.00	4.00
38.00	74.00	0.00	20.00	23.00	1.00	0.00	--	0.00	1.00	4.00	--	--
40.00	47.00	0.00	22.00	28.50	1.00	0.00	2.00	0.00	1.90	9.00	4.00	3.00
41.00	76.00	1.00	29.00	79.90	1.00	0.00	--	0.00	1.40	8.00	--	--
42.00	79.00	0.00	21.00	30.90	1.00	0.00	3.00	0.00	3.20	4.00	6.00	4.00
44.00	81.00	0.00	34.00	86.30	0.00	0.00	0.00	1.00	16.10	7.00	3.00	8.00
45.00	74.00	0.00	33.00	75.20	0.00	0.00	0.00	1.00	16.00	11.00	5.00	5.00
46.00	41.00	1.00	8.00	15.60	1.00	0.00	--	0.00	0.80	1.00	--	--
47.00	76.00	0.00	29.00	77.20	1.00	0.00	0.00	1.00	34.60	7.00	7.00	2.00
48.00	67.00	0.00	31.00	68.10	1.00	1,000.00	1,007.00	1.00	18.40	6.00	11.00	4.00

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex doses	ICU Death	ICU stay (days)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
50.00	60.00	0.00	19.00	48.00	1.00	0.00	0.00	0.00	6.20	7.00	4.00	6.00
51.00	46.00	1.00	24.00	49.70	0.00	1,000.00	1,006.00	1.00	4.60	14.00	16.00	3.00
52.00	61.00	0.00	33.00	78.60	0.00	7.00	10.00	1.00	7.70	11.00	11.00	3.00
53.00	67.00	1.00	18.00	21.20	0.00	0.00	0.00	1.00	11.10	11.00	10.00	3.00
55.00	80.00	1.00	31.00	73.30	0.00	0.00	3.00	1.00	8.20	8.00	8.00	4.00
56.00	40.00	1.00	6.00	10.20	1.00	0.00	--	0.00	0.40	6.00	--	--
57.00	60.00	0.00	27.00	60.20	1.00	0.00	--	1.00	1.80	10.00	--	--
58.00	41.00	0.00	21.00	17.80	1.00	0.00	0.00	0.00	19.70	4.00	12.00	2.00
59.00	68.00	0.00	20.00	32.30	0.00	0.00	--	0.00	0.80	5.00	--	--
60.00	57.00	0.00	24.00	67.00	0.00	0.00	--	0.00	6.50	3.00	--	--
61.00	76.00	1.00	18.00	14.40	0.00	0.00	--	0.00	0.70	5.00	--	--
62.00	74.00	0.00	20.00	19.90	1.00	0.00	0.00	0.00	0.90	1.00	2.00	3.00
63.00	62.00	0.00	18.00	44.40	1.00	1,000.00	--	0.00	1.00	2.00	--	--
64.00	66.00	0.00	34.00	81.00	0.00	0.00	--	0.00	22.40	13.00	--	--
65.00	38.00	1.00	17.00	26.20	0.00	0.00	--	0.00	18.90	9.00	--	--
67.00	68.00	1.00	31.00	80.30	0.00	1,000.00	0.00	0.00	5.00	4.00	4.00	5.00
68.00	74.00	0.00	24.00	49.70	0.00	0.00	7.00	0.00	10.70	14.00	12.00	5.00
69.00	61.00	0.00	22.00	54.90	1.00	1,000.00	1,004.00	0.00	76.40	10.00	13.00	4.00
71.00	25.00	0.00	17.00	1.20	0.00	0.00	--	0.00	2.00	3.00	--	--
72.00	51.00	0.00	21.00	12.40	0.00	1.00	1.00	0.00	15.50	11.00	7.00	12.00
73.00	69.00	0.00	12.00	20.20	0.00	0.00	--	0.00	5.80	3.00	--	--
74.00	65.00	0.00	33.00	80.40	0.00	0.00	7.00	0.00	19.60	6.00	11.00	4.00
75.00	28.00	1.00	14.00	18.50	1.00	0.00	--	0.00	0.60	10.00	--	--
77.00	54.00	1.00	17.00	1.20	0.00	0.00	--	0.00	0.80	7.00	--	--
78.00	45.00	0.00	12.00	5.50	1.00	0.00	--	0.00	0.90	4.00	--	--
79.00	41.00	0.00	7.00	7.60	1.00	0.00	0.00	0.00	9.30	4.00	3.00	3.00
80.00	38.00	0.00	16.00	23.30	1.00	1,000.00	--	0.00	0.80	9.00	--	--
81.00	71.00	0.00	25.00	35.90	1.00	0.00	1.00	0.00	8.30	5.00	6.00	3.00
82.00	80.00	1.00	17.00	12.70	0.00	0.00	1.00	0.00	10.30	1.00	1.00	2.00
83.00	46.00	1.00	11.00	10.30	1.00	0.00	--	0.00	2.90	4.00	--	--
84.00	56.00	0.00	28.00	63.90	0.00	1,000.00	1,006.00	0.00	15.40	13.00	10.00	3.00
85.00	73.00	0.00	18.00	44.40	1.00	0.00	0.00	0.00	1.40	6.00	7.00	2.00
87.00	34.00	1.00	12.00	12.40	1.00	0.00	--	0.00	2.30	6.00	--	--
88.00	81.00	0.00	17.00	23.60	1.00	1,000.00	1,004.00	1.00	9.30	9.00	9.00	5.00

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex doses	ICU Death	ICU stay (days)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
89.00	22.00	1.00	10.00	9.50	1.00	0.00	--	0.00	0.20	2.00	--	--
90.00	53.00	0.00	32.00	60.90	1.00	0.00	2.00	0.00	3.00	10.00	7.00	4.00
91.00	62.00	0.00	22.00	58.90	1.00	0.00	--	0.00	1.70	9.00	--	--
94.00	32.00	1.00	25.00	53.30	0.00	0.00	--	0.00	17.30	18.00	--	--
96.00	63.00	1.00	18.00	4.80	0.00	0.00	0.00	0.00	1.90	3.00	2.00	3.00
99.00	37.00	0.00	13.00	16.50	1.00	0.00	--	0.00	2.10	5.00	--	--
100.00	50.00	1.00	26.00	38.20	0.00	0.00	12.00	0.00	2.60	4.00	4.00	3.00
102.00	26.00	0.00	23.00	45.70	1.00	0.00	--	0.00	2.30	4.00	2.00	2.00
103.00	52.00	0.00	16.00	12.20	1.00	0.00	0.00	0.00	2.00	2.00	1.00	3.00
104.00	38.00	0.00	3.00	2.70	0.00	0.00	--	0.00	0.30	2.00	--	--
105.00	39.00	0.00	34.00	87.50	0.00	1,000.00	1,001.00	0.00	3.00	11.00	8.00	2.00
107.00	45.00	1.00	12.00	6.60	1.00	0.00	--	0.00	3.10	8.00	--	--
108.00	44.00	1.00	6.00	0.90	0.00	0.00	--	0.00	0.80	3.00	--	--
110.00	48.00	1.00	26.00	35.20	0.00	0.00	8.00	1.00	4.90	7.00	12.00	5.00
111.00	23.00	0.00	9.00	4.30	1.00	0.00	7.00	0.00	9.20	7.00	2.00	5.00
112.00	45.00	1.00	24.00	22.40	1.00	0.00	0.00	1.00	10.70	1.00	10.00	4.00
113.00	70.00	0.00	27.00	47.70	0.00	0.00	2.00	1.00	6.50	4.00	5.00	3.00
114.00	65.00	1.00	24.00	65.70	1.00	0.00	3.00	0.00	32.80	9.00	8.00	4.00
115.00	47.00	0.00	10.00	1.50	0.00	0.00	--	0.00	0.60	2.00	--	--
116.00	61.00	0.00	13.00	10.60	0.00	0.00	--	0.00	2.40	1.00	--	--
117.00	48.00	0.00	13.00	8.50	1.00	0.00	2.00	0.00	4.20	3.00	3.00	4.00
118.00	59.00	0.00	23.00	39.90	1.00	0.00	1.00	0.00	12.10	9.00	12.00	2.00
119.00	67.00	1.00	18.00	14.40	0.00	0.00	--	0.00	1.30	2.00	--	--
121.00	86.00	0.00	22.00	42.20	1.00	0.00	3.00	0.00	6.60	7.00	4.00	5.00
123.00	60.00	0.00	16.00	14.60	0.00	1,000.00	1,004.00	0.00	3.10	7.00	6.00	3.00
124.00	33.00	0.00	14.00	27.40	1.00	1.00	4.00	0.00	4.70	7.00	6.00	2.00
126.00	68.00	0.00	27.00	46.00	0.00	1,000.00	--	0.00	1.60	5.00	--	--
127.00	100.00	0.00	25.00	64.60	1.00	0.00	--	0.00	3.50	12.00	--	--
128.00	80.00	1.00	30.00	56.90	1.00	0.00	--	0.00	2.90	7.00	--	--
129.00	31.00	0.00	19.00	44.00	0.00	0.00	--	0.00	2.90	12.00	--	--
131.00	65.00	0.00	21.00	55.30	1.00	0.00	--	0.00	6.70	8.00	--	--
133.00	35.00	0.00	14.00	7.20	1.00	0.00	--	0.00	0.90	0.00	--	--
135.00	41.00	1.00	16.00	14.30	1.00	0.00	--	0.00	0.60	2.00	--	--
137.00	69.00	1.00	19.00	32.00	1.00	0.00	--	0.00	0.80	4.00	--	--

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex doses	ICU Death	ICU stay (days)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
139.00	54.00	1.00	23.00	55.80	1.00	0.00	--	0.00	2.60	8.00	--	--
140.00	74.00	0.00	21.00	41.60	1.00	0.00	--	0.00	2.10	8.00	--	--
141.00	67.00	0.00	32.00	78.00	1.00	0.00	--	0.00	19.60	10.00	2.00	11.00
143.00	52.00	0.00	10.00	17.40	1.00	0.00	--	1.00	9.80	2.00	--	--
146.00	38.00	0.00	18.00	24.20	1.00	6.00	21.00	0.00	10.30	6.00	5.00	6.00
147.00	70.00	0.00	20.00	35.50	1.00	0.00	0.00	0.00	1.50	7.00	3.00	2.00
148.00	71.00	0.00	25.00	53.30	0.00	0.00	--	0.00	0.80	5.00	--	--
149.00	74.00	0.00	15.00	--	1.00	0.00	--	1.00	13.80	5.00	--	--
150.00	62.00	0.00	21.00	38.90	0.00	0.00	--	0.00	35.20	11.00	--	--
151.00	36.00	0.00	8.00	8.60	1.00	--	--	0.00	1.20	0.00	--	--
152.00	47.00	0.00	11.00	7.60	0.00	0.00	--	0.00	2.00	3.00	--	--
153.00	77.00	1.00	27.00	46.00	0.00	--	--	0.00	3.10	4.00	--	--
154.00	49.00	0.00	26.00	4.40	0.00	0.00	--	0.00	0.90	4.00	--	--
155.00	43.00	0.00	25.00	3.80	0.00	0.00	--	0.00	0.80	8.00	--	--
156.00	41.00	0.00	33.00	78.60	0.00	0.00	--	1.00	1.80	12.00	--	--
157.00	61.00	0.00	31.00	81.40	1.00	0.00	--	0.00	11.40	14.00	--	--
158.00	56.00	0.00	11.00	22.30	1.00	--	--	0.00	9.00	3.00	--	--
159.00	60.00	1.00	19.00	11.30	1.00	0.00	--	0.00	1.50	4.00	--	--
163.00	77.00	1.00	26.00	56.70	1.00	0.00	--	0.00	1.40	10.00	--	--
164.00	62.00	1.00	14.00	9.70	0.00	0.00	--	0.00	7.00	10.00	--	--
165.00	35.00	0.00	5.00	0.20	0.00	0.00	--	0.00	1.00	1.00	--	--
166.00	63.00	0.00	13.00	13.40	1.00	0.00	--	0.00	0.60	8.00	--	--
167.00	56.00	0.00	32.00	76.00	0.00	1.00	--	0.00	2.10	8.00	--	--
168.00	52.00	1.00	26.00	56.90	0.00	3.00	--	0.00	34.10	10.00	--	--
169.00	39.00	1.00	13.00	24.60	1.00	66.00	--	0.00	24.60	14.00	--	--
171.00	67.00	0.00	27.00	60.50	0.00	0.00	--	0.00	2.30	14.00	--	--

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Globulins admission (g/l)	Globulins follow-up (g/l)	Plasma FAD admission (nmol/l)	Plasma FAD follow-up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow-up (nmol/l)
12.00	126.00	156.00	24.00	23.00	22.00	22.00	77.20	79.90	--	--
13.00	22.00	261.00	27.00	22.00	28.00	25.00	111.70	63.50	--	--
14.00	220.00	124.00	12.00	10.00	33.00	27.00	81.20	56.80	--	--
15.00	257.00	73.00	23.00	16.00	40.00	38.00	375.90	145.60	--	--
16.00	122.00	--	28.00	--	44.00	--	75.00	--	--	--
17.00	3.00	55.00	33.00	26.00	40.00	23.00	289.60	73.70	--	--
18.00	152.00	64.00	10.00	10.00	21.00	20.00	29.80	62.20	--	--
19.00	143.00	101.00	12.00	14.00	56.00	24.00	133.40	1,510.30	--	--
21.00	23.00	--	10.00	--	15.00	--	195.90	--	--	--
22.00	20.00	67.00	27.00	15.00	44.00	25.00	58.70	55.00	18.50	4.70
23.00	125.00	216.00	10.00	13.00	29.00	35.00	87.00	157.00	--	--
24.00	81.00	--	35.00	--	39.00	--	70.30	--	--	--
27.00	103.00	133.00	23.00	17.00	31.00	29.00	170.10	307.10	--	--
29.00	59.00	20.00	9.00	10.00	16.00	29.00	22.80	33.20	--	--
30.00	26.00	179.00	16.00	11.00	28.00	31.00	826.50	1,697.50	--	--
31.00	50.00	--	22.00	--	24.00	--	42.40	--	--	--
32.00	434.00	--	17.00	--	22.00	--	58.80	--	--	--
33.00	259.00	173.00	17.00	10.00	24.00	25.00	41.30	25.30	--	--
34.00	404.00	336.00	30.00	15.00	26.00	24.00	658.40	908.30	--	--
35.00	119.00	303.00	9.00	13.00	18.00	23.00	36.70	42.50	--	--
36.00	132.00	70.00	14.00	14.00	21.00	25.00	1,915.70	911.30	--	--
37.00	81.00	35.00	12.00	12.00	33.00	27.00	321.50	414.40	--	--
38.00	127.00	--	14.00	--	42.00	--	41.90	--	--	--
40.00	15.00	234.00	20.00	18.00	19.00	23.00	67.40	51.70	--	--
41.00	184.00	--	19.00	--	14.00	--	37.60	--	--	--
42.00	8.00	149.00	17.00	18.00	15.00	19.00	29.90	28.90	--	--
44.00	151.00	55.00	10.00	16.00	27.00	27.00	546.20	346.50	--	--
45.00	258.00	164.00	14.00	14.00	27.00	31.00	37.00	881.50	--	--
46.00	250.00	--	11.00	--	21.00	--	31.10	--	--	--
47.00	64.00	72.00	24.00	24.00	34.00	29.00	1,179.40	1,224.70	--	--
48.00	119.00	123.00	10.00	9.00	47.00	38.00	529.60	5,478.50	--	--
50.00	180.00	141.00	23.00	15.00	8.00	27.00	52.40	58.20	34.20	6.90
51.00	56.00	58.00	14.00	19.00	29.00	28.00	53.70	89.60	11.50	7.10

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Globulins admission (g/l)	Globulins follow-up (g/l)	Plasma FAD admission (nmol/l)	Plasma FAD follow-up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow-up (nmol/l)
52.00	565.00	257.00	9.00	9.00	34.00	35.00	67.20	100.40	--	--
53.00	148.00	131.00	9.00	9.00	25.00	24.00	61.80	56.30	--	--
55.00	107.00	71.00	13.00	9.00	28.00	26.00	30.40	43.80	--	3.60
56.00	37.00	--	16.00	--	17.00	--	28.60	--	4.90	--
57.00	181.00	--	10.00	--	27.00	--	10.40	--	--	--
58.00	6.00	36.00	19.00	12.00	20.00	27.00	35.60	22.70	7.70	5.20
59.00	32.00	--	36.00	--	30.00	--	69.30	--	--	--
60.00	166.00	--	26.00	--	49.00	--	52.70	--	--	--
61.00	380.00	--	26.00	--	39.00	--	58.70	--	--	--
62.00	88.00	180.00	12.00	14.00	26.00	29.00	28.70	32.80	5.40	4.30
63.00	117.00	--	14.00	--	13.00	--	18.80	--	--	--
64.00	311.00	--	18.00	--	46.00	--	31.00	--	--	--
65.00	163.00	--	23.00	--	31.00	--	41.20	--	--	--
67.00	438.00	52.00	20.00	16.00	15.00	20.00	24.10	26.70	--	--
68.00	67.00	139.00	11.00	11.00	26.00	25.00	19.60	38.90	--	8.20
69.00	179.00	356.00	14.00	16.00	22.00	25.00	10.30	36.00	--	10.70
71.00	2.00	--	38.00	--	37.00	--	42.20	--	--	--
72.00	40.00	89.00	32.00	15.00	26.00	41.00	78.20	86.30	6.10	12.10
73.00	2.00	--	31.00	--	31.00	--	38.20	--	--	--
74.00	77.00	28.00	15.00	13.00	33.00	29.00	--	115.40	--	6.80
75.00	184.00	--	11.00	--	26.00	--	28.70	--	--	--
77.00	2.00	--	20.00	--	27.00	--	33.20	--	--	--
78.00	177.00	--	27.00	--	34.00	--	53.00	--	--	--
79.00	2.00	251.00	24.00	21.00	20.00	23.00	77.60	51.10	12.40	6.90
80.00	126.00	--	14.00	--	44.00	--	54.50	--	--	--
81.00	8.00	284.00	10.00	21.00	13.00	23.00	43.60	68.70	--	--
82.00	4.00	86.00	29.00	29.00	31.00	33.00	19.20	57.30	2.80	4.60
83.00	318.00	--	21.00	--	27.00	--	42.20	--	--	--
84.00	63.00	80.00	9.00	9.00	20.00	23.00	27.40	26.70	21.90	19.30
85.00	176.00	198.00	11.00	9.00	15.00	18.00	25.30	34.80	3.60	4.10
87.00	110.00	--	18.00	--	22.00	--	39.30	--	24.40	--
88.00	108.00	104.00	12.00	11.00	39.00	40.00	29.50	43.90	--	--
89.00	31.00	--	16.00	--	17.00	--	41.90	--	--	--
90.00	159.00	38.00	11.00	29.00	30.00	9.00	20.90	39.70	--	--

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Globulins admission (g/l)	Globulins follow-up (g/l)	Plasma FAD admission (nmol/l)	Plasma FAD follow-up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow-up (nmol/l)
91.00	268.00	--	14.00	--	26.00	--	--	--	--	--
94.00	147.00	--	14.00	--	33.00	--	61.40	--	--	--
96.00	76.00	27.00	29.00	28.00	35.00	28.00	46.40	55.00	--	--
99.00	287.00	--	22.00	--	30.00	--	33.40	--	--	--
100.00	253.00	171.00	13.00	9.00	28.00	24.00	20.30	12.80	--	--
102.00	28.00	198.00	19.00	19.00	19.00	20.00	26.20	26.20	28.10	23.10
103.00	--	69.00	42.00	27.00	31.00	22.00	53.10	35.00	--	--
104.00	1.00	--	34.00	--	37.00	--	73.40	--	28.40	--
105.00	25.00	23.00	30.00	29.00	36.00	28.00	25.50	19.10	29.30	--
107.00	261.00	--	17.00	--	22.00	--	45.30	--	--	--
108.00	48.00	--	35.00	--	35.00	--	44.90	--	28.70	--
110.00	199.00	153.00	13.00	11.00	35.00	23.00	18.20	71.80	--	16.70
111.00	6.00	245.00	28.00	13.00	25.00	24.00	42.70	25.50	--	--
112.00	2.00	165.00	20.00	9.00	22.00	17.00	64.70	18.70	19.40	--
113.00	14.00	205.00	31.00	19.00	29.00	24.00	51.40	24.10	30.70	--
114.00	49.00	98.00	16.00	9.00	21.00	18.00	30.40	12.40	--	34.10
115.00	1.00	--	39.00	--	24.00	--	67.80	--	17.30	--
116.00	3.00	--	24.00	--	24.00	--	34.90	--	6.80	--
117.00	9.00	88.00	32.00	23.00	23.00	22.00	64.40	36.00	19.60	--
118.00	252.00	204.00	12.00	9.00	21.00	19.00	16.60	22.10	--	--
119.00	2.00	--	25.00	--	25.00	--	68.20	--	16.60	--
121.00	203.00	88.00	15.00	15.00	22.00	24.00	13.70	30.50	--	--
123.00	2.00	207.00	45.00	28.00	38.00	30.00	85.80	50.50	7.70	--
124.00	97.00	149.00	15.00	14.00	25.00	30.00	18.00	39.40	11.60	--
126.00	3.00	--	21.00	--	25.00	--	158.60	--	75.60	--
127.00	316.00	--	17.00	--	42.00	--	17.40	--	--	--
128.00	51.00	--	16.00	--	30.00	--	34.60	--	--	--
129.00	63.00	--	20.00	--	33.00	--	37.80	--	--	--
131.00	165.00	--	21.00	--	26.00	--	19.90	--	--	--
133.00	35.00	--	16.00	--	20.00	--	47.70	--	7.20	--
135.00	221.00	--	18.00	--	40.00	--	40.50	--	--	--
137.00	218.00	--	11.00	--	23.00	--	43.70	--	--	--
139.00	72.00	--	14.00	--	19.00	--	48.30	--	--	--
140.00	51.00	--	9.00	--	17.00	--	99.80	--	--	--

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Globulins admission (g/l)	Globulins follow-up (g/l)	Plasma FAD admission (nmol/l)	Plasma FAD follow-up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow-up (nmol/l)
141.00	143.00	80.00	9.00	9.00	31.00	33.00	40.10	56.20	--	--
143.00	39.00	--	14.00	--	46.00	--	61.50	--	--	--
146.00	73.00	22.00	26.00	18.00	12.00	27.00	58.00	85.50	--	--
147.00	203.00	216.00	32.00	24.00	24.00	27.00	57.60	59.90	--	--
148.00	17.00	--	23.00	--	31.00	--	78.40	--	--	--
149.00	94.00	--	13.00	--	27.00	--	26.10	--	--	--
150.00	295.00	--	24.00	--	31.00	--	41.80	--	--	--
151.00	229.00	--	13.00	--	34.00	--	41.80	--	--	--
152.00	26.00	--	35.00	--	30.00	--	100.40	--	--	--
153.00	45.00	--	19.00	--	31.00	--	52.80	--	--	--
154.00	39.00	--	33.00	--	29.00	--	84.50	--	--	--
155.00	52.00	--	19.00	--	49.00	--	45.30	--	--	--
156.00	316.00	--	20.00	--	53.00	--	48.00	--	--	--
157.00	294.00	--	13.00	--	31.00	--	21.40	--	--	--
158.00	163.00	--	10.00	--	24.00	--	45.80	--	--	--
159.00	137.00	--	15.00	--	24.00	--	43.00	--	--	--
163.00	269.00	--	12.00	--	26.00	--	34.60	--	--	--
164.00	291.00	--	24.00	--	34.00	--	45.80	--	--	--
165.00	0.60	--	47.00	--	28.00	--	87.60	--	--	--
166.00	75.00	--	13.00	--	24.00	--	66.90	--	--	--
167.00	74.00	--	23.00	--	20.00	--	58.90	--	--	--
168.00	131.00	--	11.00	--	27.00	--	39.40	--	--	--
169.00	273.00	--	14.00	--	36.00	--	75.80	--	--	--
171.00	203.00	--	20.00	--	32.00	--	49.50	--	--	--

Patient No.	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow-up (nmol/l)	Plasma FAD/ Riboflavin admission	Plasma FAD/ Riboflavin follow-up	Red cell FAD admission (pmol/g Hb)	Red cell FAD follow-up (pmol/g Hb)	Red cell FMN admission (pmol/g Hb)	Red cell FMN follow-up (pmol/g Hb)	Red cell riboflavin admission (pmol/g Hb)	Red cell riboflavin follow-up (pmol/g Hb)
12.00	32.70	39.50	2.36	2.02	1.76	1.70	0.14	0.14	0.05	0.05
13.00	42.70	103.90	2.62	0.61	1.53	1.54	0.16	0.14	0.06	0.05
14.00	170.30	300.00	0.48	0.19	1.82	1.91	0.14	0.12	0.09	0.08
15.00	--	--	--	--	1.46	1.42	--	--	0.03	--
16.00	77.20	--	0.97	--	1.05	--	0.08	--	0.03	--
17.00	--	--	--	--	1.33	1.24	0.08	0.08	0.04	0.02
18.00	7.80	3.00	3.82	20.73	1.13	1.08	0.07	0.06	0.01	0.01
19.00	--	--	--	--	1.69	1.90	--	--	0.04	0.12
21.00	53.00	--	3.70	--	1.42	--	0.09	--	0.03	--
22.00	279.00	172.10	0.21	0.32	2.09	2.00	0.22	0.21	0.22	0.14
23.00	--	--	--	--	1.63	1.67	0.09	0.09	0.02	0.03
24.00	62.70	--	1.12	--	2.29	--	--	--	0.05	--
27.00	--	--	--	--	1.63	1.88	--	--	0.10	0.10
29.00	198.70	66.20	0.11	0.50	1.57	1.86	0.11	0.13	0.07	0.06
30.00	--	--	--	--	1.97	2.02	--	--	0.14	0.27
31.00	13.50	--	3.14	--	1.30	--	0.08	--	0.03	--
32.00	18.20	--	3.23	--	1.38	--	0.09	--	0.03	--
33.00	--	--	--	--	1.20	1.26	0.08	0.08	0.01	0.01
34.00	--	--	--	--	1.86	2.04	0.13	0.18	0.19	0.10
35.00	--	--	--	--	1.35	1.68	0.09	0.10	0.01	0.01
36.00	--	--	--	--	2.17	2.03	0.14	0.13	0.17	0.06
37.00	--	--	--	--	1.58	1.74	0.10	0.11	0.07	0.08
38.00	45.60	--	0.92	--	1.70	--	0.11	--	0.06	--
40.00	--	--	--	--	1.41	1.33	0.09	0.09	0.01	0.01
41.00	--	--	--	--	1.20	--	0.06	--	0.04	--
42.00	43.00	40.10	0.70	0.72	1.29	--	0.08	--	0.02	--
44.00	--	--	--	--	2.04	2.15	0.17	0.17	0.07	0.06
45.00	--	--	--	--	2.21	2.19	0.14	0.15	0.02	0.06
46.00	31.90	--	0.97	--	1.21	--	0.08	--	0.01	--
47.00	--	--	--	--	1.85	2.15	0.15	0.16	0.09	0.17

Patient No.	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow-up (nmol/l)	Plasma FAD/ Riboflavin admission	Plasma FAD/ Riboflavin follow-up	Red cell FAD admission (pmol/g Hb)	Red cell FAD follow-up (pmol/g Hb)	Red cell FMN admission (pmol/g Hb)	Red cell FMN follow-up (pmol/g Hb)	Red cell riboflavin admission (pmol/g Hb)	Red cell riboflavin follow-up (pmol/g Hb)
48.00	--	--	--	--	2.14	2.55	0.16	0.18	0.11	0.62
50.00	44.30	29.10	1.18	2.00	1.49	1.46	0.10	0.10	0.04	0.02
51.00	106.50	330.60	0.50	0.27	1.80	1.89	0.16	0.15	0.11	0.17
52.00	748.10	910.80	0.09	0.11	2.30	2.30	0.11	0.10	0.17	0.24
53.00	--	--	--	--	1.04	0.96	0.05	0.05	0.01	0.00
55.00	4.10	237.70	7.41	0.18	1.22	2.00	0.08	0.14	--	0.12
56.00	16.60	--	1.72	--	1.45	--	0.07	--	0.14	--
57.00	52.50	--	0.20	--	1.33	--	0.13	--	0.04	--
58.00	15.40	12.40	2.31	1.83	1.16	1.14	0.07	0.07	0.02	0.02
59.00	53.70	--	1.29	--	1.22	--	0.07	--	0.01	--
60.00	11.80	--	4.47	--	1.17	--	0.05	--	0.00	--
61.00	38.00	--	1.54	--	1.21	--	0.06	--	0.01	--
62.00	6.60	9.60	4.35	3.42	1.20	1.09	0.06	0.05	0.01	0.01
63.00	48.00	--	0.39	--	1.80	--	0.26	--	0.09	--
64.00	10.00	--	3.10	--	1.06	--	0.06	--	0.01	--
65.00	57.30	--	0.72	--	1.91	--	0.36	--	0.03	--
67.00	34.00	39.80	0.71	0.67	1.72	1.68	0.19	0.19	0.08	0.07
68.00	5.90	577.10	3.32	0.07	1.54	2.30	0.11	0.17	0.02	0.20
69.00	571.90	616.80	0.02	0.06	1.34	2.26	0.12	0.22	0.03	0.25
71.00	8.60	--	4.91	--	0.97	--	0.05	--	0.01	--
72.00	55.70	559.90	1.40	0.15	1.78	2.40	0.09	0.13	0.03	0.21
73.00	40.70	--	0.94	--	1.38	--	0.14	--	0.05	--
74.00	--	1,166.10	--	0.10	1.44	1.96	0.13	0.17	0.04	0.28
75.00	53.90	--	0.53	--	2.10	--	0.48	--	0.09	--
77.00	24.80	--	1.34	--	1.81	--	0.13	--	0.03	--
78.00	139.00	--	0.38	--	1.93	--	0.17	--	0.06	--
79.00	18.40	17.40	4.22	2.94	1.24	1.21	0.07	0.07	0.01	0.01
80.00	140.80	--	0.39	--	2.23	--	0.28	--	0.14	--
81.00	7.10	33.00	6.14	2.08	1.56	1.66	0.09	0.09	0.01	0.03
82.00	3.00	14.50	6.40	3.95	1.31	1.68	0.08	0.11	0.01	0.02
83.00	7.80	--	5.41	--	1.49	--	0.08	--	0.01	--
84.00	62.00	356.00	0.44	0.08	1.75	2.51	0.14	0.20	0.04	0.17
85.00	6.70	8.00	3.78	4.35	1.30	1.33	0.09	0.10	0.05	0.03

Patient No.	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow-up (nmol/l)	Plasma FAD/ Riboflavin admission	Plasma FAD/ Riboflavin follow-up	Red cell FAD admission (pmol/g Hb)	Red cell FAD follow-up (pmol/g Hb)	Red cell FMN admission (pmol/g Hb)	Red cell FMN follow-up (pmol/g Hb)	Red cell riboflavin admission (pmol/g Hb)	Red cell riboflavin follow-up (pmol/g Hb)
87.00	65.60	--	0.60	--	1.62	--	0.12	--	0.03	--
88.00	43.30	401.00	0.68	0.11	1.35	2.21	0.07	0.14	0.03	0.15
89.00	6.80	--	6.16	--	1.17	--	0.08	--	0.01	--
90.00	11.70	74.30	1.79	0.53	2.06	1.88	0.11	0.11	0.03	0.06
91.00	--	--	--	--	1.25	--	0.08	--	0.04	--
94.00	14.30	--	4.29	--	1.87	--	0.14	--	0.02	--
96.00	20.20	11.20	2.30	4.91	2.12	2.00	0.15	0.14	0.06	0.06
99.00	43.40	--	0.77	--	1.48	--	0.09	--	0.01	--
100.00	337.50	172.10	0.06	0.07	2.56	2.50	0.33	0.25	0.21	0.12
102.00	27.00	36.80	0.97	0.71	1.31	1.27	0.08	0.07	0.02	0.02
103.00	43.10	26.30	1.23	1.33	1.59	1.59	0.16	0.16	0.07	0.07
104.00	33.80	--	2.17	--	1.31	--	0.06	--	0.02	--
105.00	133.80	114.20	0.19	0.17	1.80	1.70	0.19	0.19	0.19	0.13
107.00	29.60	--	1.53	--	1.67	--	0.17	--	0.06	--
108.00	11.80	--	3.81	--	1.23	--	0.05	--	0.01	--
110.00	--	516.30	--	0.14	1.13	2.63	0.06	0.17	0.01	0.16
111.00	24.30	20.90	1.76	1.22	1.37	1.80	0.06	0.11	0.01	0.02
112.00	28.60	54.00	2.26	0.35	1.15	1.33	0.12	0.14	0.03	0.05
113.00	68.60	62.20	0.75	0.39	1.31	1.37	0.06	0.06	0.01	0.01
114.00	--	66.20	--	0.19	1.14	1.40	0.06	0.08	0.01	0.02
115.00	7.30	--	9.29	--	1.02	--	0.05	--	0.01	--
116.00	13.60	--	2.57	--	1.29	--	--	--	0.01	--
117.00	39.90	44.00	1.61	0.82	1.53	1.58	--	--	0.02	0.03
118.00	193.10	29.00	0.09	0.76	1.85	1.48	--	--	0.04	0.02
119.00	24.00	--	2.84	--	1.33	--	0.08	--	0.02	--
121.00	14.00	26.20	0.98	1.16	1.54	1.79	0.14	0.16	0.03	0.05
123.00	115.00	69.40	0.75	0.73	1.77	1.88	--	--	0.05	0.06
124.00	55.80	97.90	0.32	0.40	1.56	1.78	--	--	0.02	0.01
126.00	458.00	--	0.35	--	1.23	--	--	--	0.04	--
127.00	374.20	--	0.05	--	2.58	--	--	--	0.23	--
128.00	14.30	--	2.42	--	1.75	--	0.09	--	0.03	--
129.00	188.00	--	0.20	--	1.88	--	--	--	0.04	--
131.00	69.70	--	0.29	--	1.68	--	--	--	0.04	--

Patient No.	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow-up (nmol/l)	Plasma FAD/ Riboflavin admission	Plasma FAD/ Riboflavin follow-up	Red cell FAD admission (pmol/g Hb)	Red cell FAD follow-up (pmol/g Hb)	Red cell FMN admission (pmol/g Hb)	Red cell FMN follow-up (pmol/g Hb)	Red cell riboflavin admission (pmol/g Hb)	Red cell riboflavin follow-up (pmol/g Hb)
133.00	40.20	--	1.19	--	1.55	--	--	--	0.02	--
135.00	81.50	--	0.50	--	1.91	--	--	--	0.03	--
137.00	4.50	--	9.71	--	1.84	--	0.10	--	0.12	--
139.00	48.60	--	0.99	--	2.02	--	0.11	--	0.03	--
140.00	21.90	--	4.56	--	2.13	--	0.13	--	0.09	--
141.00	9.20	199.10	4.36	0.28	1.53	2.11	0.08	0.14	0.02	0.08
143.00	34.60	--	1.78	--	1.84	--	0.12	--	0.08	--
146.00	196.30	288.50	0.30	0.30	2.13	2.22	0.13	0.13	0.07	0.07
147.00	6.50	7.50	8.86	7.99	1.23	1.17	0.06	0.06	0.01	0.02
148.00	26.20	--	2.99	--	1.54	--	0.09	--	0.01	--
149.00	27.60	--	0.95	--	1.36	--	0.08	--	0.04	--
150.00	5.50	--	7.60	--	1.42	--	0.07	--	0.01	--
151.00	5.50	--	7.60	--	1.24	--	0.06	--	0.01	--
152.00	18.60	--	5.40	--	1.29	--	0.07	--	0.03	--
153.00	23.00	--	2.30	--	1.37	--	0.07	--	0.00	--
154.00	90.50	--	0.93	--	1.82	--	0.15	--	0.11	--
155.00	26.50	--	1.71	--	1.44	--	0.09	--	0.03	--
156.00	7.80	--	6.15	--	1.22	--	0.06	--	0.01	--
157.00	228.60	--	0.09	--	1.37	--	0.08	--	0.02	--
158.00	62.20	--	0.74	--	1.58	--	0.12	--	0.09	--
159.00	21.10	--	2.04	--	1.43	--	0.07	--	0.01	--
163.00	9.40	--	3.68	--	1.15	--	0.05	--	0.00	--
164.00	10.60	--	4.32	--	1.47	--	0.08	--	0.01	--
165.00	19.80	--	4.42	--	1.27	--	0.06	--	0.01	--
166.00	29.20	--	2.29	--	1.47	--	0.09	--	0.05	--
167.00	7.30	--	8.07	--	1.39	--	0.09	--	0.02	--
168.00	307.40	--	0.13	--	1.82	--	0.11	--	0.05	--
169.00	85.60	--	0.89	--	1.77	--	0.14	--	0.07	--
171.00	20.30	--	2.44	--	1.77	--	0.13	--	0.06	--

Patient No.	Red cell FAD/ Riboflavin admission	Red cell FAD/ Riboflavin follow-up	Alkaline phosphatase admission (IU/l)	Alkaline phosphatase follow-up (IU/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
12.00	33.86	37.79	115.00	120.00	13.90	12.60
13.00	27.66	29.59	277.00	206.00	9.90	7.30
14.00	20.93	23.13	538.00	406.00	7.50	9.20
15.00	41.92	--	146.00	241.00	10.10	10.90
16.00	32.81	--	234.00	--	12.30	--
17.00	33.13	51.80	344.00	213.00	14.90	10.00
18.00	85.21	104.25	253.00	177.00	12.90	9.90
19.00	41.67	16.08	184.00	159.00	7.10	8.40
21.00	46.66	--	1,221.00	--	10.10	--
22.00	9.65	14.49	226.00	136.00	10.10	--
23.00	91.75	58.88	170.00	307.00	6.70	10.20
24.00	42.46	--	250.00	--	12.80	--
27.00	16.21	18.26	261.00	441.00	12.10	10.80
29.00	21.92	30.03	168.00	204.00	8.90	9.00
30.00	14.41	7.61	1,089.00	659.00	10.10	7.80
31.00	44.84	--	126.00	--	12.20	--
32.00	45.98	--	331.00	--	9.80	--
33.00	133.75	105.61	107.00	280.00	12.00	10.60
34.00	9.80	21.12	96.00	220.00	12.50	8.70
35.00	226.38	201.10	69.00	121.00	9.50	8.70
36.00	12.84	34.75	238.00	543.00	8.70	9.50
37.00	23.42	22.66	264.00	255.00	13.30	13.50
38.00	29.93	--	398.00	--	9.20	--
40.00	143.02	114.52	35.00	39.00	7.20	8.90
41.00	33.49	--	54.00	--	11.60	--
42.00	65.15	--	26.00	41.00	9.30	10.50
44.00	29.73	34.91	31.00	88.00	10.90	10.00
45.00	135.91	37.48	158.00	564.00	7.70	10.10
46.00	108.60	--	71.00	--	8.80	--
47.00	19.89	12.91	276.00	264.00	8.50	8.60
48.00	19.16	4.13	121.00	86.00	9.60	10.10
50.00	40.72	74.07	36.00	94.00	--	9.60
51.00	16.12	11.12	24.00	99.00	8.90	9.70

Patient No.	Red cell FAD/ Riboflavin admission	Red cell FAD/ Riboflavin follow-up	Alkaline phosphatase admission (IU/l)	Alkaline phosphatase follow-up (IU/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
52.00	13.33	9.58	111.00	258.00	8.40	7.20
53.00	134.75	200.75	96.00	158.00	9.40	8.20
55.00	--	16.54	70.00	116.00	8.20	8.50
56.00	10.42	--	42.00	--	8.60	--
57.00	35.49	--	43.00	--	9.40	--
58.00	56.95	69.23	36.00	22.00	10.00	8.30
59.00	94.88	--	52.00	--	15.20	--
60.00	320.24	--	85.00	--	--	--
61.00	81.90	--	76.00	--	9.40	--
62.00	136.29	92.98	48.00	55.00	9.20	10.30
63.00	19.38	--	67.00	--	9.90	--
64.00	133.48	--	83.00	--	8.70	--
65.00	59.53	--	58.00	--	9.10	--
67.00	20.64	24.97	212.00	125.00	9.80	11.80
68.00	77.10	11.38	28.00	125.00	11.00	8.80
69.00	39.69	9.18	47.00	81.00	9.10	9.30
71.00	127.88	--	95.00	--	15.50	--
72.00	52.28	11.53	79.00	181.00	10.50	8.20
73.00	28.38	--	97.00	--	10.70	--
74.00	36.32	7.01	820.00	644.00	11.80	11.50
75.00	22.09	--	51.00	--	7.70	--
77.00	57.88	--	46.00	--	8.90	--
78.00	32.69	--	148.00	--	10.00	--
79.00	85.25	86.13	47.00	76.00	13.30	12.00
80.00	15.44	--	183.00	--	7.90	--
81.00	144.55	65.14	27.00	52.00	9.20	7.90
82.00	88.89	72.64	72.00	72.00	11.10	9.80
83.00	234.07	--	81.00	--	11.80	--
84.00	48.45	14.62	124.00	127.00	11.20	11.10
85.00	25.33	52.65	16.00	17.00	11.70	--
87.00	63.46	--	79.00	--	7.40	--
88.00	51.28	14.72	77.00	91.00	--	9.00
89.00	88.75	--	67.00	--	9.40	--
90.00	75.43	33.11	69.00	81.00	8.50	7.90

Patient No.	Red cell FAD/ Riboflavin admission	Red cell FAD/ Riboflavin follow-up	Alkaline phosphatase admission (IU/l)	Alkaline phosphatase follow-up (IU/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
91.00	30.68	--	55.00	--	10.70	--
94.00	77.64	--	108.00	--	9.20	--
96.00	36.20	32.32	86.00	108.00	9.70	9.00
99.00	113.55	--	105.00	--	13.70	--
100.00	12.43	20.64	183.00	132.00	7.60	8.40
102.00	63.03	76.31	39.00	60.00	8.50	7.70
103.00	21.34	22.36	71.00	80.00	13.20	--
104.00	64.97	--	88.00	--	11.70	--
105.00	9.51	13.25	94.00	98.00	7.00	7.70
107.00	30.24	--	71.00	--	10.50	--
108.00	141.58	--	66.00	--	12.70	--
110.00	175.86	16.87	31.00	113.00	9.00	8.20
111.00	153.26	94.66	99.00	52.00	16.20	6.80
112.00	37.01	28.02	64.00	43.00	15.40	8.90
113.00	207.10	242.66	64.00	40.00	14.90	10.00
114.00	81.40	62.45	100.00	86.00	10.70	7.70
115.00	124.49	--	67.00	--	14.20	--
116.00	111.09	--	50.00	--	9.70	--
117.00	95.05	46.82	69.00	54.00	12.90	9.30
118.00	46.45	90.92	88.00	86.00	8.10	9.20
119.00	77.68	--	73.00	--	11.60	--
121.00	50.16	39.37	56.00	166.00	10.00	10.20
123.00	32.94	29.32	148.00	80.00	10.50	10.20
124.00	94.48	161.65	83.00	107.00	7.00	--
126.00	29.57	--	74.00	--	9.20	--
127.00	11.43	--	278.00	--	8.50	--
128.00	60.10	--	73.00	--	8.80	--
129.00	48.96	--	265.00	--	11.30	--
131.00	47.94	--	74.00	--	10.60	--
133.00	86.30	--	102.00	--	11.00	--
135.00	58.77	--	399.00	--	7.30	--
137.00	15.50	--	155.00	--	--	--
139.00	59.24	--	28.00	--	--	--
140.00	23.42	--	2,326.00	--	--	--

Patient No.	Red cell FAD/ Riboflavin admission	Red cell FAD/ Riboflavin follow-up	Alkaline phosphatase admission (IU/l)	Alkaline phosphatase follow-up (IU/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
141.00	65.92	26.96	104.00	143.00	--	--
143.00	22.29	--	178.00	--	--	--
146.00	30.59	33.65	61.00	73.00	--	--
147.00	92.69	65.28	51.00	46.00	--	--
148.00	111.33	--	68.00	--	--	--
149.00	35.46	--	68.00	--	--	--
150.00	99.38	--	106.00	--	--	--
151.00	214.18	--	143.00	--	--	--
152.00	51.09	--	153.00	--	--	--
153.00	410.25	--	91.00	--	--	--
154.00	16.53	--	88.00	--	--	--
155.00	54.92	--	66.00	--	--	--
156.00	97.23	--	97.00	--	--	--
157.00	81.14	--	58.00	--	--	--
158.00	17.04	--	57.00	--	--	--
159.00	108.24	--	154.00	--	--	--
163.00	423.55	--	75.00	--	--	--
164.00	135.30	--	56.00	--	--	--
165.00	94.76	--	69.00	--	--	--
166.00	27.63	--	283.00	--	--	--
167.00	65.81	--	56.00	--	--	--
168.00	34.76	--	73.00	--	--	--
169.00	24.09	--	418.00	--	--	--
171.00	27.89	--	34.00	--	--	--

12.8 Appendix 8 Chapter 4 data

Appendix 8. Characteristics and biochemical measurements of controls (Chapter 4)

Control No.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma PLP (nmol/l)	Plasma PL (nmol/l)	Plasma PLP/PL	Red cell PLP (pmol/g Hb)	Red cell PL (pmol/g Hb)	Red Cell PLP/PL
1.00	66.00	1.00	6.00	40.00	7.00	5.71	391.00	71.00	5.51
2.00	48.00	1.00	6.00	65.00	14.00	4.64	504.00	105.00	4.80
3.00	62.00	1.00	6.00	43.00	8.00	5.38	365.00	101.00	3.61
4.00	53.00	1.00	6.00	44.00	10.00	4.40	372.00	61.00	6.10
5.00	67.00	1.00	6.00	67.00	12.00	5.58	538.00	97.00	5.55
6.00	38.00	1.00	6.00	92.00	16.00	5.75	563.00	97.00	5.80
7.00	61.00	1.00	6.00	98.00	20.00	4.90	551.00	83.00	6.64
8.00	35.00	1.00	6.00	66.00	15.00	4.40	505.00	55.00	9.18
9.00	53.00	1.00	6.00	33.00	6.00	5.50	351.00	53.00	6.62
10.00	72.00	1.00	6.00	30.00	7.00	4.29	342.00	53.00	6.45
11.00	35.00	1.00	6.00	97.00	23.00	4.22	510.00	114.00	4.47
12.00	67.00	1.00	6.00	114.00	23.00	4.96	626.00	148.00	4.23
13.00	70.00	1.00	6.00	42.00	8.00	5.25	336.00	56.00	6.00
14.00	58.00	1.00	6.00	40.00	8.00	5.00	280.00	33.00	8.48
15.00	58.00	1.00	6.00	80.00	19.00	4.21	411.00	43.00	9.56
16.00	58.00	1.00	6.00	75.00	14.00	5.36	463.00	57.00	8.12
17.00	64.00	1.00	6.00	30.00	5.00	6.00	278.00	33.00	8.42
18.00	51.00	1.00	6.00	36.00	9.00	4.00	301.00	72.00	4.18
19.00	39.00	1.00	6.00	79.00	15.00	5.27	499.00	119.00	4.19
20.00	53.00	1.00	6.00	74.00	15.00	4.93	557.00	134.00	4.16
21.00	55.00	1.00	6.00	69.00	12.00	5.75	465.00	150.00	3.10
22.00	47.00	1.00	6.00	74.00	19.00	3.89	466.00	83.00	5.61
23.00	31.00	1.00	6.00	162.00	33.00	4.91	746.00	248.00	3.01
24.00	69.00	1.00	6.00	68.00	12.00	5.67	475.00	156.00	3.04
25.00	39.00	1.00	6.00	107.00	19.00	5.63	501.00	109.00	4.60
26.00	45.00	1.00	6.00	40.00	7.00	5.71	391.00	62.00	6.31
27.00	72.00	1.00	6.00	49.00	10.00	4.90	387.00	72.00	5.38

Control No.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma PLP (nmol/l)	Plasma PL (nmol/l)	Plasma PLP/PL	Red cell PLP (pmol/g Hb)	Red cell PL (pmol/g Hb)	Red Cell PLP/PL
28.00	48.00	1.00	6.00	54.00	15.00	3.60	413.00	73.00	5.66
29.00	44.00	1.00	6.00	41.00	10.00	4.10	394.00	49.00	8.04
30.00	43.00	1.00	6.00	33.00	7.00	4.71	317.00	40.00	7.93
31.00	47.00	1.00	6.00	55.00	11.00	5.00	279.00	39.00	7.15
32.00	49.00	1.00	6.00	35.00	9.00	3.89	351.00	57.00	6.16
33.00	71.00	1.00	6.00	41.00	10.00	4.10	347.00	84.00	4.13
34.00	51.00	1.00	6.00	41.00	10.00	4.10	341.00	25.00	13.64
35.00	49.00	1.00	6.00	194.00	40.00	4.85	815.00	150.00	5.43
36.00	56.00	1.00	6.00	60.00	11.00	5.45	356.00	99.00	3.60
37.00	47.00	1.00	6.00	46.00	9.00	5.11	447.00	85.00	5.26
38.00	45.00	1.00	6.00	49.00	12.00	4.08	369.00	75.00	4.92
39.00	66.00	1.00	6.00	130.00	30.00	4.33	723.00	201.00	3.60
40.00	63.00	1.00	6.00	34.00	9.00	3.78	417.00	118.00	3.53
41.00	73.00	1.00	6.00	37.00	14.00	2.64	339.00	75.00	4.52
42.00	47.00	1.00	6.00	55.00	18.00	3.06	403.00	119.00	3.39
43.00	41.00	1.00	6.00	40.00	9.00	4.44	351.00	59.00	5.95
44.00	60.00	1.00	6.00	30.00	6.00	5.00	308.00	73.00	4.22
45.00	54.00	1.00	6.00	32.00	8.00	4.00	329.00	76.00	4.33
46.00	68.00	1.00	6.00	38.00	9.00	4.22	340.00	49.00	6.94
47.00	50.00	1.00	6.00	44.00	10.00	4.40	350.00	33.00	10.61
48.00	45.00	1.00	6.00	50.00	10.00	5.00	382.00	59.00	6.47
49.00	41.00	1.00	6.00	70.00	13.00	5.38	467.00	97.00	4.81
50.00	73.00	1.00	6.00	30.00	7.00	4.29	307.00	58.00	5.29
51.00	47.00	1.00	6.00	35.00	7.00	5.00	307.00	62.00	4.95
52.00	65.00	1.00	6.00	140.00	32.00	4.38	742.00	221.00	3.36
53.00	61.00	1.00	6.00	44.00	7.00	6.29	371.00	27.00	13.74
54.00	54.00	1.00	6.00	49.00	9.00	5.44	381.00	33.00	11.55
55.00	46.00	1.00	6.00	36.00	7.00	5.14	318.00	31.00	10.26
56.00	63.00	1.00	6.00	37.00	8.00	4.63	339.00	44.00	7.70
57.00	72.00	1.00	6.00	19.00	7.00	2.71	234.00	54.00	4.33
58.00	38.00	1.00	6.00	64.00	22.00	2.91	402.00	75.00	5.36
59.00	59.00	1.00	6.00	35.00	9.00	3.89	329.00	58.00	5.67
60.00	50.00	0.00	6.00	39.00	10.00	3.90	350.00	67.00	5.22
61.00	52.00	0.00	6.00	38.00	15.00	2.53	318.00	26.00	12.23

Control No.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma PLP (nmol/l)	Plasma PL (nmol/l)	Plasma PLP/PL	Red cell PLP (pmol/g Hb)	Red cell PL (pmol/g Hb)	Red Cell PLP/PL
62.00	48.00	0.00	6.00	53.00	19.00	2.79	403.00	105.00	3.84
63.00	53.00	0.00	6.00	33.00	9.00	3.67	318.00	86.00	3.70
64.00	45.00	0.00	6.00	78.00	27.00	2.89	477.00	144.00	3.31
65.00	38.00	0.00	6.00	65.00	14.00	4.64	445.00	97.00	4.59
66.00	57.00	0.00	6.00	154.00	32.00	4.81	774.00	227.00	3.41
67.00	48.00	0.00	6.00	49.00	13.00	3.77	370.00	26.00	14.23
68.00	57.00	0.00	6.00	43.00	14.00	3.07	371.00	27.00	13.74
69.00	39.00	0.00	6.00	36.00	10.00	3.60	328.00	33.00	9.94
70.00	64.00	0.00	6.00	58.00	11.00	5.27	413.00	53.00	7.79
71.00	57.00	0.00	6.00	87.00	11.00	7.91	530.00	78.00	6.79
72.00	37.00	0.00	6.00	70.00	14.00	5.00	466.00	93.00	5.01
73.00	57.00	0.00	6.00	34.00	8.00	4.25	318.00	112.00	2.84
74.00	47.00	0.00	6.00	62.00	11.00	5.64	424.00	59.00	7.19
75.00	71.00	0.00	6.00	26.00	7.00	3.71	297.00	34.00	8.74
76.00	42.00	0.00	6.00	32.00	8.00	4.00	318.00	33.00	9.64
77.00	49.00	0.00	6.00	45.00	11.00	4.09	372.00	42.00	8.86
78.00	35.00	0.00	6.00	33.00	10.00	3.30	318.00	33.00	9.64
79.00	69.00	0.00	6.00	40.00	10.00	4.00	349.00	84.00	4.15
80.00	62.00	0.00	6.00	32.00	6.00	5.33	318.00	51.00	6.24
81.00	71.00	0.00	6.00	51.00	10.00	5.10	381.00	91.00	4.19
82.00	38.00	0.00	6.00	129.00	10.00	12.90	688.00	105.00	6.55
83.00	62.00	0.00	6.00	33.00	8.00	4.13	318.00	44.00	7.23
84.00	58.00	0.00	6.00	31.00	8.00	3.88	318.00	44.00	7.23
85.00	66.00	0.00	6.00	42.00	10.00	4.20	360.00	42.00	8.57
86.00	53.00	0.00	6.00	65.00	11.00	5.91	435.00	69.00	6.30
87.00	38.00	0.00	6.00	49.00	11.00	4.45	382.00	93.00	4.11
88.00	57.00	0.00	6.00	62.00	10.00	6.20	424.00	92.00	4.61
89.00	51.00	0.00	6.00	44.00	11.00	4.00	360.00	52.00	6.92
90.00	40.00	0.00	6.00	164.00	23.00	7.13	657.00	100.00	6.57
91.00	45.00	0.00	6.00	39.00	6.00	6.50	349.00	92.00	3.79
92.00	39.00	0.00	6.00	57.00	10.00	5.70	413.00	84.00	4.92
93.00	48.00	0.00	6.00	41.00	16.00	2.56	465.00	80.00	5.81
94.00	70.00	0.00	6.00	64.00	15.00	4.27	345.00	75.00	4.60
95.00	42.00	0.00	6.00	66.00	13.00	5.08	356.00	38.00	9.37

Control No.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Plasma PLP (nmol/l)	Plasma PL (nmol/l)	Plasma PLP/PL	Red cell PLP (pmol/g Hb)	Red cell PL (pmol/g Hb)	Red Cell PLP/PL
96.00	55.00	0.00	6.00	49.00	10.00	4.90	391.00	27.00	14.48
97.00	48.00	0.00	6.00	49.00	12.00	4.08	342.00	35.00	9.77
98.00	61.00	0.00	6.00	30.00	8.00	3.75	272.00	38.00	7.16
99.00	60.00	0.00	6.00	52.00	10.00	5.20	311.00	27.00	11.52
100.00	57.00	0.00	6.00	52.00	9.00	5.78	316.00	46.00	6.87
101.00	42.00	0.00	6.00	87.00	17.00	5.12	489.00	49.00	9.98
102.00	38.00	0.00	6.00	42.00	10.00	4.20	360.00	34.00	10.59
103.00	39.00	0.00	6.00	60.00	9.00	6.67	424.00	59.00	7.19
104.00	68.00	0.00	6.00	46.00	8.00	5.75	371.00	26.00	14.27
105.00	45.00	0.00	6.00	130.00	29.00	4.48	699.00	84.00	8.32
106.00	56.00	0.00	6.00	48.00	10.00	4.80	371.00	78.00	4.76
107.00	70.00	0.00	6.00	73.00	6.00	12.17	476.00	129.00	3.69
108.00	39.00	0.00	6.00	50.00	6.00	8.33	381.00	44.00	8.66
109.00	70.00	0.00	6.00	82.00	8.00	10.25	508.00	119.00	4.27
110.00	41.00	0.00	6.00	104.00	5.00	20.80	582.00	142.00	4.10
111.00	44.00	0.00	6.00	101.00	20.00	5.05	573.00	85.00	6.74
112.00	51.00	0.00	6.00	134.00	3.00	44.67	700.00	53.00	13.21
113.00	43.00	0.00	6.00	63.00	11.00	5.73	434.00	69.00	6.29
114.00	53.00	0.00	6.00	62.00	6.00	10.33	434.00	68.00	6.38
115.00	44.00	0.00	6.00	55.00	11.00	5.00	402.00	62.00	6.48
116.00	70.00	0.00	6.00	100.00	8.00	12.50	572.00	91.00	6.29
117.00	55.00	0.00	6.00	154.00	8.00	19.25	775.00	243.00	3.19
118.00	51.00	0.00	6.00	74.00	11.00	6.73	476.00	112.00	4.25
119.00	47.00	0.00	6.00	66.00	8.00	8.25	445.00	82.00	5.43
120.00	54.00	0.00	6.00	100.00	12.00	8.33	572.00	108.00	5.30
121.00	63.00	0.00	6.00	72.00	7.00	10.29	466.00	76.00	6.13
122.00	66.00	0.00	6.00	76.00	9.00	8.44	478.00	82.00	5.83
123.00	58.00	0.00	6.00	64.00	10.00	6.40	423.00	51.00	8.29
124.00	59.00	0.00	6.00	58.00	7.00	8.29	403.00	42.00	9.60
125.00	45.00	0.00	6.00	74.00	9.00	8.22	467.00	116.00	4.03
126.00	60.00	0.00	6.00	60.00	11.00	5.45	402.00	33.00	12.18

Appendix 8. Clinical and biochemical characteristics of critically ill patients (Chapter 4)

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II score	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro ICU Pabrinex doses	Pro+ICU Pabrinex doses	ICU Alive/Dead (0/1)	ICU stay (days)	Day of follow-up sample
12.00	61.00	0.00	29.00	76.60	0.00	0.00	0.00	0.00	7.40	2.00
13.00	43.00	1.00	12.00	11.70	1.00	0.00	2.00	0.00	48.10	3.00
14.00	71.00	1.00	18.00	28.90	1.00	0.00	0.00	1.00	12.90	2.00
15.00	53.00	1.00	19.00	32.20	0.00	0.00	1.00	0.00	4.80	5.00
16.00	61.00	0.00	23.00	28.50	0.00	0.00		0.00	1.00	
17.00	18.00	0.00	12.00	20.20	0.00	0.00	3.00	1.00	6.20	4.00
18.00	67.00	0.00	18.00	44.40	1.00	0.00	0.00	1.00	59.50	4.00
19.00	50.00	0.00	21.00	38.90	0.00	0.00	3.00	1.00	46.30	4.00
21.00	61.00	0.00	34.00	76.80	1.00	0.00		0.00	2.00	
22.00	52.00	0.00	13.00	9.90	0.00	95.00	101.00	0.00	3.70	4.00
23.00	71.00	0.00	23.00	42.50	1.00	9.00	10.00	1.00	20.90	7.00
24.00	77.00	1.00	21.00	35.00	0.00	0.00		0.00	2.00	
27.00	76.00	1.00	23.00	46.00	0.00	1.00	3.00	0.00	21.70	3.00
29.00	70.00	0.00	16.00	20.20	1.00	4.00	10.00	0.00	5.10	4.00
30.00	73.00	1.00	25.00	53.30	1.00	0.00	2.00	1.00	15.50	3.00
31.00	62.00	0.00	19.00	27.10	1.00	0.00		0.00	0.50	
32.00	55.00	1.00	15.00	21.00	1.00	0.00		0.00	2.20	
33.00	80.00	1.00	31.00	84.20	1.00	0.00	0.00	1.00	12.00	5.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	0.00	29.40	4.00
35.00	20.00	0.00	18.00	39.70	1.00	0.00	1.00	1.00	44.00	3.00
36.00	43.00	1.00	38.00	92.60	0.00	5.00	10.00	0.00	25.50	4.00
37.00	60.00	0.00	27.00	63.10	0.00	0.00	0.00	0.00	21.80	4.00
38.00	74.00	0.00	20.00	23.00	1.00	0.00		0.00	1.00	
40.00	47.00	0.00	22.00	28.50	1.00	0.00	2.00	0.00	1.90	3.00
41.00	76.00	1.00	29.00	79.90	1.00	0.00		0.00	1.40	
42.00	79.00	0.00	21.00	30.90	1.00	0.00	3.00	0.00	3.20	4.00
44.00	81.00	0.00	34.00	86.30	0.00	0.00	0.00	1.00	16.10	8.00
45.00	74.00	0.00	33.00	75.20	0.00	0.00	0.00	1.00	16.00	5.00
46.00	41.00	1.00	8.00	15.60	1.00	0.00		0.00	0.80	
47.00	76.00	0.00	29.00	77.20	1.00	0.00	0.00	1.00	34.60	2.00
48.00	67.00	0.00	31.00	68.10	1.00	1,000.00	1,007.00	1.00	18.40	4.00
50.00	60.00	0.00	19.00	48.00	1.00	0.00	0.00	0.00	6.20	6.00

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II score	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro ICU Pabrinex doses	Pro+ICU Pabrinex doses	ICU Alive/Dead (0/1)	ICU stay (days)	Day of follow-up sample
51.00	46.00	1.00	24.00	49.70	0.00	1,000.00	1,006.00	1.00	4.60	3.00
52.00	61.00	0.00	33.00	78.60	0.00	7.00	10.00	1.00	7.70	3.00
53.00	67.00	1.00	18.00	21.20	0.00	0.00	0.00	1.00	11.10	3.00
55.00	80.00	1.00	31.00	73.30	0.00	0.00	3.00	1.00	8.20	4.00
56.00	40.00	1.00	6.00	10.20	1.00	0.00		0.00	0.40	
57.00	60.00	0.00	27.00	60.20	1.00	0.00		1.00	1.80	
58.00	41.00	0.00	21.00	17.80	1.00	0.00	0.00	0.00	19.70	2.00
59.00	68.00	0.00	20.00	32.30	0.00	0.00		0.00	0.80	
60.00	57.00	0.00	24.00	67.00	0.00	0.00		0.00	6.50	
61.00	76.00	1.00	18.00	14.40	0.00	0.00		0.00	0.70	
62.00	74.00	0.00	20.00	19.90	1.00	0.00	0.00	0.00	0.90	3.00
63.00	62.00	0.00	18.00	44.40	1.00	1,000.00		0.00	1.00	
64.00	66.00	0.00	34.00	81.00	0.00	0.00		0.00	22.40	
65.00	38.00	1.00	17.00	26.20	0.00	0.00		0.00	8.90	
67.00	68.00	1.00	31.00	80.30	0.00	1,000.00	0.00	0.00	5.00	5.00
68.00	74.00	0.00	24.00	49.70	0.00	0.00	7.00	0.00	10.70	5.00
69.00	61.00	0.00	22.00	54.90	1.00	1,000.00	1,004.00	0.00	76.40	4.00
71.00	25.00	0.00	17.00	1.20	0.00	0.00		0.00	2.00	
72.00	51.00	0.00	21.00	12.40	0.00	1.00	1.00	0.00	15.50	12.00
73.00	69.00	0.00	12.00	20.20	0.00	0.00		0.00	5.80	
74.00	65.00	0.00	33.00	80.40	0.00	0.00	7.00	0.00	19.60	4.00
75.00	28.00	1.00	14.00	18.50	1.00	0.00		0.00	0.60	
77.00	54.00	1.00	17.00	1.20	0.00	0.00		0.00	0.80	
78.00	45.00	0.00	12.00	5.50	1.00	0.00		0.00	0.90	
79.00	41.00	0.00	7.00	7.60	1.00	0.00	0.00	0.00	9.30	3.00
80.00	38.00	0.00	16.00	23.30	1.00	1,000.00		0.00	0.80	
83.00	46.00	1.00	11.00	10.30	1.00	0.00		0.00	2.90	
85.00	73.00	0.00	18.00	44.40	1.00	0.00	0.00	0.00	1.40	2.00
87.00	34.00	1.00	12.00	12.40	1.00	0.00		0.00	2.30	
88.00	81.00	0.00	17.00	23.60	1.00	1,000.00	1,004.00	1.00	9.30	5.00
89.00	22.00	1.00	10.00	9.50	1.00	0.00		0.00	0.20	
90.00	53.00	0.00	32.00	60.90	1.00	0.00	2.00	0.00	3.00	4.00
91.00	62.00	0.00	22.00	58.90	1.00	0.00		0.00	1.70	
94.00	32.00	1.00	25.00	53.30	0.00	0.00		0.00	17.30	
96.00	63.00	1.00	18.00	4.80	0.00	0.00	0.00	0.00	1.90	3.00
99.00	37.00	0.00	13.00	16.50	1.00	0.00		0.00	2.10	

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II score	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro ICU Pabrinex doses	Pro+ICU Pabrinex doses	ICU Alive/Dead (0/1)	ICU stay (days)	Day of follow-up sample
100.00	50.00	1.00	26.00	38.20	0.00	0.00	12.00	0.00	2.60	3.00
101.00	35.00	1.00	9.00	4.70	1.00	0.00		0.00	0.80	
102.00	26.00	0.00	23.00	45.70	1.00	0.00		0.00	2.30	2.00
103.00	52.00	0.00	16.00	12.20	1.00	0.00	0.00	0.00	2.00	3.00
104.00	38.00	0.00	3.00	2.70	0.00	0.00		0.00	0.30	
105.00	39.00	0.00	34.00	87.50	0.00	1,000.00	1,001.00	0.00	3.00	2.00
107.00	45.00	1.00	12.00	6.60	1.00	0.00		0.00	3.10	
108.00	44.00	1.00	6.00	0.90	0.00	0.00		0.00	0.80	
110.00	48.00	1.00	26.00	35.20	0.00	0.00	8.00	1.00	4.90	5.00
111.00	23.00	0.00	9.00	4.30	1.00	0.00	7.00	0.00	9.20	5.00
112.00	45.00	1.00	24.00	22.40	1.00	0.00	0.00	1.00	10.70	4.00
113.00	70.00	0.00	27.00	47.70	0.00	0.00	2.00	1.00	6.50	3.00
114.00	65.00	1.00	24.00	65.70	1.00	0.00	3.00	1.00	6.50	4.00
115.00	47.00	0.00	10.00	1.50	0.00	0.00		0.00	0.60	
116.00	61.00	0.00	13.00	10.60	0.00	0.00		0.00	2.40	
117.00	48.00	0.00	13.00	8.50	1.00	0.00	2.00	0.00	4.20	4.00
118.00	59.00	0.00	23.00	39.90	1.00	0.00	1.00	0.00	12.10	2.00
119.00	67.00	1.00	18.00	14.40	0.00	0.00		0.00	1.30	
121.00	86.00	0.00	22.00	42.20	1.00	0.00	3.00	0.00	6.60	5.00
123.00	60.00	0.00	16.00	14.60	0.00	1,000.00	1,004.00	0.00	3.10	3.00
124.00	33.00	0.00	14.00	27.40	1.00	1.00	4.00	0.00	4.70	2.00
126.00	68.00	0.00	27.00	46.00	0.00	1,000.00		0.00	1.60	
127.00	100.00	0.00	25.00	64.60	1.00	0.00		0.00	3.50	
128.00	80.00	1.00	30.00	56.90	1.00	0.00		0.00	2.90	
129.00	31.00	0.00	19.00	44.00	0.00	0.00		0.00	2.90	
131.00	65.00	0.00	21.00	55.30	1.00	0.00		0.00	6.70	
133.00	35.00	0.00	14.00	7.20	1.00	0.00		0.00	0.90	
135.00	41.00	1.00	16.00	14.30	1.00	0.00		0.00	0.60	

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow-up (nmol/l)	Plasma PL admission (nmol/l)	Plasma PL follow-up (nmol/l)	Plasma PLP/PL admission	Plasma PLP/PL follow-up
12.00	126.00	156.00	24.00	23.00	23.00		16.90	12.10	3.00	4.70	5.63	2.57
13.00	22.00	261.00	27.00	22.00	5.00	8.00	49.20	17.60	10.00	6.60	4.92	2.67
14.00	220.00	124.00	12.00	10.00	24.90	17.60	5.10	3.10	9.80	12.00	0.52	0.26
15.00	257.00	73.00	23.00	16.00	11.40	6.60	48.20	37.40	359.20	14.30	0.13	2.62
16.00	122.00		28.00		5.70		67.80		1,111.60		0.06	
17.00	3.00	55.00	33.00	26.00	11.40	7.70	98.00	30.00	9.00		10.89	
18.00	152.00	64.00	10.00	10.00	12.10	25.00	8.50	6.60	12.00		0.71	
19.00	143.00	101.00	12.00	14.00	17.00	8.00	13.10	59.10	3.80	41.10	3.45	1.44
21.00	23.00		10.00		14.00		332.60		27.60		12.05	
22.00	20.00	67.00	27.00	15.00	24.80		197.50	71.50	246.30	87.60	0.80	0.82
23.00	125.00	216.00	10.00	13.00	8.30	10.70	15.80	9.30	4.70	3.40	3.36	2.74
24.00	81.00		35.00		31.00		39.40		6.90		5.71	
27.00	103.00	133.00	23.00	17.00	3.80	13.70	21.70	30.30	2.30	12.20	9.43	2.48
29.00	59.00	20.00	9.00	10.00	10.60	22.00	26.70	19.80	75.10	18.40	0.36	1.08
30.00	26.00	179.00	16.00	11.00	16.70	18.30	12.60	15.10	14.80	42.60	0.85	0.35
31.00	50.00		22.00		9.20		17.20		3.10		5.55	
32.00	434.00		17.00		14.70		3.80		4.10		0.93	
33.00	259.00	173.00	17.00	10.00	10.00	19.40	6.80	2.30	0.90	3.10	7.56	0.74
34.00	404.00	336.00	30.00	15.00	10.10	8.70	69.10	19.70	89.80	42.10	0.77	0.47
35.00	119.00	303.00	9.00	13.00	6.30	8.40	6.90	11.60	1.60	7.50	4.31	1.55
36.00	132.00	70.00	14.00	14.00	13.80	29.50	33.10	7.60	155.10	20.10	0.21	0.38
37.00	81.00	35.00	12.00	12.00	31.30	22.00	141.40	27.00	2.90	5.70	48.76	4.74
38.00	127.00		14.00				6.00		0.60		10.00	
40.00	15.00	234.00	20.00	18.00	3.80	8.00	13.90	30.30	2.70	10.90	5.15	2.78
41.00	184.00		19.00		16.40		9.00		9.50		0.95	
42.00	8.00	149.00	17.00	18.00	10.00		21.40	45.90	4.70	16.30	4.55	2.82
44.00	151.00	55.00	10.00	16.00	10.70	17.40	37.10	26.20	5.50	10.40	6.75	2.52
45.00	258.00	164.00	14.00	14.00	18.90	10.60	154.40	7.20	2.50	6.00	61.76	1.20
46.00	250.00		11.00		20.20		4.80		2.40		2.00	
47.00	64.00	72.00	24.00	24.00	12.80	13.30	295.70	357.20	18.30	30.30	16.16	11.79
48.00	119.00	123.00	10.00	9.00		23.50	9.40	16.90	19.20	752.70	0.49	0.02
50.00	180.00	141.00	23.00	15.00	8.90	17.70	7.90	5.00	0.50	2.10	15.80	2.38

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow-up (nmol/l)	Plasma PL admission (nmol/l)	Plasma PL follow-up (nmol/l)	Plasma PLP/PL admission	Plasma PLP/PL follow-up
51.00	56.00	58.00	14.00	19.00		10.10	28.80	45.50	285.50	634.50	0.10	0.07
52.00	565.00	257.00	9.00	9.00	48.60	66.00	38.10	70.10	109.70	259.20	0.35	0.27
53.00	148.00	131.00	9.00	9.00	4.40	6.10	1.70	1.50	1.30	2.00	1.31	0.75
55.00	107.00	71.00	13.00	9.00		24.00	1.20	22.00	0.90	109.90	1.33	0.20
56.00	37.00		16.00		9.60		4.00		1.00		4.00	
57.00	181.00		10.00		6.60		10.30		8.90		1.16	
58.00	6.00	36.00	19.00	12.00			37.40	28.10	6.50	3.90	5.75	7.21
59.00	32.00		36.00		11.10		151.80		1,106.40		0.14	
60.00	166.00		26.00		12.90		13.20		5.30		2.49	
61.00	380.00		26.00				10.90		6.10		1.79	
62.00	88.00	180.00	12.00	14.00	12.50	12.10	6.40	6.00	8.10	6.10	0.79	0.98
63.00	117.00		14.00		5.60		15.90		7.10		2.24	
64.00	311.00		18.00		11.60		34.40		2.20		15.64	
65.00	163.00		23.00		16.10		63.10		12.60		5.01	
67.00	438.00	52.00	20.00	16.00	7.30		2.60	9.30	1.30	6.60	2.00	1.41
68.00	67.00	139.00	11.00	11.00	3.60		9.20	13.10	0.90	250.60	10.22	0.05
69.00	179.00	356.00	14.00	16.00	2.00		17.60	121.70	2.40	134.50	7.33	0.90
71.00	2.00		38.00		11.50		89.80		3.60		24.94	
72.00	40.00	89.00	32.00	15.00	35.40	37.60	169.60	20.60	864.70	1.20	0.20	17.17
73.00	2.00		31.00				27.30		9.00		3.03	
74.00	77.00	28.00	15.00	13.00	7.70	8.70	45.60	42.50	9.00	114.60	5.07	0.37
75.00	184.00		11.00		4.10		20.70		0.70		29.57	
77.00	2.00		20.00		7.10		43.80		5.00		8.76	
78.00	177.00		27.00		13.20		30.60		1.70		18.00	
79.00	2.00	251.00	24.00	21.00	8.40	16.00	65.20	26.70	1.80	4.10	36.22	6.51
80.00	126.00		14.00		34.20		21.10		9.50		2.22	
83.00	318.00		21.00		8.70		8.60		2.90		2.97	
85.00	176.00	198.00	11.00	9.00	3.70	7.20	10.40	10.20	6.10	4.70	1.70	2.17
87.00	110.00		18.00		7.30		9.60		2.00		4.80	
88.00	108.00	104.00	12.00	11.00	11.20	19.30	17.90	24.30	117.20	28.60	0.15	0.85
89.00	31.00		16.00		11.90		6.80		2.60		2.62	
90.00	159.00	38.00	11.00	29.00			2.20	45.20	9.00	46.00	0.24	0.98
91.00	268.00		14.00				14.10		3.00		4.70	

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow-up (nmol/l)	Plasma PL admission (nmol/l)	Plasma PL follow-up (nmol/l)	Plasma PLP/PL admission	Plasma PLP/PL follow-up
94.00	147.00		14.00		17.60		23.00		9.00		2.56	
96.00	76.00	27.00	29.00	28.00			9.00	9.00	9.00	9.00	1.00	1.00
99.00	287.00		22.00		16.40		111.00		258.00		0.43	
100.00	253.00	171.00	13.00	9.00	15.30	29.50	49.00	44.00	912.00	55.00	0.05	0.80
101.00	231.00		25.00		5.70		9.00		9.00		1.00	
102.00	28.00	198.00	19.00	19.00			33.00	54.00	9.00		3.67	
103.00		69.00	42.00	27.00	10.70	11.40	34.00	84.00	15.00		2.27	
104.00	1.00		34.00				28.00		10.00		2.80	
105.00	25.00	23.00	30.00	29.00	11.60	11.30	50.00	101.00	45.00	160.00	1.11	0.63
107.00	261.00		17.00		13.60		9.00		9.00		1.00	
108.00	48.00		35.00		14.30		9.00		9.00		1.00	
110.00	199.00	153.00	13.00	11.00	31.20	22.00	9.00	63.00	9.00	1,057.00	1.00	0.06
111.00	6.00	245.00	28.00	13.00	11.90	3.90	126.00	93.00	9.00	15.00	14.00	6.20
112.00	2.00	165.00	20.00	9.00	19.30		16.00	9.00	9.00	9.00	1.78	1.00
113.00	14.00	205.00	31.00	19.00			25.00	24.00	9.00	22.00	2.78	1.09
114.00	49.00	98.00	16.00	9.00	13.10	22.50	19.00	9.00	8.00	122.00	2.38	0.07
115.00	1.00		39.00		12.50		9.00		9.00		1.00	
116.00	3.00		24.00		7.80		31.00		12.00		2.58	
117.00	9.00	88.00	32.00	23.00	15.20	5.60	60.00	155.00	9.00	25.00	6.67	6.20
118.00	252.00	204.00	12.00	9.00	13.70	9.00	20.00	10.00	30.00	9.00	0.67	1.11
119.00	2.00		25.00				18.00		9.00		2.00	
121.00	203.00	88.00	15.00	15.00			12.00	28.00	9.00		1.33	
123.00	2.00	207.00	45.00	28.00	9.70	8.30	306.00	74.00	519.00		0.59	
124.00	97.00	149.00	15.00	14.00	16.20	33.30	38.00	37.00	11.00	19.00	3.45	1.95
126.00	3.00		21.00				127.00		1,346.00		0.09	
127.00	316.00		17.00				9.00		9.00		1.00	
128.00	51.00		16.00		24.70		9.00		9.00		1.00	
129.00	63.00		20.00				22.00		744.00		0.03	
131.00	165.00		21.00				15.00		9.00		1.67	
133.00	35.00		16.00				155.00		84.00		1.85	
135.00	221.00		18.00		16.00		9.00		9.00		1.00	

Patient No.	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow-up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red Cell PLP/PL admission	Red cell PLP/PL follow-up	Red cell PL Follow-up (pmol/g Hb)	White cell PLP admission (pmol/10 (6) cells)	White cell PLP follow-up (pmol/10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow-up (pmol/10 (6) cells)
12.00	348.39	270.11	59.14	5.89	3.41	79.31	1.98	1.97	0.75	0.77
13.00	345.35	446.74	70.93	4.87	2.13	209.78	2.67	2.55	0.79	1.31
14.00	624.69	398.84	129.63	4.82	3.15	126.74	2.30	2.09	1.15	1.45
15.00	2,282.98	131.76	12,431.91	0.18	1.29	102.35	4.06	2.69	6.88	0.68
16.00	7,870.37		23,635.80	0.33			8.24		5.35	
17.00	331.03	168.67	101.15	3.27	2.00	84.34	3.29	2.48	0.48	0.43
18.00	216.30	155.91	54.35	3.98	2.38	65.59	2.34	2.08	0.70	0.73
19.00	334.18	1,558.43	45.57	7.33	1.74	894.38	2.22	5.18	0.43	4.34
21.00	563.10		279.76	2.01			3.01		1.30	
22.00	25,583.33	4,232.91	53,479.76	0.48	1.56	2,716.46	6.16		10.29	
23.00	396.30	196.20	101.23	3.91	3.60	54.43	3.13	2.15	0.66	0.42
24.00	241.98		103.70	2.33			2.52		1.03	
27.00	116.28	548.28	39.53	2.94	1.24	442.53	2.19	2.35	0.73	1.71
29.00	2,274.16	1,725.93	4,038.20	0.56	1.88	918.52	4.38	3.57	6.08	3.58
30.00	173.91	1,204.49	90.22	1.93	1.46	823.60	2.42	4.09	1.22	7.17
31.00	158.89		36.67	4.33			2.31		0.32	
32.00	317.24		40.23	7.89			1.30		0.24	
33.00	176.25	204.88	18.75	9.40	3.82	53.66	1.39	1.95	0.34	0.98
34.00	3,922.58	2,146.15	3,627.96	1.08	1.19	1,798.72	5.18	5.88	5.29	3.52
35.00	131.03	184.72	35.63	3.68	2.22	83.33	1.93	2.79	0.38	0.69
36.00	2,983.13	853.01	7,756.63	0.38	2.11	403.61	2.46	2.12	9.79	3.07
37.00	205.26	280.52	50.00	4.11	3.93	71.43	1.74	1.90	0.56	0.73
38.00	310.26		32.05	9.68						
40.00	155.42	346.51	18.07	8.60	2.98	116.28	3.47	1.94	0.35	0.56
41.00	198.77		30.86	6.44			2.18		0.34	
42.00	240.45		41.57	5.78			2.64		0.38	
44.00	720.99	447.06	97.53	7.39	4.75	94.12	1.85	2.07	0.60	1.04
45.00	325.93	207.32	105.56	3.09			2.05	1.88	0.72	0.48
46.00	211.63		26.74	7.91			1.75		0.34	
47.00	316.88	514.08	64.94	4.88	9.36	54.93	2.12	2.14	0.54	0.55
48.00	1,013.92	22,001.47	241.77	4.19	0.76	28,867.65		3.81		9.22
50.00	313.92	304.29					1.50	1.62	0.52	0.60
51.00	11,113.89	33,797.44	13,498.61	0.82	1.31	25,896.15	6.15	4.85	4.66	7.28

Patient No.	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow-up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red Cell PLP/PL admission	Red cell PLP/PL follow-up	Red cell PL Follow-up (pmol/g Hb)	White cell PLP admission (pmol/10 (6) cells)	White cell PLP follow-up (pmol/10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow-up (pmol/10 (6) cells)
52.00	7,519.74	2,924.32	3,788.16	1.99	2.04	1,432.43	1.97	1.54	6.15	5.95
53.00	168.67	142.86	28.92	5.83	6.00	23.81	1.44	1.48	0.26	0.37
55.00	191.80	5,881.43			1.17	5,027.14		4.67		6.65
56.00	260.98		23.17	11.26			1.64		0.17	
57.00	208.33		15.48	13.46			0.37		0.07	
58.00	241.05	186.79	58.95	4.09	3.81	49.06				
59.00	8,840.00		22,254.44	0.40			7.21		4.39	
60.00	138.71		21.51	6.45			1.68		0.18	
61.00	181.25		27.50	6.59						
62.00	176.74	78.16					1.94	1.38	0.27	0.17
63.00	321.35		51.69	6.22			2.79		0.56	
64.00	139.74		24.36	5.74			1.35		0.31	
65.00	642.50		166.25	3.86			1.46		1.04	
67.00	111.39	51.09	17.72	6.29			1.58		0.44	
68.00	213.98	5,347.31	26.88	7.96	0.62	8,679.57	1.32			
69.00	265.88	2,214.63	24.71	10.76	1.36	1,624.39	1.76			
71.00	103.57		14.29	7.25			2.49		0.30	
72.00	13,716.88	321.43	12,640.26	1.09	4.22	76.19	3.98	1.81	8.06	1.48
73.00	150.00		57.14	2.63						
74.00	254.55	3,167.47					2.26	7.24	0.28	6.46
75.00	153.85		6.41	24.00			2.07		0.37	
77.00	263.46		44.23	5.96			2.57		0.24	
78.00	279.07		44.19	6.32			2.05		0.38	
79.00	157.14	109.18	25.51	6.16	8.92	12.24	3.56	1.86	0.53	0.54
80.00	798.80		90.36	8.84			2.68		1.09	
83.00	104.55		31.82	3.29			1.65		0.17	
85.00	201.20	241.57			7.96	30.34	1.64	1.84	0.34	0.40
87.00	177.89		26.32	6.76			1.36		0.20	
88.00	2,785.37	2,453.25	2,850.00	0.98	2.30	1,067.53	3.83	2.68	4.02	3.93
89.00	204.85		41.75	4.91			2.05		0.43	
90.00	241.46	1,728.74	39.02	6.19	0.86	2,013.79				
91.00	209.00		28.00	7.46						
94.00	243.68						1.02			

Patient No.	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow-up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red Cell PLP/PL admission	Red cell PLP/PL follow-up	Red cell PL Follow-up (pmol/g Hb)	White cell PLP admission (pmol/10 (6) cells)	White cell PLP follow-up (pmol/10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow-up (pmol/10 (6) cells)
96.00	245.83	118.18	16.67	14.75	3.96	29.87				
99.00	2,581.63		4,807.14	0.54			6.10		7.13	
100.00	10,787.95	3,750.00	38,222.89	0.28	1.08	3,465.91	3.63	5.70	7.78	10.32
101.00										
102.00	177.89	123.96	35.79	4.97	4.10	30.21				
103.00	149.44	501.18	38.20	3.91	1.66	301.18	2.64	2.14	0.46	1.37
104.00	219.15		60.64	3.61						
105.00	808.79	1,734.83	263.74	3.07	0.81	2,152.81	2.14	4.80	0.97	4.41
107.00	232.98						1.89		0.53	
108.00	147.47		75.76	1.95			2.32		0.50	
110.00	186.21	2,019.44			0.68	2,950.00	0.89	3.48	0.18	5.35
111.00	300.00	1,230.00	38.82	7.73	3.71	331.25	3.20	3.43	0.49	1.18
112.00	160.67	224.71	21.35	7.53	5.97	37.65	2.47		0.53	
113.00	265.22	732.04	40.22	6.59	2.20	333.01				
114.00	196.77	1,715.46			0.88	1,959.79	1.43	3.34	0.33	6.95
115.00	127.00		23.00	5.52			2.18		0.35	
116.00	383.87									
117.00	222.68	651.96								
118.00	594.38	210.34								
119.00	127.55		21.43	5.95						
121.00	209.30	958.62	29.07	7.20	2.18	440.23				
123.00	11,912.94	4,267.71								
124.00	846.15	1,089.66								
126.00	21,893.41									
127.00	342.68									
128.00	285.23						1.63		0.35	
129.00	23,853.33									
131.00	348.31									
133.00	1,989.00									
135.00	454.88									

Patient No.	White cell PLP/PL admission	White cell PLP/PL follow-up	Alkaline phosphatase admission (U/l)	Alkaline phosphatase follow-up (U/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
12.00	2.64	2.55	115.00	120.00	13.90	12.60
13.00	3.36	1.94	277.00	206.00	9.90	7.30
14.00	1.99	1.44	538.00	406.00	7.50	9.20
15.00	0.59	3.96	146.00	241.00	10.10	10.90
16.00	1.54		234.00		12.30	
17.00	6.91	5.70	344.00	213.00	14.90	10.00
18.00	3.34	2.83	253.00	177.00	12.90	9.90
19.00	5.14	1.20	184.00	159.00	7.10	8.40
21.00	2.32		1,221.00		10.10	
22.00	0.60		226.00	136.00	10.10	
23.00	4.72	5.11	170.00	307.00	6.70	10.20
24.00	2.45		250.00		12.80	
27.00	3.00	1.38	261.00	441.00	12.10	10.80
29.00	0.72	1.00	168.00	204.00	8.90	9.00
30.00	1.99	0.57	1,089.00	659.00	10.10	7.80
31.00	7.21		126.00		12.20	
32.00	5.45		331.00		9.80	
33.00	4.08	1.98	107.00	280.00	12.00	10.60
34.00	0.98	1.67	96.00	220.00	12.50	8.70
35.00	5.07	4.06	69.00	121.00	9.50	8.70
36.00	0.25	0.69	238.00	543.00	8.70	9.50
37.00	3.11	2.61	264.00	255.00	13.30	13.50
38.00			398.00		9.20	
40.00	9.83	3.49	35.00	39.00	7.20	8.90
41.00	6.38		54.00		11.60	
42.00	7.05		26.00	41.00	9.30	10.50
44.00	3.08	1.98	31.00	88.00	10.90	10.00
45.00	2.84	3.96	158.00	564.00	7.70	10.10
46.00	5.12		71.00		8.80	
47.00	3.94	3.92	276.00	264.00	8.50	8.60
48.00		0.41	121.00	86.00	9.60	10.10
50.00	2.88	2.70	36.00	94.00		9.60

Patient No.	White cell PLP/PL admission	White cell PLP/PL follow-up	Alkaline phosphatase admission (U/l)	Alkaline phosphatase follow-up (U/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
51.00	1.32	0.67	24.00	99.00	8.90	9.70
52.00	0.32	0.26	111.00	258.00	8.40	7.20
53.00	5.57	4.04	96.00	158.00	9.40	8.20
55.00		0.70	70.00	116.00	8.20	8.50
56.00	9.42		42.00		8.60	
57.00	5.25		43.00		9.40	
58.00			36.00	22.00	10.00	8.30
59.00	1.64		52.00		15.20	
60.00	9.54		85.00			
61.00			76.00		9.40	
62.00	7.18	8.07	48.00	55.00	9.20	10.30
63.00	4.97		67.00		9.90	
64.00	4.36		83.00		8.70	
65.00	1.40		58.00		9.10	
67.00	3.56		212.00	125.00	9.80	11.80
68.00			28.00	125.00	11.00	8.80
69.00			47.00	81.00	9.10	9.30
71.00	8.35		95.00		15.50	
72.00	0.49	1.22	79.00	181.00	10.50	8.20
73.00			97.00		10.70	
74.00	7.96	1.12	820.00	644.00	11.80	11.50
75.00	5.55		51.00		7.70	
77.00	10.65		46.00		8.90	
78.00	5.47		148.00		10.00	
79.00	6.66	3.43	47.00	76.00	13.30	12.00
80.00	2.46		183.00		7.90	
83.00	9.81		81.00		11.80	
85.00	4.82	4.55	16.00	17.00	11.70	
87.00	6.91		79.00		7.40	
88.00	0.95	0.68	77.00	91.00		9.00
89.00	4.75		67.00		9.40	
90.00			69.00	81.00	8.50	7.90
91.00			55.00		10.70	

Patient No.	White cell PLP/PL admission	White cell PLP/PL follow-up	Alkaline phosphatase admission (U/l)	Alkaline phosphatase follow-up (U/l)	Haemoglobin admission (g/dl)	Haemoglobin follow-up (g/dl)
94.00			108.00		9.20	
96.00			86.00	108.00	9.70	9.00
99.00	0.86		105.00		13.70	
100.00	0.47	0.55	183.00	132.00	7.60	8.40
101.00			40.00		10.10	
102.00			39.00	60.00	8.50	7.70
103.00	5.75	1.56	71.00	80.00	13.20	
104.00			88.00		11.70	
105.00	2.21	1.09	94.00	98.00	7.00	7.70
107.00	3.55		71.00		10.50	
108.00	4.69		66.00		12.70	
110.00	5.07	0.65	31.00	113.00	9.00	8.20
111.00	6.53	2.90	99.00	52.00	16.20	6.80
112.00	4.68		64.00	43.00	15.40	8.90
113.00			64.00	40.00	14.90	10.00
114.00	4.33	0.48	100.00	86.00	10.70	7.70
115.00	6.31		67.00		14.20	
116.00			50.00		9.70	
117.00			69.00	54.00	12.90	9.30
118.00			88.00	86.00	8.10	9.20
119.00			73.00		11.60	
121.00			56.00	166.00	10.00	10.20
123.00			148.00	80.00	10.50	10.20
124.00			83.00	107.00	7.00	
126.00			74.00		9.20	
127.00			278.00		8.50	
128.00	4.71		73.00		8.80	
129.00			265.00		11.30	
131.00			74.00		10.60	
133.00			102.00		11.00	
135.00			399.00		7.30	

12.9 Appendix 9 Chapter 5 data

Appendix 9. Characteristics and biochemical measurements of controls (Chapter 5)

Control no.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Total protein (g/l)	Plasma α-tocopherol (umol/l)	Plasma β-carotene (ug/l)	Free MDA (umol/l)	MDA/ Total Protein (umol/g)	Plasma ascorbic acid (umol/l)
1.00	51.00	0.00	6.00	46.00	77.00	24.00	100.00	0.04	0.01	3
2.00	53.00	1.00	6.00	41.00	73.00	29.00	272.00	0.04	0.01	57
3.00	43.00	0.00	6.00	45.00	76.00	25.00	186.00	0.05	0.00	23
4.00	47.00	0.00	6.00	48.00	78.00	23.00	344.00	0.05	0.00	21
5.00	55.00	1.00	6.00	44.00	76.00	27.00	150.00	0.05	0.01	34
6.00	59.00	0.00	6.00	41.00	70.00	38.00	249.00	0.05	0.01	.
7.00	69.00	1.00	6.00	38.00	71.00	40.00	312.00	0.05	0.01	.
8.00	45.00	1.00	6.00	45.00	78.00	21.00	271.00	0.06	0.01	48
9.00	48.00	0.00	8.00	44.00	73.00	31.00	122.00	0.07	0.01	35
10.00	57.00	0.00	6.00	43.00	72.00	23.00	146.00	0.07	0.01	24
11.00	58.00	1.00	6.00	48.00	76.00	37.00	157.00	0.07	0.01	71
12.00	73.00	1.00	6.00	39.00	69.00	37.00	210.00	0.07	0.01	29
13.00	51.00	0.00	6.00	44.00	74.00	26.00	708.00	0.07	0.01	6
14.00	62.00	0.00	6.00	41.00	70.00	31.00	258.00	0.07	0.01	57
15.00	44.00	0.00	6.00	42.00	69.00	33.00	155.00	0.07	0.01	9
16.00	64.00	1.00	6.00	41.00	76.00	41.00	288.00	0.07	0.01	.
17.00	59.00	1.00	6.00	43.00	72.00	42.00	595.00	0.08	0.01	43
18.00	57.00	1.00	6.00	44.00	73.00	29.00	167.00	0.08	0.01	20
19.00	45.00	1.00	6.00	47.00	70.00	30.00	157.00	0.08	0.01	41
20.00	52.00	0.00	6.00	43.00	72.00	20.00	152.00	0.08	0.01	24
21.00	64.00	0.00	6.00	43.00	72.00	31.00	328.00	0.08	0.01	.
22.00	51.00	1.00	6.00	42.00	71.00	24.00	109.00	0.08	0.01	82
23.00	50.00	0.00	6.00	44.00	70.00	37.00	100.00	0.08	0.01	54
24.00	60.00	0.00	6.00	49.00	80.00	27.00	123.00	0.08	0.01	51
25.00	47.00	0.00	6.00	44.00	72.00	22.00	147.00	0.08	0.01	3
26.00	53.00	0.00	6.00	46.00	71.00	31.00	102.00	0.09	0.01	10
27.00	55.00	1.00	6.00	43.00	72.00	33.00	314.00	0.09	0.01	20

Control no.	Age	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Total protein (g/l)	Plasma α-tocopherol (umol/l)	Plasma β-carotene (ug/l)	Free MDA (umol/l)	MDA/ Total Protein (umol/g)	Plasma ascorbic acid (umol/l)
28.00	44.00	0.00	6.00	48.00	80.00	24.00	73.00	0.09	0.01	43
29.00	44.00	1.00	6.00	50.00	80.00	28.00	80.00	0.09	0.01	2
30.00	56.00	0.00	6.00	47.00	81.00	28.00	87.00	0.09	0.01	47
31.00	49.00	1.00	6.00	47.00	78.00	31.00	126.00	0.09	0.01	18
32.00	55.00	1.00	6.00	46.00	76.00	14.00	228.00	0.09	0.01	4
33.00	52.00	0.00	6.00	41.00	69.00	32.00	202.00	0.09	0.01	43
34.00	57.00	1.00	6.00	45.00	76.00	25.00	134.00	0.09	0.01	4
35.00	56.00	0.00	6.00	44.00	74.00	29.00	57.00	0.09	0.01	5
36.00	71.00	1.00	6.00	41.00	70.00	33.00	255.00	0.09	0.01	.
37.00	61.00	0.00	6.00	44.00	77.00	37.00	188.00	0.10	0.01	.
38.00	56.00	0.00	6.00	43.00	66.00	39.00	366.00	0.10	0.02	41

Appendix 9. Characteristics and biochemical measurements of patients (Chapter 5)

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicte mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
13.00	43.00	1.00	12.00	11.70	1.00	0.00	2.00	0.00	5.00	9.00	3.00
14.00	71.00	1.00	18.00	28.90	1.00	0.00	0.00	1.00	8.00	9.00	2.00
15.00	53.00	1.00	19.00	32.20	0.00	0.00	1.00	0.00	8.00	2.00	5.00
16.00	61.00	0.00	23.00	28.50	0.00	0.00	--	0.00	6.00	--	--
18.00	67.00	0.00	18.00	44.40	1.00	0.00	0.00	1.00	10.00	10.00	4.00
19.00	50.00	0.00	21.00	38.90	0.00	0.00	3.00	1.00	10.00	9.00	4.00
21.00	61.00	0.00	34.00	76.80	1.00	0.00	--	0.00	8.00	--	--
22.00	52.00	0.00	13.00	9.90	0.00	95.00	101.00	0.00	2.00	3.00	4.00
23.00	71.00	0.00	23.00	42.50	1.00	9.00	10.00	1.00	8.00	4.00	7.00
24.00	77.00	1.00	21.00	35.00	0.00	0.00	--	0.00	4.00	--	--
27.00	76.00	1.00	23.00	46.00	0.00	1.00	3.00	0.00	5.00	4.00	3.00
30.00	73.00	1.00	25.00	53.30	1.00	0.00	2.00	1.00	11.00	7.00	3.00
31.00	62.00	0.00	19.00	27.10	1.00	0.00	--	0.00	11.00	--	--
32.00	55.00	1.00	15.00	21.00	1.00	0.00	--	0.00	6.00	--	--
33.00	80.00	1.00	31.00	84.20	1.00	0.00	0.00	1.00	7.00	8.00	5.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	0.00	6.00	7.00	4.00
35.00	20.00	0.00	18.00	39.70	1.00	0.00	1.00	1.00	10.00	7.00	3.00
36.00	43.00	1.00	38.00	92.60	0.00	5.00	10.00	0.00	13.00	10.00	4.00
40.00	47.00	0.00	22.00	28.50	1.00	0.00	2.00	0.00	9.00	4.00	3.00
41.00	76.00	1.00	29.00	79.90	1.00	0.00	--	0.00	8.00	--	--
42.00	79.00	0.00	21.00	30.90	1.00	0.00	3.00	0.00	4.00	6.00	4.00
45.00	74.00	0.00	33.00	75.20	0.00	0.00	0.00	1.00	11.00	5.00	5.00
47.00	76.00	0.00	29.00	77.20	1.00	0.00	0.00	1.00	7.00	7.00	2.00
50.00	60.00	0.00	19.00	48.00	1.00	0.00	0.00	0.00	7.00	4.00	6.00
51.00	46.00	1.00	24.00	49.70	0.00	1,000.00	1,006.00	1.00	14.00	16.00	3.00
52.00	61.00	0.00	33.00	78.60	0.00	7.00	10.00	1.00	11.00	11.00	3.00
53.00	67.00	1.00	18.00	21.20	0.00	0.00	0.00	1.00	11.00	10.00	3.00
56.00	40.00	1.00	6.00	10.20	1.00	0.00	--	0.00	6.00	--	--
60.00	57.00	0.00	24.00	67.00	0.00	0.00	--	0.00	3.00	--	--
62.00	74.00	0.00	20.00	19.90	1.00	0.00	0.00	0.00	1.00	2.00	3.00
63.00	62.00	0.00	18.00	44.40	1.00	1,000.00	--	0.00	2.00	--	--
64.00	66.00	0.00	34.00	81.00	0.00	0.00	--	0.00	13.00	--	--

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicte mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
65.00	38.00	1.00	17.00	26.20	0.00	0.00	--	0.00	9.00	--	--
67.00	68.00	1.00	31.00	80.30	0.00	1,000.00	0.00	0.00	4.00	4.00	5.00
71.00	25.00	0.00	17.00	1.20	0.00	0.00	--	0.00	3.00	--	--
72.00	51.00	0.00	21.00	12.40	0.00	1.00	1.00	0.00	11.00	7.00	12.00
73.00	69.00	0.00	12.00	20.20	0.00	0.00	--	0.00	3.00	--	--
74.00	65.00	0.00	33.00	80.40	0.00	0.00	7.00	0.00	6.00	11.00	4.00
75.00	28.00	1.00	14.00	18.50	1.00	0.00	--	0.00	10.00	--	--
79.00	41.00	0.00	7.00	7.60	1.00	0.00	0.00	0.00	4.00	3.00	3.00
80.00	38.00	0.00	16.00	23.30	1.00	1,000.00	--	0.00	9.00	--	--
81.00	71.00	0.00	25.00	35.90	1.00	0.00	1.00	0.00	5.00	6.00	3.00
82.00	80.00	1.00	17.00	12.70	0.00	0.00	1.00	0.00	1.00	1.00	2.00
83.00	46.00	1.00	11.00	10.30	1.00	0.00	--	0.00	4.00	--	--
84.00	56.00	0.00	28.00	63.90	0.00	1,000.00	1,006.00	0.00	13.00	10.00	3.00
85.00	73.00	0.00	18.00	44.40	1.00	0.00	0.00	0.00	6.00	7.00	2.00
87.00	34.00	1.00	12.00	12.40	1.00	0.00	--	0.00	6.00	--	--
89.00	22.00	1.00	10.00	9.50	1.00	0.00	--	0.00	2.00	--	--
99.00	37.00	0.00	13.00	16.50	1.00	0.00	--	0.00	5.00	--	--
100.00	50.00	1.00	26.00	38.20	0.00	0.00	12.00	0.00	4.00	4.00	3.00
101.00	35.00	1.00	9.00	4.70	1.00	0.00	--	0.00	5.00	--	--
102.00	26.00	0.00	23.00	45.70	1.00	0.00	--	0.00	4.00	2.00	2.00
103.00	52.00	0.00	16.00	12.20	1.00	0.00	0.00	0.00	2.00	1.00	3.00
104.00	38.00	0.00	3.00	2.70	0.00	0.00	--	0.00	2.00	--	--
105.00	39.00	0.00	34.00	87.50	0.00	1,000.00	1,001.00	0.00	11.00	8.00	2.00
107.00	45.00	1.00	12.00	6.60	1.00	0.00	--	0.00	8.00	--	--
108.00	44.00	1.00	6.00	0.90	0.00	0.00	--	0.00	3.00	--	--
110.00	48.00	1.00	26.00	35.20	0.00	0.00	8.00	1.00	7.00	12.00	5.00
111.00	23.00	0.00	9.00	4.30	1.00	0.00	7.00	0.00	7.00	2.00	5.00
113.00	70.00	0.00	27.00	47.70	0.00	0.00	2.00	1.00	4.00	5.00	3.00
114.00	65.00	1.00	24.00	65.70	1.00	0.00	3.00	0.00	9.00	8.00	4.00
115.00	47.00	0.00	10.00	1.50	0.00	0.00	--	0.00	2.00	--	--
116.00	61.00	0.00	13.00	10.60	0.00	0.00	--	0.00	1.00	--	--
117.00	48.00	0.00	13.00	8.50	1.00	0.00	2.00	0.00	3.00	3.00	4.00
118.00	59.00	0.00	23.00	39.90	1.00	0.00	1.00	0.00	9.00	12.00	2.00
119.00	67.00	1.00	18.00	14.40	0.00	0.00	--	0.00	2.00	--	--
121.00	86.00	0.00	22.00	42.20	1.00	0.00	3.00	0.00	7.00	4.00	5.00
123.00	60.00	0.00	16.00	14.60	0.00	1,000.00	1,004.00	0.00	7.00	6.00	3.00

Patient No.	Age	Male/ Female (0/1)	APACHE II	Predicte mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	SOFA score admission	SOFA score follow-up	Follow-up sample (days)
126.00	68.00	0.00	27.00	46.00	0.00	1,000.00	--	0.00	5.00	--	--
128.00	80.00	1.00	30.00	56.90	1.00	0.00	--	0.00	7.00	--	--
133.00	35.00	0.00	14.00	7.20	1.00	0.00	--	0.00	0.00	--	--
135.00	41.00	1.00	16.00	14.30	1.00	0.00	--	0.00	2.00	--	--
137.00	69.00	1.00	19.00	32.00	1.00	0.00	--	0.00	4.00	--	--
139.00	54.00	1.00	23.00	55.80	1.00	0.00	--	0.00	8.00	--	--
140.00	74.00	0.00	21.00	41.60	1.00	0.00	--	0.00	8.00	--	--
141.00	67.00	0.00	32.00	78.00	1.00	0.00	--	0.00	10.00	2.00	11.00
143.00	52.00	0.00	10.00	17.40	1.00	0.00	--	1.00	2.00	--	--
147.00	70.00	0.00	20.00	35.50	1.00	0.00	0.00	0.00	7.00	3.00	2.00
150.00	62.00	0.00	21.00	38.90	0.00	0.00	--	0.00	11.00	--	--
154.00	49.00	0.00	26.00	4.40	0.00	0.00	--	0.00	4.00	--	--
155.00	43.00	0.00	25.00	3.80	0.00	0.00	--	0.00	8.00	--	--
157.00	61.00	0.00	31.00	81.40	1.00	0.00	--	0.00	14.00	--	--
164.00	62.00	1.00	14.00	9.70	0.00	0.00	--	0.00	10.00	--	--

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma α-tocopherol admission (umol/l)	Red cell α-tocopherol admission (umol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Red cell α-tocopherol follow-up (umol/g Hb)
13.00	22.00	261.00	27.00	22.00	55.00	47.00	5.00	8.00	15.35	11.30	11.98	11.53
14.00	220.00	124.00	12.00	10.00	45.00	37.00	24.90	17.60	16.26	22.20	10.49	22.17
15.00	257.00	73.00	23.00	16.00	63.00	54.00	11.40	6.60	14.48	3.40	26.88	25.78
16.00	122.00	--	28.00	--	72.00	--	5.70	--	15.68	16.48	--	--
18.00	152.00	64.00	10.00	10.00	31.00	30.00	12.10	25.00	6.27	16.01	14.47	11.94
19.00	143.00	101.00	12.00	14.00	68.00	38.00	17.00	8.00	17.70	18.62	15.40	12.15
21.00	23.00	--	10.00	--	25.00	--	14.00	--	16.03	16.00	--	--
22.00	20.00	67.00	27.00	15.00	71.00	40.00	24.80	--	13.74	18.73	16.90	--
23.00	125.00	216.00	10.00	13.00	39.00	48.00	8.30	10.70	7.93	13.47	18.48	18.62
24.00	81.00	--	35.00	--	74.00	--	31.00	--	26.95	22.18	--	--
27.00	103.00	133.00	23.00	17.00	54.00	46.00	3.80	13.70	24.42	19.14	21.91	25.37
30.00	26.00	179.00	16.00	11.00	44.00	42.00	16.70	18.30	33.79	39.28	23.73	36.95
31.00	50.00	--	22.00	--	46.00	--	9.20	--	14.80	17.83	--	--
32.00	434.00	--	17.00	--	39.00	--	14.70	--	10.30	11.39	--	--
33.00	259.00	173.00	17.00	10.00	41.00	35.00	10.00	19.40	11.83	16.94	13.34	19.80
34.00	404.00	336.00	30.00	15.00	56.00	39.00	10.10	8.70	19.65	15.33	23.17	34.15
35.00	119.00	303.00	9.00	13.00	27.00	36.00	6.30	8.40	10.16	26.99	16.07	21.66
36.00	132.00	70.00	14.00	14.00	35.00	39.00	13.80	29.50	6.44	12.38	14.56	27.64
40.00	15.00	234.00	20.00	18.00	39.00	41.00	3.80	8.00	17.38	21.05	21.31	20.02
41.00	184.00	--	19.00	--	33.00	--	16.40	--	10.05	25.22	--	--
42.00	8.00	149.00	17.00	18.00	32.00	37.00	10.00	--	12.37	15.59	14.77	22.05
45.00	258.00	164.00	14.00	14.00	41.00	45.00	18.90	10.60	15.25	16.23	19.08	19.03
47.00	64.00	72.00	24.00	24.00	58.00	53.00	12.80	13.30	14.47	18.55	12.24	18.77
50.00	180.00	141.00	23.00	15.00	31.00	42.00	8.90	17.70	9.98	18.10	15.88	15.54
51.00	56.00	58.00	14.00	19.00	43.00	47.00	--	10.10	8.40	--	12.09	--
52.00	565.00	257.00	9.00	9.00	43.00	44.00	48.60	66.00	7.83	16.99	8.64	11.91
53.00	148.00	131.00	9.00	9.00	34.00	33.00	4.40	6.10	16.34	20.19	19.99	23.66
56.00	37.00	--	16.00	--	33.00	--	9.60	--	11.20	17.05	--	--
60.00	166.00	--	26.00	--	75.00	--	12.90	--	30.79	21.47	--	--
62.00	88.00	180.00	12.00	14.00	38.00	43.00	12.50	12.10	14.40	18.07	17.15	14.28
63.00	117.00	--	14.00	--	27.00	--	5.60	--	9.63	13.00	--	--

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma α-tocopherol admission (umol/l)	Red cell α-tocopherol admission (umol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Red cell α-tocopherol follow-up (umol/g Hb)
64.00	311.00	--	18.00	--	64.00	--	11.60	--	19.42	20.23	--	--
65.00	163.00	--	23.00	--	54.00	--	16.10	--	10.05	20.07	--	--
67.00	438.00	52.00	20.00	16.00	35.00	36.00	7.30	--	12.43	29.75	25.83	48.37
71.00	2.00	--	38.00	--	75.00	--	11.50	--	21.98	17.68	--	--
72.00	40.00	89.00	32.00	15.00	58.00	56.00	35.40	37.60	16.40	9.77	33.61	38.31
73.00	2.00	--	31.00	--	62.00	--	--	--	19.99	16.66	--	--
74.00	77.00	28.00	15.00	13.00	48.00	42.00	7.70	8.70	11.23	13.46	12.79	23.73
75.00	184.00	--	11.00	--	37.00	--	4.10	--	20.40	17.85	--	--
79.00	2.00	251.00	24.00	21.00	44.00	44.00	8.40	16.00	17.25	25.28	21.55	24.34
80.00	126.00	--	14.00	--	58.00	--	34.20	--	11.19	--	--	--
81.00	8.00	284.00	10.00	21.00	23.00	44.00	5.30	7.60	6.08	21.71	16.42	28.07
82.00	4.00	86.00	29.00	29.00	60.00	62.00	20.00	21.40	33.45	21.44	24.02	23.52
83.00	318.00	--	21.00	--	48.00	--	8.70	--	17.97	12.79	--	--
84.00	63.00	80.00	9.00	9.00	29.00	32.00	7.80	19.60	5.33	16.02	8.20	8.18
85.00	176.00	198.00	11.00	9.00	26.00	27.00	3.70	7.20	8.06	22.33	8.94	20.74
87.00	110.00	--	18.00	--	40.00	--	7.30	--	16.63	18.91	--	--
89.00	31.00	--	16.00	--	33.00	--	11.90	--	19.22	19.83	--	--
99.00	287.00	--	22.00	--	52.00	--	16.40	--	15.00	11.60	--	--
100.00	253.00	171.00	13.00	9.00	41.00	33.00	15.30	29.50	19.00	22.67	12.00	30.64
101.00	231.00	--	25.00	--	46.00	--	5.70	--	15.00	16.68	--	--
102.00	28.00	198.00	19.00	19.00	38.00	39.00	--	--	11.00	23.38	14.00	15.70
103.00	--	69.00	42.00	27.00	73.00	49.00	10.70	11.40	41.00	31.49	29.00	38.97
104.00	1.00	--	34.00	--	71.00	--	--	--	36.00	13.43	--	--
105.00	25.00	23.00	30.00	29.00	66.00	57.00	11.60	11.30	13.00	21.66	10.00	8.76
107.00	261.00	--	17.00	--	39.00	--	13.60	--	16.00	12.87	--	--
108.00	48.00	--	35.00	--	70.00	--	14.30	--	23.00	--	--	--
110.00	199.00	153.00	13.00	11.00	48.00	34.00	31.20	22.00	17.00	23.88	27.00	19.26
111.00	6.00	245.00	28.00	13.00	53.00	37.00	11.90	3.90	26.00	22.55	18.00	22.30
113.00	14.00	205.00	31.00	19.00	60.00	43.00	--	--	17.00	13.13	9.00	14.98
114.00	49.00	98.00	16.00	9.00	37.00	27.00	13.10	22.50	15.00	16.57	10.00	23.03
115.00	1.00	--	39.00	--	63.00	--	12.50	--	24.00	--	--	--
116.00	3.00	--	24.00	--	48.00	--	7.80	--	12.00	--	--	--
117.00	9.00	88.00	32.00	23.00	55.00	45.00	15.20	5.60	36.00	--	29.00	--
118.00	252.00	204.00	12.00	9.00	33.00	28.00	13.70	9.00	7.00	16.69	9.00	16.45

Patient No.	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	White cell counts admission (10 (9) cells)	White cell counts follow-up (10 (9) cells)	Plasma α-tocopherol admission (umol/l)	Red cell α-tocopherol admission (umol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Red cell α-tocopherol follow-up (umol/g Hb)
119.00	2.00	--	25.00	--	50.00	--	--	--	32.00	--	--	--
121.00	203.00	88.00	15.00	15.00	37.00	39.00	--	--	8.00	--	15.00	--
123.00	2.00	207.00	45.00	28.00	83.00	58.00	9.70	8.30	27.00	19.07	24.00	22.45
126.00	3.00	--	21.00	--	46.00	--	--	--	20.00	--	--	--
128.00	51.00	--	16.00	--	46.00	--	24.70	--	17.00	--	--	--
133.00	35.00	--	16.00	--	36.00	--	--	--	6.00	--	--	--
135.00	221.00	--	18.00	--	58.00	--	16.00	--	25.00	--	--	--
137.00	218.00	--	11.00	--	34.00	--	--	--	20.00	--	--	--
139.00	72.00	--	14.00	--	33.00	--	--	--	6.00	--	--	--
140.00	51.00	--	9.00	--	26.00	--	--	--	5.00	--	--	--
141.00	143.00	80.00	9.00	9.00	40.00	42.00	--	--	6.00	--	22.00	--
143.00	39.00	--	14.00	--	60.00	--	--	--	22.00	--	--	--
147.00	203.00	216.00	32.00	24.00	56.00	51.00	--	--	12.00	--	11.00	--
150.00	295.00	--	24.00	--	55.00	--	--	--	12.00	--	--	--
154.00	39.00	--	33.00	--	62.00	--	--	--	23.00	--	--	--
155.00	52.00	--	19.00	--	68.00	--	--	--	11.00	--	--	--
157.00	294.00	--	13.00	--	44.00	--	--	--	6.00	--	--	--
164.00	291.00	--	24.00	--	58.00	--	--	--	12.00	--	--	--

Patient No.	Plasma β-carotene admission (ug/l)	Plasma β-carotene follow-up (ug/l)	Free MDA admission (umol/l)	Free MDA follow-up (umol/l)	MDA/Total Protein admission (umol/g)	MDA/Total Protein follow-up (umol/g)	Plasma ascorbic acid admission (umol/l)	Plasma ascorbic acid follow-up (umol/l)	White cell ascorbic acid admission (umol/10 (9) cells)	White cell ascorbic acid follow-up (umol/10 (9) cells)
13.00	0.50	10.90	0.16	0.17	0.02	0.01	24	2	1.9	2.64
14.00	8.90	0.60	0.58	0.16	0.01	0.02	0.9	3	2.45	3.12
15.00	3.90	0.40	0.17	0.14	0.02	0.01	16	0.9	2.93	2.1
16.00	20.40	--	0.20	--	0.01	--	1	.	1.39	.
18.00	0.30	2.80	0.19	0.17	0.03	0.02	4	3	1.91	1.45
19.00	117.90	60.30	0.26	0.40	0.01	0.03	37	15	1.76	2.17
21.00	10.20	--	0.62	--	0.09	--	2	.	0.69	.
22.00	39.00	39.00	0.38	--	0.01	--	136	25	2.57	.
23.00	28.30	115.40	0.19	0.11	0.01	0.01	20	10	3.02	1.11
24.00	115.60	--	0.37	--	0.02	--	21	.	1.81	.
27.00	67.60	183.50	0.22	0.25	0.02	0.02	13	28	3.39	1.41
30.00	40.10	25.30	0.97	0.56	0.02	0.02	11	.	1.56	.
31.00	66.90	--	0.21	--	0.01	--	3	.	1.91	.
32.00	9.30	--	0.23	--	0.02	--	3	.	1.32	.
33.00	39.00	57.80	0.31	0.61	0.02	0.03	8	5	1.7	2.1
34.00	0.60	69.50	0.14	0.13	0.01	0.01	59	25	2.91	2.69
35.00	34.30	54.40	5.96	9.41	0.53	0.57	0.9	4	2.63	2.5
36.00	14.40	30.40	0.20	0.33	0.02	0.04	59	16	3.81	3.53
40.00	31.70	11.10	0.06	0.03	0.01	0.01	20	11	1.93	2.2
41.00	45.20	--	0.05	--	0.01	--	19	.	3.3	.
42.00	57.10	76.40	--	--	--	--	0.9	4	1.82	.
45.00	15.60	50.20	0.06	0.08	0.01	0.01	1	2	0.75	0.84
47.00	31.20	22.50	0.07	0.13	0.01	0.01	0.9	0.9	0.6	0.67
50.00	25.90	23.30	0.15	0.09	0.01	0.01	50	3	2.35	2.43
51.00	23.90	46.70	0.10	0.13	0.01	0.01	11	7	3.06	4.86
52.00	0.60	0.40	0.18	0.21	0.01	0.01	102	60	.	.
53.00	0.90	1.50	0.10	0.09	0.01	0.01	0.9	1	.	.
56.00	29.70	--	--	--	0.01	--	4	.	.	.
60.00	85.00	--	0.07	--	0.01	--	12	.	.	.

Patient No.	Plasma β-carotene admission (ug/l)	Plasma β-carotene follow-up (ug/l)	Free MDA admission (umol/l)	Free MDA follow-up (umol/l)	MDA/Total Protein admission (umol/g)	MDA/Total Protein follow-up (umol/g)	Plasma ascorbic acid admission (umol/l)	Plasma ascorbic acid follow-up (umol/l)	White cell ascorbic acid admission (umol/10 (9) cells)	White cell ascorbic acid follow-up (umol/10 (9) cells)
62.00	53.10	85.00	0.05	0.71	0.02	0.02	0.9	.	.	.
63.00	20.90	--	0.06	--	0.02	--	0.9	.	.	.
64.00	65.60	--	0.02	--	0.01	--	4	.	.	.
65.00	--	--	0.08	--	0.01	--	14	.	.	.
67.00	22.00	126.00	0.26	0.08	0.02	0.02	11	9	.	.
71.00	16.00	--	0.04	--	0.01	--	6	.	.	.
72.00	0.50	187.20	--	--	--	--	79	.	.	.
73.00	51.50	--	0.05	--	0.02	--	0.9	.	.	.
74.00	0.30	0.50	--	--	--	--	2	69	.	.
75.00	18.20	--	0.18	--	0.03	--	0.9	.	.	.
79.00	41.90	22.10	--	--	--	--	0.9	2	.	.
80.00	10.60	--	--	--	--	--	2	.	1.47	.
81.00	14.60	21.60	0.08	0.07	0.02	0.01	10	6	1.54	1.46
82.00	401.80	248.20	--	--	--	--	0.9	22	.	.
83.00	91.60	--	0.04	--	0.02	--	1	.	1.6	.
84.00	0.70	14.40	0.13	0.15	0.03	0.03	1	16	1.57	1.42
85.00	9.00	13.40	--	--	--	--	1	0.9	.	.
87.00	141.30	--	0.02	--	0.01	--	3	.	.	.
89.00	62.60	--	--	--	--	--	12	.	1.94	.
99.00	9.00	--	0.05	--	0.01	--	29	.	1.79	.
100.00	9.00	9.00	0.19	0.12	0.02	0.01	2	24	1.95	5.8
101.00	46.00	--	0.07	--	0.01	--	17	.	2.12	.
102.00	44.00	56.00	--	--	--	--	9	5	.	.
103.00	167.00	218.00	--	--	--	--	35	18	.	.
104.00	32.00	--	--	--	--	--	0.9	.	.	.
105.00	52.00	32.00	0.15	0.17	0.01	0.01	5	0.9	8.77	1.9
107.00	311.00	--	0.08	--	0.02	--	16	.	.	.
108.00	48.00	--	0.10	--	0.01	--	3	.	1.42	.
110.00	11.00	16.00	0.09	0.12	0.01	0.02	5	17	1.5	2.54
111.00	50.00	50.00	--	--	--	--	2	3	1.99	4.08
113.00	25.00	9.00	0.35	0.05	0.03	0.01	2	2	.	.
114.00	53.00	18.00	0.12	0.16	0.02	0.02	0.9	2	0.46	1.02

Patient No.	Plasma β-carotene admission (ug/l)	Plasma β-carotene follow-up (ug/l)	Free MDA admission (umol/l)	Free MDA follow-up (umol/l)	MDA/Total Protein admission (umol/g)	MDA/Total Protein follow-up (umol/g)	Plasma ascorbic acid admission (umol/l)	Plasma ascorbic acid follow-up (umol/l)	White cell ascorbic acid admission (umol/10 (9) cells)	White cell ascorbic acid follow-up (umol/10 (9) cells)
115.00	51.00	--	0.05	--	0.02	--	17	.	0.99	.
116.00	9.00	--	0.06	--	0.02	--	0.9	.	0.74	.
117.00	16.00	15.00	0.06	0.06	0.03	0.01	2	2	.	.
118.00	9.00	9.00	0.05	0.17	0.02	0.02	0.9	0.9	.	.
119.00	140.00	--	0.06	--	0.03	--	11	.	.	.
121.00	9.00	27.00	0.08	0.06	0.02	0.01	2	7	.	.
123.00	24.00	73.00	0.04	0.10	0.01	0.01	2	20	.	.
126.00	14.00	--	0.06	--	0.01	--	10	.	.	.
128.00	39.00	--	0.10	--	0.02	--	2	.	.	.
133.00	9.00	--	0.08	--	0.02	--	17	.	.	.
135.00	176.00	--	0.06	--	0.01	--	5	.	2.04	.
137.00	48.00	--	0.04	--	0.01	--	4	.	.	.
139.00	9.00	--	0.04	--	0.02	--	11	.	.	.
140.00	9.00	--	0.08	--	0.02	--	1	.	.	.
141.00	9.00	13.00	0.12	0.11	0.02	0.01	0.9	0.9	.	.
143.00	518.00	--	0.08	--	0.01	--	3	.	.	.
147.00	23.00	24.00	0.05	0.05	0.01	0.01	12	10	.	.
150.00	9.00	--	0.06	--	0.01	--	5	.	.	.
154.00	10.00	--	0.09	--	0.01	--	58	.	.	.
155.00	92.00	--	0.19	--	0.02	--	28	.	.	.
157.00	9.00	--	0.22	--	0.01	--	29	.	.	.
164.00	8.00	--	0.05	--	0.01	--	0.9	.	.	.

12.10 Appendix 10 Chapter 6 data

Appendix 10. Characteristics and biochemical measurements of controls (Chapter 6)

Control no.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)
1.00	42.00	0.00	6.00	44.00	0.70	4.20	20.00	4.76	28.57
2.00	42.00	0.00	6.00	44.00	1.60	5.90	27.00	4.58	16.88
3.00	42.00	0.00	6.00	45.00	1.25	4.95	24.00	4.85	19.20
4.00	43.00	0.00	6.00	45.00	0.50	5.40	25.00	4.63	50.00
5.00	44.00	1.00	6.00	50.00	1.50	6.40	28.00	4.38	18.67
6.00	44.00	0.00	6.00	42.00	2.00	5.85	33.00	5.64	16.50
7.00	44.00	0.00	6.00	48.00	1.35	4.80	24.00	5.00	17.78
8.00	45.00	1.00	6.00	45.00	0.55	4.25	21.00	4.94	38.18
9.00	45.00	1.00	6.00	47.00	1.25	6.15	30.00	4.88	24.00
10.00	47.00	0.00	6.00	48.00	0.65	5.80	23.00	3.97	35.38
11.00	47.00	0.00	6.00	44.00	1.05	5.65	22.00	3.89	20.95
12.00	48.00	0.00	8.00	44.00	1.45	5.75	31.00	5.39	21.38
13.00	49.00	1.00	6.00	47.00	2.50	5.05	31.00	6.14	12.40
14.00	50.00	0.00	6.00	44.00	0.55	5.55	37.00	6.67	67.27
15.00	51.00	1.00	6.00	42.00	1.25	4.50	24.00	5.33	19.20
16.00	51.00	0.00	6.00	44.00	0.55	5.15	26.00	5.05	47.27
17.00	51.00	0.00	6.00	46.00	1.00	4.85	24.00	4.95	24.00
18.00	52.00	0.00	6.00	43.00	1.35	5.40	20.00	3.70	14.81
19.00	52.00	0.00	6.00	41.00	1.70	5.35	32.00	5.98	18.82
20.00	53.00	0.00	6.00	46.00	1.25	6.10	31.00	5.08	24.80
21.00	53.00	1.00	6.00	41.00	0.75	5.00	29.00	5.80	38.67
22.00	55.00	1.00	6.00	44.00	2.10	5.20	27.00	5.19	12.86
23.00	55.00	1.00	6.00	46.00	0.50	3.85	14.00	3.64	28.00
24.00	55.00	1.00	6.00	43.00	0.95	4.50	33.00	7.33	34.74
25.00	56.00	0.00	6.00	47.00	1.50	5.55	28.00	5.05	18.67
26.00	56.00	0.00	6.00	44.00	4.05	5.75	29.00	5.04	7.16

Control no.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)
27.00	56.00	0.00	6.00	43.00	1.65	6.15	39.00	6.34	23.64
28.00	57.00	0.00	6.00	43.00	0.85	6.50	23.00	3.54	27.06
29.00	57.00	1.00	6.00	45.00	1.45	5.90	25.00	4.24	17.24
30.00	57.00	1.00	6.00	44.00	0.65	5.25	29.00	5.52	44.62
31.00	58.00	1.00	6.00	48.00	1.30	5.25	37.00	7.05	28.46
32.00	59.00	1.00	6.00	43.00	0.90	6.90	42.00	6.09	46.67
33.00	59.00	0.00	6.00	41.00	--	4.90	38.00	7.76	--
34.00	60.00	0.00	6.00	49.00	1.05	7.30	27.00	3.70	25.71
35.00	61.00	0.00	6.00	44.00	--	5.50	37.00	6.73	--
36.00	62.00	0.00	6.00	41.00	1.20	6.20	31.00	5.00	25.83
37.00	64.00	1.00	6.00	41.00	--	4.90	41.00	8.37	--
38.00	64.00	0.00	6.00	43.00	--	5.35	31.00	5.79	--
39.00	69.00	1.00	6.00	38.00	--	6.10	40.00	6.56	--
40.00	71.00	1.00	6.00	41.00	--	5.70	33.00	5.79	--
41.00	73.00	1.00	6.00	39.00	--	5.30	37.00	6.98	--
42.00	29.00	1.00	--	--	1.10	5.30	24.00	4.53	21.82
43.00	33.00	1.00	--	--	1.10	4.80	25.00	5.21	22.73
44.00	60.00	1.00	--	--	2.35	5.30	29.00	5.47	12.34
45.00	66.00	0.00	--	--	1.20	7.00	25.00	3.57	20.83
46.00	58.00	0.00	--	--	2.30	6.40	30.00	4.69	13.04
47.00	30.00	1.00	--	--	1.50	5.20	27.00	5.19	18.00
48.00	51.00	0.00	--	--	1.70	4.10	30.00	7.32	17.65
49.00	50.00	1.00	--	--	0.60	5.70	24.00	4.21	40.00
50.00	47.00	0.00	--	--	0.65	6.40	30.00	4.69	46.15
51.00	41.00	1.00	--	--	1.25	4.80	28.00	5.83	22.40
52.00	47.00	0.00	--	--	1.35	6.40	24.00	3.75	17.78
53.00	61.00	0.00	--	--	1.60	6.30	29.00	4.60	18.13
54.00	31.00	0.00	--	--	1.20	3.70	18.00	4.86	15.00
55.00	60.00	0.00	--	--	1.40	7.80	31.00	3.97	22.14
56.00	69.00	1.00	--	--	1.50	5.10	30.00	5.88	20.00
57.00	61.00	0.00	--	--	2.80	7.50	41.00	5.47	14.64
58.00	71.00	1.00	--	--	1.10	4.50	26.00	5.78	23.64
59.00	56.00	1.00	--	--	1.18	7.80	47.00	6.03	39.83

Control no.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)
60.00	57.00	1.00	--	--	0.70	4.30	24.00	5.58	34.29
61.00	79.00	0.00	--	--	1.10	5.90	36.00	6.10	32.73
62.00	47.00	0.00	--	--	1.60	6.70	27.00	4.03	16.88
63.00	69.00	1.00	--	--	1.30	4.50	31.00	6.89	23.85
64.00	75.00	1.00	--	--	1.00	3.80	27.00	7.11	27.00
65.00	69.00	1.00	--	--	1.20	5.70	27.00	4.74	22.50
66.00	73.00	1.00	--	--	1.30	3.10	27.00	8.71	20.77
67.00	51.00	0.00	--	--	3.60	7.30	40.00	5.48	11.11

Appendix 10. Characteristics and biochemical measurements of patients (Chapter 6)

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital stay (days)
12.00	61.00	0.00	29.00	76.60	0.00	0.00	0.00	0.00	7.40	13.40
13.00	43.00	1.00	12.00	11.70	1.00	0.00	2.00	0.00	48.10	50.10
14.00	71.00	1.00	18.00	28.90	1.00	0.00	0.00	1.00	12.90	12.90
15.00	53.00	1.00	19.00	32.20	0.00	0.00	1.00	0.00	4.80	10.80
16.00	61.00	0.00	23.00	28.50	0.00	0.00	--	0.00	1.00	10.00
17.00	18.00	0.00	12.00	20.20	0.00	0.00	3.00	1.00	6.20	6.20
18.00	67.00	0.00	18.00	44.40	1.00	0.00	0.00	1.00	59.50	59.50
19.00	50.00	0.00	21.00	38.90	0.00	0.00	3.00	1.00	46.30	46.30
21.00	61.00	0.00	34.00	76.80	1.00	0.00	--	0.00	2.00	53.00
22.00	52.00	0.00	13.00	9.90	0.00	95.00	101.00	0.00	3.70	77.00
23.00	71.00	0.00	23.00	42.50	1.00	9.00	10.00	1.00	20.90	20.90
24.00	77.00	1.00	21.00	35.00	0.00	0.00	--	0.00	2.00	6.00
27.00	76.00	1.00	23.00	46.00	0.00	1.00	3.00	0.00	21.70	21.70
29.00	70.00	0.00	16.00	20.20	1.00	4.00	10.00	0.00	5.10	85.00
30.00	73.00	1.00	25.00	53.30	1.00	0.00	2.00	1.00	15.50	15.50
31.00	62.00	0.00	19.00	27.10	1.00	0.00	--	0.00	0.50	25.00
32.00	55.00	1.00	15.00	21.00	1.00	0.00	--	0.00	2.20	22.20
33.00	80.00	1.00	31.00	84.20	1.00	0.00	0.00	1.00	12.00	12.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	0.00	29.40	85.00
35.00	20.00	0.00	18.00	39.70	1.00	0.00	1.00	1.00	44.00	44.00
36.00	43.00	1.00	38.00	92.60	0.00	5.00	10.00	0.00	25.50	27.50
37.00	60.00	0.00	27.00	63.10	0.00	0.00	0.00	0.00	21.80	29.00
38.00	74.00	0.00	20.00	23.00	1.00	0.00	--	0.00	1.00	150.00
40.00	47.00	0.00	22.00	28.50	1.00	0.00	2.00	0.00	1.90	19.90
41.00	76.00	1.00	29.00	79.90	1.00	0.00	--	0.00	1.40	21.40
42.00	79.00	0.00	21.00	30.90	1.00	0.00	3.00	0.00	3.20	27.00
44.00	81.00	0.00	34.00	86.30	0.00	0.00	0.00	1.00	16.10	16.10
45.00	74.00	0.00	33.00	75.20	0.00	0.00	0.00	1.00	16.00	16.00
47.00	76.00	0.00	29.00	77.20	1.00	0.00	0.00	1.00	34.60	36.80
48.00	67.00	0.00	31.00	68.10	1.00	1,000.00	1,007.00	1.00	18.40	18.40
50.00	60.00	0.00	19.00	48.00	1.00	0.00	0.00	0.00	6.20	124.20
52.00	61.00	0.00	33.00	78.60	0.00	7.00	10.00	1.00	7.70	7.70

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital stay (days)
53.00	67.00	1.00	18.00	21.20	0.00	0.00	0.00	1.00	11.10	11.10
55.00	80.00	1.00	31.00	73.30	0.00	0.00	3.00	1.00	8.20	8.20
56.00	40.00	1.00	6.00	10.20	1.00	0.00	--	0.00	0.40	0.40
57.00	60.00	0.00	27.00	60.20	1.00	0.00	--	1.00	1.80	1.80
58.00	41.00	0.00	21.00	17.80	1.00	0.00	0.00	0.00	19.70	36.70
59.00	68.00	0.00	20.00	32.30	0.00	0.00	--	0.00	0.80	12.00
60.00	57.00	0.00	24.00	67.00	0.00	0.00	--	0.00	6.50	59.50
61.00	76.00	1.00	18.00	14.40	0.00	0.00	--	0.00	0.70	22.70
62.00	74.00	0.00	20.00	19.90	1.00	0.00	0.00	0.00	0.90	253.00
63.00	62.00	0.00	18.00	44.40	1.00	1,000.00	--	0.00	1.00	508.00
64.00	66.00	0.00	34.00	81.00	0.00	0.00	--	0.00	22.40	46.00
65.00	38.00	1.00	17.00	26.20	0.00	0.00	--	0.00	18.90	37.00
67.00	68.00	1.00	31.00	80.30	0.00	1,000.00	0.00	0.00	5.00	8.00
68.00	74.00	0.00	24.00	49.70	0.00	0.00	7.00	0.00	10.70	27.70
69.00	61.00	0.00	22.00	54.90	1.00	1,000.00	1,004.00	0.00	76.40	122.40
71.00	25.00	0.00	17.00	1.20	0.00	0.00	--	0.00	2.00	3.50
72.00	51.00	0.00	21.00	12.40	0.00	1.00	1.00	0.00	15.50	40.00
73.00	69.00	0.00	12.00	20.20	0.00	0.00	--	0.00	5.80	23.00
74.00	65.00	0.00	33.00	80.40	0.00	0.00	7.00	0.00	19.60	40.00
75.00	28.00	1.00	14.00	18.50	1.00	0.00	--	0.00	0.60	58.00
77.00	54.00	1.00	17.00	1.20	0.00	0.00	--	0.00	0.80	12.00
79.00	41.00	0.00	7.00	7.60	1.00	0.00	0.00	0.00	9.30	19.00
81.00	71.00	0.00	25.00	35.90	1.00	0.00	1.00	0.00	8.30	20.00
82.00	80.00	1.00	17.00	12.70	0.00	0.00	1.00	0.00	10.30	29.00
83.00	46.00	1.00	11.00	10.30	1.00	0.00	--	0.00	2.90	10.00
84.00	56.00	0.00	28.00	63.90	0.00	1,000.00	1,006.00	0.00	15.40	35.00
85.00	73.00	0.00	18.00	44.40	1.00	0.00	0.00	0.00	1.40	160.00
87.00	34.00	1.00	12.00	12.40	1.00	0.00	--	0.00	2.30	12.00
88.00	81.00	0.00	17.00	23.60	1.00	1,000.00	1,004.00	1.00	9.30	10.00
89.00	22.00	1.00	10.00	9.50	1.00	0.00	--	0.00	0.20	1.00
90.00	53.00	0.00	32.00	60.90	1.00	0.00	2.00	0.00	3.00	36.00
91.00	62.00	0.00	22.00	58.90	1.00	0.00	--	0.00	1.70	14.00
94.00	32.00	1.00	25.00	53.30	0.00	0.00	--	0.00	17.30	24.00
96.00	63.00	1.00	18.00	4.80	0.00	0.00	0.00	0.00	1.90	15.00
99.00	37.00	0.00	13.00	16.50	1.00	0.00	--	0.00	2.10	11.00
100.00	50.00	1.00	26.00	38.20	0.00	0.00	12.00	0.00	2.60	119.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/ Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro+ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital stay (days)
101.00	35.00	1.00	9.00	4.70	1.00	0.00	--	0.00	0.80	6.00
102.00	26.00	0.00	23.00	45.70	1.00	0.00	--	0.00	2.30	77.00
103.00	52.00	0.00	16.00	12.20	1.00	0.00	0.00	0.00	2.00	48.00
104.00	38.00	0.00	3.00	2.70	0.00	0.00	--	0.00	0.30	5.00
105.00	39.00	0.00	34.00	87.50	0.00	1,000.00	1,001.00	0.00	3.00	58.00
107.00	45.00	1.00	12.00	6.60	1.00	0.00	--	0.00	3.10	48.00
110.00	48.00	1.00	26.00	35.20	0.00	0.00	8.00	1.00	4.90	6.00
111.00	23.00	0.00	9.00	4.30	1.00	0.00	7.00	0.00	9.20	48.00
112.00	45.00	1.00	24.00	22.40	1.00	0.00	0.00	1.00	10.70	12.00
113.00	70.00	0.00	27.00	47.70	0.00	0.00	2.00	1.00	6.50	7.00
114.00	65.00	1.00	24.00	65.70	1.00	0.00	3.00	0.00	32.80	61.00
118.00	59.00	0.00	23.00	39.90	1.00	0.00	1.00	0.00	12.10	24.00
123.00	60.00	0.00	16.00	14.60	0.00	1,000.00	1,004.00	0.00	3.10	23.00
129.00	31.00	0.00	19.00	44.00	0.00	0.00	--	0.00	2.90	54.00

Patient no.	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)
12.00	7.00	7.00	2.00	126.00	156.00	24.00	23.00	0.61	0.43	1.70	1.73
13.00	5.00	9.00	3.00	22.00	261.00	27.00	22.00	2.80	1.95	4.27	2.08
14.00	8.00	9.00	2.00	220.00	124.00	12.00	10.00	1.55	0.82	2.19	1.28
15.00	8.00	2.00	5.00	257.00	73.00	23.00	16.00	1.52	--	2.75	--
16.00	6.00	--	--	122.00	--	28.00	--	0.75	--	3.76	--
17.00	2.00	0.00	4.00	3.00	55.00	33.00	26.00	0.71	1.93	2.83	2.27
18.00	10.00	10.00	4.00	152.00	64.00	10.00	10.00	0.80	2.75	0.55	1.47
19.00	10.00	9.00	4.00	143.00	101.00	12.00	14.00	0.72	1.77	3.06	2.09
21.00	8.00	--	--	23.00	--	10.00	--	1.60	--	2.60	--
22.00	2.00	3.00	4.00	20.00	67.00	27.00	15.00	1.10	--	3.00	--
23.00	8.00	4.00	7.00	125.00	216.00	10.00	13.00	0.50	0.80	1.10	1.60
24.00	4.00	--	--	81.00	--	35.00	--	0.80	--	4.90	--
27.00	5.00	4.00	3.00	103.00	133.00	23.00	17.00	1.80	0.80	3.90	2.50
29.00	4.00	4.00	4.00	59.00	20.00	9.00	10.00	0.80	1.10	0.90	1.80
30.00	11.00	7.00	3.00	26.00	179.00	16.00	11.00	1.20	0.60	3.80	3.20
31.00	11.00	--	--	50.00	--	22.00	--	0.60	--	2.50	--
32.00	6.00	--	--	434.00	--	17.00	--	1.10	--	2.00	--
33.00	7.00	8.00	5.00	259.00	173.00	17.00	10.00	0.80	1.00	2.10	1.70
34.00	6.00	7.00	4.00	404.00	336.00	30.00	15.00	2.01	1.31	1.25	1.94
35.00	10.00	7.00	3.00	119.00	303.00	9.00	13.00	0.70	1.28	1.27	1.74
36.00	13.00	10.00	4.00	132.00	70.00	14.00	14.00	0.68	1.02	0.53	1.94
37.00	10.00	9.00	4.00	81.00	35.00	12.00	12.00	0.38	0.65	0.84	1.35
38.00	4.00	--	--	127.00	--	14.00	--	1.66	--	2.40	--
40.00	9.00	4.00	3.00	15.00	234.00	20.00	18.00	--	2.10	--	2.00
41.00	8.00	--	--	184.00	--	19.00	--	0.64	--	1.51	--
42.00	4.00	6.00	4.00	8.00	149.00	17.00	18.00	0.50	0.80	2.10	2.40
44.00	7.00	3.00	8.00	151.00	55.00	10.00	16.00	0.44	1.18	0.98	3.44
45.00	11.00	5.00	5.00	258.00	164.00	14.00	14.00	0.69	1.18	2.41	2.07
47.00	7.00	7.00	2.00	64.00	72.00	24.00	24.00	0.80	0.70	2.20	1.80
48.00	6.00	11.00	4.00	119.00	123.00	10.00	9.00	0.70	0.80	0.90	0.90
50.00	7.00	4.00	6.00	180.00	141.00	23.00	15.00	1.20	1.10	1.50	1.50

Patient no.	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)
52.00	11.00	11.00	3.00	565.00	257.00	9.00	9.00	0.70	0.90	1.00	1.40
53.00	11.00	10.00	3.00	148.00	131.00	9.00	9.00	2.50	2.60	1.70	1.90
55.00	8.00	8.00	4.00	107.00	71.00	13.00	9.00	0.30	0.20	1.10	0.60
56.00	6.00	--	--	37.00	--	16.00	--	1.07	--	1.42	--
57.00	10.00	--	--	181.00	--	10.00	--	0.55	--	0.40	--
58.00	4.00	12.00	2.00	6.00	36.00	19.00	12.00	0.95	0.39	2.12	1.13
59.00	5.00	--	--	32.00	--	36.00	--	5.05	--	4.23	--
60.00	3.00	--	--	166.00	--	26.00	--	2.53	--	4.56	--
61.00	5.00	--	--	380.00	--	26.00	--	2.13	--	4.60	--
62.00	1.00	2.00	3.00	88.00	180.00	12.00	14.00	--	0.93	--	2.10
63.00	2.00	--	--	117.00	--	14.00	--	1.34	--	1.49	--
64.00	13.00	--	--	311.00	--	18.00	--	1.47	--	1.68	--
65.00	9.00	--	--	163.00	--	23.00	--	1.12	--	1.36	--
67.00	4.00	4.00	5.00	438.00	52.00	20.00	16.00	0.87	1.40	1.21	2.47
68.00	14.00	12.00	5.00	67.00	139.00	11.00	11.00	1.68	1.48	1.44	1.93
69.00	10.00	13.00	4.00	179.00	356.00	14.00	16.00	0.53	1.63	0.71	0.96
71.00	3.00	--	--	2.00	--	38.00	--	2.10	--	3.20	--
72.00	11.00	7.00	12.00	40.00	89.00	32.00	15.00	3.40	1.50	3.30	3.10
73.00	3.00	--	--	2.00	--	31.00	--	1.80	--	3.30	--
74.00	6.00	11.00	4.00	77.00	28.00	15.00	13.00	0.90	1.00	1.30	3.80
75.00	10.00	--	--	184.00	--	11.00	--	2.80	--	2.00	--
77.00	7.00	--	--	2.00	--	20.00	--	0.50	--	2.10	--
79.00	4.00	3.00	3.00	2.00	251.00	24.00	21.00	--	1.00	--	1.80
81.00	5.00	6.00	3.00	8.00	284.00	10.00	21.00	0.60	1.90	1.10	2.20
82.00	1.00	1.00	2.00	4.00	86.00	29.00	29.00	0.70	0.40	4.00	2.60
83.00	4.00	--	--	318.00	--	21.00	--	0.80	--	3.10	--
84.00	13.00	10.00	3.00	63.00	80.00	9.00	9.00	0.90	2.20	0.90	1.10
85.00	6.00	7.00	2.00	176.00	198.00	11.00	9.00	0.30	0.50	1.20	1.20
87.00	6.00	--	--	110.00	--	18.00	--	0.70	--	2.60	--
88.00	9.00	9.00	5.00	108.00	104.00	12.00	11.00	0.80	0.60	1.70	1.70
89.00	2.00	--	--	31.00	--	16.00	--	1.50	--	2.70	--
90.00	10.00	7.00	4.00	159.00	38.00	11.00	29.00	1.60	1.80	1.60	1.90
91.00	9.00	--	--	268.00	--	14.00	--	1.30	--	1.60	--
94.00	18.00	--	--	147.00	--	14.00	--	2.60	--	1.90	--

Patient no.	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)	Albumin admission (g/l)	Albumin follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)
96.00	3.00	2.00	3.00	76.00	27.00	29.00	28.00	1.60	1.10	4.40	4.40
99.00	5.00	--	--	287.00	--	22.00	--	1.06	--	2.50	--
100.00	4.00	4.00	3.00	253.00	171.00	13.00	9.00	2.40	0.50	2.30	1.40
101.00	5.00	--	--	231.00	--	25.00	--	0.70	--	2.20	--
102.00	4.00	2.00	2.00	28.00	198.00	19.00	19.00	0.60	2.40	1.70	1.80
103.00	2.00	1.00	3.00	--	69.00	42.00	27.00	3.30	--	4.10	--
104.00	2.00	--	--	1.00	--	34.00	--	4.70	--	6.40	--
105.00	11.00	8.00	2.00	25.00	23.00	30.00	29.00	1.00	0.60	2.00	1.90
107.00	8.00	--	--	261.00	--	17.00	--	--	--	--	--
110.00	7.00	12.00	5.00	199.00	153.00	13.00	11.00	1.10	3.20	1.60	2.10
111.00	7.00	2.00	5.00	6.00	245.00	28.00	13.00	2.00	2.10	4.00	1.60
112.00	1.00	10.00	4.00	2.00	165.00	20.00	9.00	1.20	0.80	5.20	1.70
113.00	4.00	5.00	3.00	14.00	205.00	31.00	19.00	1.30	0.40	2.70	1.40
114.00	9.00	8.00	4.00	49.00	98.00	16.00	9.00	1.40	0.70	2.20	1.00
118.00	9.00	12.00	2.00	252.00	204.00	12.00	9.00	0.70	1.10	0.70	1.00
123.00	7.00	6.00	3.00	2.00	207.00	45.00	28.00	1.90	0.80	4.80	3.90
129.00	12.00	--	--	63.00	--	20.00	--	--	--	--	--

Patient no.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)	Red cell α-tocopherol admission (pmol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma α-tocopherol/ triglycerides follow-up (umol/mmol)	Red cell α-tocopherol follow-up (pmol/g Hb)
12.00	14.36	8.45	23.54	17.58	12.70	7.34	29.53	19.51
13.00	15.35	3.59	5.48	11.30	11.98	5.76	6.14	11.53
14.00	16.26	7.42	10.49	22.20	10.49	8.20	12.79	22.17
15.00	14.48	5.27	9.53	3.40	26.88	--	--	25.78
16.00	15.68	4.17	20.91	16.48	--	--	--	--
17.00	17.00	6.01	23.94	19.66	17.00	7.49	8.81	13.93
18.00	6.27	11.40	7.84	16.01	14.47	9.84	5.26	11.94
19.00	17.70	5.78	24.58	18.62	15.40	7.37	8.70	12.15
21.00	16.03	6.17	10.02	16.00	--	--	--	--
22.00	13.74	4.58	12.49	18.73	16.90	--	--	--
23.00	7.93	7.21	15.86	13.47	18.48	11.55	23.10	18.62
24.00	26.95	5.50	33.69	22.18	--	--	--	--
27.00	24.42	6.26	13.57	19.14	21.91	8.76	27.39	25.37
29.00	10.91	12.12	13.64	19.67	16.56	9.20	15.05	21.26
30.00	33.79	8.89	28.16	39.28	23.73	7.42	39.55	36.95
31.00	14.80	5.92	24.67	17.83	--	--	--	--
32.00	10.30	5.15	9.36	11.39	--	--	--	--
33.00	11.83	5.63	14.79	16.94	13.34	7.85	13.34	19.80
34.00	19.65	15.72	9.78	15.33	23.17	11.94	17.69	34.15
35.00	10.16	8.00	14.51	26.99	16.07	9.24	12.55	21.66
36.00	6.44	12.15	9.47	12.38	14.56	7.51	14.27	27.64
37.00	6.25	7.44	16.45	16.08	10.86	8.04	16.71	19.80
38.00	20.99	8.75	12.64	18.59	--	--	--	--
40.00	17.38	--	--	21.05	21.31	10.66	10.15	20.02
41.00	10.05	6.66	15.70	25.22	--	--	--	--
42.00	12.37	5.89	24.74	15.59	14.77	6.15	18.46	22.05
44.00	8.31	8.48	18.89	18.50	35.61	10.35	30.18	26.89
45.00	15.25	6.33	22.10	16.23	19.08	9.22	16.17	19.03
47.00	14.47	6.58	18.09	18.55	12.24	6.80	17.49	18.77
48.00	8.06	8.96	11.51	24.40	7.13	7.92	8.91	15.01

Patient no.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)	Red cell α-tocopherol admission (pmol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma α-tocopherol/ triglycerides follow-up (umol/mmol)	Red cell α-tocopherol follow-up (pmol/g Hb)
50.00	9.98	6.65	8.32	18.10	15.88	10.59	14.44	15.54
52.00	7.83	7.83	11.19	16.99	8.64	6.17	9.60	11.91
53.00	16.34	9.61	6.54	20.19	19.99	10.52	7.69	23.66
55.00	8.12	7.38	27.07	26.96	7.12	11.87	35.60	24.43
56.00	11.20	7.89	10.47	17.05	--	--	--	--
57.00	3.55	8.88	6.45	21.03	--	--	--	--
58.00	11.37	5.36	11.97	17.17	5.95	5.27	15.26	18.57
59.00	33.30	7.87	6.59	21.69	--	--	--	--
60.00	30.79	6.75	12.17	21.47	--	--	--	--
61.00	31.14	6.77	14.62	27.94	--	--	--	--
62.00	14.40	--	--	18.07	17.15	8.17	18.44	14.28
63.00	9.63	6.46	7.19	13.00	--	--	--	--
64.00	19.42	11.56	13.21	20.23	--	--	--	--
65.00	10.05	7.39	8.97	20.07	--	--	--	--
67.00	12.43	10.27	14.29	29.75	25.83	10.46	18.45	48.37
68.00	10.01	6.95	5.96	19.01	18.30	9.48	12.36	30.88
69.00	5.31	7.48	10.02	16.86	11.39	11.86	6.99	15.25
71.00	21.98	6.87	10.47	17.68	--	--	--	--
72.00	16.40	4.97	4.82	9.77	33.61	10.84	22.41	38.31
73.00	19.99	6.06	11.11	16.66	--	--	--	--
74.00	11.23	8.64	12.48	13.46	12.79	3.37	12.79	23.73
75.00	20.40	10.20	7.29	17.85	--	--	--	--
77.00	13.23	6.30	26.46	26.49	--	--	--	--
79.00	17.25	--	--	25.28	21.55	11.97	21.55	24.34
81.00	6.08	5.53	10.13	21.71	16.42	7.46	8.64	28.07
82.00	33.45	8.36	47.79	21.44	24.02	9.24	60.05	23.52
83.00	17.97	5.80	22.46	12.79	--	--	--	--
84.00	5.33	5.92	5.92	16.02	8.20	7.45	3.73	8.18
85.00	8.06	6.72	26.87	22.33	8.94	7.45	17.88	20.74
87.00	16.63	6.40	23.76	18.91	--	--	--	--
88.00	12.70	7.47	15.88	23.14	17.83	10.49	29.72	32.48
89.00	19.22	7.12	12.81	19.83	--	--	--	--
90.00	15.93	9.96	9.96	22.65	17.29	9.10	9.61	27.27

Patient no.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol/ triglycerides admission (umol/mmol)	Red cell α-tocopherol admission (pmol/g Hb)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma α-tocopherol/ triglycerides follow-up (umol/mmol)	Red cell α-tocopherol follow-up (pmol/g Hb)
91.00	15.56	9.73	11.97	18.69	--	--	--	--
94.00	14.00	7.37	5.38	13.45	--	--	--	--
96.00	27.00	6.14	16.88	29.52	27.00	6.14	24.55	29.33
99.00	15.00	6.00	14.15	11.60	--	--	--	--
100.00	19.00	8.26	7.92	22.67	12.00	8.57	24.00	30.64
101.00	15.00	6.82	21.43	16.68	--	--	--	--
102.00	11.00	6.47	18.33	23.38	14.00	7.78	5.83	15.70
103.00	41.00	10.00	12.42	31.49	29.00	--	--	38.97
104.00	36.00	5.63	7.66	13.43	--	--	--	--
105.00	13.00	6.50	13.00	21.66	10.00	5.26	16.67	8.76
107.00	16.00	--	--	12.87	--	--	--	--
110.00	17.00	10.63	15.45	23.88	27.00	12.86	8.44	19.26
111.00	26.00	6.50	13.00	22.55	18.00	11.25	8.57	22.30
112.00	28.00	5.38	23.33	24.97	12.00	7.06	15.00	25.82
113.00	17.00	6.30	13.08	13.13	9.00	6.43	22.50	14.98
114.00	15.00	6.82	10.71	16.57	10.00	10.00	14.29	23.03
118.00	7.00	10.00	10.00	16.69	9.00	9.00	8.18	16.45
123.00	27.00	5.63	14.21	19.07	24.00	6.15	30.00	22.45
129.00	12.00	--	--	5.15	--	--	--	--

12.11 Appendix 11 Chapter 7 data

Appendix 11. Characteristics and biochemical measurements of controls (Chapter 7)

Control no.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Total protein (g/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Plasma retinol (umol/l)	Plasma α-tocopherol (umol/l)	Plasma α-tocopherol/ cholesterol (umol/mmol)
1.00	59.00	1.00	6.00	43.00	72.00	0.90	6.90	1.30	42.00	6.09
2.00	53.00	1.00	6.00	41.00	73.00	0.75	5.00	1.60	29.00	5.80
3.00	45.00	1.00	6.00	45.00	78.00	0.55	4.25	1.60	21.00	4.94
4.00	48.00	0.00	8.00	44.00	73.00	1.45	5.75	1.60	31.00	5.39
5.00	57.00	1.00	6.00	44.00	73.00	0.65	5.25	1.60	29.00	5.52
6.00	52.00	0.00	6.00	43.00	72.00	1.35	5.40	1.60	20.00	3.70
7.00	71.00	1.00	6.00	41.00	70.00	--	5.70	1.60	33.00	5.79
8.00	55.00	1.00	6.00	43.00	72.00	0.95	4.50	1.70	33.00	7.33
9.00	55.00	1.00	6.00	46.00	76.00	0.50	3.85	1.70	14.00	3.64
10.00	57.00	1.00	6.00	45.00	76.00	1.45	5.90	1.70	25.00	4.24
11.00	53.00	0.00	6.00	46.00	71.00	1.25	6.10	1.80	31.00	5.08
12.00	61.00	0.00	6.00	44.00	77.00	--	5.50	1.80	37.00	6.73
13.00	73.00	1.00	6.00	39.00	69.00	--	5.30	1.90	37.00	6.98
14.00	51.00	0.00	6.00	44.00	74.00	0.55	5.15	1.90	26.00	5.05
15.00	64.00	0.00	6.00	43.00	72.00	--	5.35	1.90	31.00	5.79
16.00	51.00	0.00	6.00	46.00	77.00	1.00	4.85	2.00	24.00	4.95
17.00	47.00	0.00	6.00	48.00	78.00	0.65	5.80	2.00	23.00	3.97
18.00	69.00	1.00	6.00	38.00	71.00	--	6.10	2.00	40.00	6.56
19.00	64.00	1.00	6.00	41.00	76.00	--	4.90	2.10	41.00	8.37
20.00	44.00	1.00	6.00	50.00	80.00	1.50	6.40	2.10	28.00	4.38
21.00	56.00	0.00	6.00	47.00	81.00	1.50	5.55	2.10	28.00	5.05
22.00	44.00	0.00	6.00	48.00	80.00	1.35	4.80	2.20	24.00	5.00
23.00	56.00	0.00	6.00	43.00	66.00	1.65	6.15	2.20	39.00	6.34
24.00	55.00	1.00	6.00	44.00	76.00	2.10	5.20	2.30	27.00	5.19
25.00	59.00	0.00	6.00	41.00	70.00	--	4.90	2.30	38.00	7.78
26.00	44.00	0.00	6.00	42.00	69.00	2.00	5.85	2.30	33.00	5.64
27.00	52.00	0.00	6.00	41.00	69.00	1.70	5.35	2.40	32.00	5.98

Control no.	Age (yrs)	Male/ Female (0/1)	C-reactive protein (mg/l)	Albumin (g/l)	Total protein (g/l)	Triglycerides (mmol/l)	Cholesterol (mmol/l)	Plasma retinol (umol/l)	Plasma α-tocopherol (umol/l)	Plasma α-tocopherol/ cholesterol (umol/mmol)
28.00	43.00	0.00	6.00	45.00	76.00	0.50	5.40	2.50	25.00	4.63
29.00	51.00	1.00	6.00	42.00	71.00	1.25	4.50	2.50	24.00	5.33
30.00	58.00	1.00	6.00	48.00	76.00	1.30	5.25	2.60	37.00	7.05
31.00	47.00	0.00	6.00	44.00	72.00	1.05	5.65	2.70	22.00	3.89
32.00	49.00	1.00	6.00	47.00	78.00	2.50	5.05	2.70	31.00	6.14
33.00	62.00	0.00	6.00	41.00	70.00	1.20	6.20	2.80	31.00	5.00
34.00	56.00	0.00	6.00	44.00	74.00	4.05	5.75	2.80	29.00	5.04
35.00	57.00	0.00	6.00	43.00	72.00	0.85	6.50	2.90	23.00	3.54
36.00	45.00	1.00	6.00	47.00	70.00	1.25	6.15	3.30	30.00	4.88
37.00	50.00	0.00	6.00	44.00	70.00	0.55	5.55	3.40	37.00	6.67
38.00	60.00	0.00	6.00	49.00	80.00	1.05	7.30	3.60	27.00	3.70

Control no.	Plasma lutein (ug/l)	Plasma lutein/ cholesterol (ug/mmol)	Plasma lycopene (ug/l)	Plasma lycopene/ cholesterol (ug/mmol)	Plasma α-carotene (ug/l)	Plasma α-carotene/ cholesterol (ug/mmol)	Plasma β-carotene (ug/l)	Plasma β-carotene/ cholesterol (ug/mmol)	Total MDA (umol/l)	Free MDA (umol/l)	Free/ Total MDA	MDA/ Total Protein (umol/g)
1.00	329.00	47.68	277.00	40.14	27.00	3.91	595.00	86.23	0.95	0.08	0.08	0.01
2.00	118.00	23.60	245.00	49.00	14.00	2.80	272.00	54.40	0.59	0.04	0.07	0.01
3.00	82.00	19.29	171.00	40.24	39.00	9.18	271.00	63.76	0.47	0.06	0.13	0.01
4.00	149.00	25.91	207.00	36.00	19.00	3.30	122.00	21.22	0.53	0.07	0.12	0.01
5.00	229.00	43.62	286.00	54.48	27.00	5.14	167.00	31.81	0.60	0.08	0.13	0.01
6.00	58.00	10.74	181.00	33.52	14.00	2.59	152.00	28.15	0.66	0.08	0.12	0.01
7.00	89.00	15.61	168.00	29.47	11.00	1.93	255.00	44.74	0.89	0.09	0.10	0.01
8.00	174.00	38.67	379.00	84.22	55.00	12.22	314.00	69.78	0.71	0.09	0.12	0.01
9.00	137.00	35.58	189.00	49.09	26.00	6.75	228.00	59.22	1.00	0.09	0.09	0.01
10.00	115.00	19.49	268.00	45.42	10.00	1.69	134.00	22.71	0.71	0.09	0.13	0.01
11.00	135.00	22.13	304.00	49.84	17.00	2.79	102.00	16.72	0.71	0.09	0.12	0.01
12.00	81.00	14.73	144.00	26.18	11.00	2.00	188.00	34.18	1.10	0.10	0.09	0.01
13.00	97.00	18.30	179.00	33.77	12.00	2.26	210.00	39.62	0.65	0.07	0.10	0.01
14.00	138.00	26.80	483.00	93.79	63.00	12.23	708.00	137.48	0.47	0.07	0.15	0.01
15.00	188.00	35.14	211.00	39.44	18.00	3.36	328.00	61.31	0.73	0.08	0.11	0.01
16.00	69.00	14.23	178.00	36.70	10.00	2.06	100.00	20.62	0.60	0.04	0.06	0.01
17.00	130.00	22.41	138.00	23.79	40.00	6.90	344.00	59.31	0.36	0.05	0.13	0.00
18.00	105.00	17.21	171.00	28.03	16.00	2.62	312.00	51.15	0.44	0.05	0.12	0.01
19.00	110.00	22.45	185.00	37.76	23.00	4.69	288.00	58.78	0.61	0.07	0.12	0.01
20.00	62.00	9.69	180.00	28.13	15.00	2.34	80.00	12.50	0.95	0.09	0.09	0.01
21.00	129.00	23.24	218.00	39.28	16.00	2.88	87.00	15.68	0.71	0.09	0.12	0.01
22.00	301.00	62.71	64.00	13.33	10.00	2.08	73.00	15.21	0.89	0.09	0.10	0.01
23.00	207.00	33.66	187.00	30.41	75.00	12.20	366.00	59.51	1.06	0.10	0.09	0.02
24.00	111.00	21.35	93.00	17.88	10.00	1.92	150.00	28.85	0.40	0.05	0.13	0.01
25.00	116.00	23.67	199.00	40.61	15.00	3.06	249.00	50.82	0.65	0.05	0.08	0.01
26.00	85.00	14.53	153.00	26.15	15.00	2.56	155.00	26.50	0.53	0.07	0.13	0.01
27.00	268.00	50.09	224.00	41.87	26.00	4.86	202.00	37.76	0.70	0.09	0.13	0.01
28.00	185.00	34.26	228.00	42.22	23.00	4.26	186.00	34.44	0.37	0.05	0.12	0.00
29.00	165.00	36.67	139.00	30.89	65.00	14.44	109.00	24.22	0.83	0.08	0.10	0.01
30.00	278.00	52.95	248.00	47.24	18.00	3.43	157.00	29.90	0.61	0.07	0.11	0.01
31.00	71.00	12.57	339.00	60.00	10.00	1.77	147.00	26.02	0.96	0.08	0.09	0.01

Control no.	Plasma lutein (ug/l)	Plasma lutein/ cholesterol (ug/mmol)	Plasma lycopene (ug/l)	Plasma lycopene/ cholesterol (ug/mmol)	Plasma α-carotene (ug/l)	Plasma α-carotene/ cholesterol (ug/mmol)	Plasma β-carotene (ug/l)	Plasma β-carotene/ cholesterol (ug/mmol)	Total MDA (umol/l)	Free MDA (umol/l)	Free/ Total MDA	MDA/ Total Protein (umol/g)
32.00	98.00	19.41	199.00	39.41	10.00	1.98	126.00	24.95	0.87	0.09	0.10	0.01
33.00	207.00	33.39	215.00	34.68	50.00	8.06	258.00	41.61	0.55	0.07	0.13	0.01
34.00	75.00	13.04	104.00	18.09	10.00	1.74	57.00	9.91	0.83	0.09	0.11	0.01
35.00	102.00	15.69	257.00	39.54	18.00	2.77	146.00	22.46	0.70	0.07	0.09	0.01
36.00	181.00	29.43	87.00	14.15	16.00	2.60	157.00	25.53	0.63	0.08	0.12	0.01
37.00	182.00	32.79	266.00	47.93	65.00	11.71	100.00	18.02	0.84	0.08	0.10	0.01
38.00	219.00	30.00	505.00	69.18	10.00	1.37	123.00	16.85	0.98	0.08	0.08	0.01

Appendix 11. Characteristics and biochemical measurements of patients (Chapter 7)

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)
12.00	61.00	0.00	29.00	76.60	0.00	7.00	7.00	2.00	126.00	156.00
13.00	43.00	1.00	12.00	11.70	1.00	5.00	9.00	3.00	22.00	261.00
14.00	71.00	1.00	18.00	28.90	1.00	8.00	9.00	2.00	220.00	124.00
15.00	53.00	1.00	19.00	32.20	0.00	8.00	2.00	5.00	257.00	73.00
16.00	61.00	0.00	23.00	28.50	0.00	6.00	--	--	122.00	--
18.00	67.00	0.00	18.00	44.40	1.00	10.00	10.00	4.00	152.00	64.00
19.00	50.00	0.00	21.00	38.90	0.00	10.00	9.00	4.00	143.00	101.00
21.00	61.00	0.00	34.00	76.80	1.00	8.00	--	--	23.00	--
22.00	52.00	0.00	13.00	9.90	0.00	2.00	3.00	4.00	20.00	67.00
23.00	71.00	0.00	23.00	42.50	1.00	8.00	4.00	7.00	125.00	216.00
24.00	77.00	1.00	21.00	35.00	0.00	4.00	--	--	81.00	--
27.00	76.00	1.00	23.00	46.00	0.00	5.00	4.00	3.00	103.00	133.00
29.00	70.00	0.00	16.00	20.20	1.00	4.00	4.00	4.00	59.00	20.00
30.00	73.00	1.00	25.00	53.30	1.00	11.00	7.00	3.00	26.00	179.00
31.00	62.00	0.00	19.00	27.10	1.00	11.00	--	--	50.00	--
32.00	55.00	1.00	15.00	21.00	1.00	6.00	--	--	434.00	--
33.00	80.00	1.00	31.00	84.20	1.00	7.00	8.00	5.00	259.00	173.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	4.00	404.00	336.00
35.00	20.00	0.00	18.00	39.70	1.00	10.00	7.00	3.00	119.00	303.00
36.00	43.00	1.00	38.00	92.60	0.00	13.00	10.00	4.00	132.00	70.00
37.00	60.00	0.00	27.00	63.10	0.00	10.00	9.00	4.00	81.00	35.00
38.00	74.00	0.00	20.00	23.00	1.00	4.00	--	--	127.00	--
40.00	47.00	0.00	22.00	28.50	1.00	9.00	4.00	3.00	15.00	234.00
41.00	76.00	1.00	29.00	79.90	1.00	8.00	--	--	184.00	--
42.00	79.00	0.00	21.00	30.90	1.00	4.00	6.00	4.00	8.00	149.00
44.00	81.00	0.00	34.00	86.30	0.00	7.00	3.00	8.00	151.00	55.00
45.00	74.00	0.00	33.00	75.20	0.00	11.00	5.00	5.00	258.00	164.00
46.00	41.00	1.00	8.00	15.60	1.00	1.00	--	--	250.00	--
47.00	76.00	0.00	29.00	77.20	1.00	7.00	7.00	2.00	64.00	72.00
48.00	67.00	0.00	31.00	68.10	1.00	6.00	11.00	4.00	119.00	123.00
50.00	60.00	0.00	19.00	48.00	1.00	7.00	4.00	6.00	180.00	141.00

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)
51.00	46.00	1.00	24.00	49.70	0.00	14.00	16.00	3.00	56.00	58.00
52.00	61.00	0.00	33.00	78.60	0.00	11.00	11.00	3.00	565.00	257.00
53.00	67.00	1.00	18.00	21.20	0.00	11.00	10.00	3.00	148.00	131.00
55.00	80.00	1.00	31.00	73.30	0.00	8.00	8.00	4.00	107.00	71.00
56.00	40.00	1.00	6.00	10.20	1.00	6.00	--	--	37.00	--
57.00	60.00	0.00	27.00	60.20	1.00	10.00	--	--	181.00	--
58.00	41.00	0.00	21.00	17.80	1.00	4.00	12.00	2.00	6.00	36.00
59.00	68.00	0.00	20.00	32.30	0.00	5.00	--	--	32.00	--
60.00	57.00	0.00	24.00	67.00	0.00	3.00	--	--	166.00	--
61.00	76.00	1.00	18.00	14.40	0.00	5.00	--	--	380.00	--
62.00	74.00	0.00	20.00	19.90	1.00	1.00	2.00	3.00	88.00	180.00
63.00	62.00	0.00	18.00	44.40	1.00	2.00	--	--	117.00	--
64.00	66.00	0.00	34.00	81.00	0.00	13.00	--	--	311.00	--
67.00	68.00	1.00	31.00	80.30	0.00	4.00	4.00	5.00	438.00	52.00
68.00	74.00	0.00	24.00	49.70	0.00	14.00	12.00	5.00	67.00	139.00
69.00	61.00	0.00	22.00	54.90	1.00	10.00	13.00	4.00	179.00	356.00
71.00	25.00	0.00	17.00	1.20	0.00	3.00	--	--	2.00	--
72.00	51.00	0.00	21.00	12.40	0.00	11.00	7.00	12.00	40.00	89.00
73.00	69.00	0.00	12.00	20.20	0.00	3.00	--	--	2.00	--
74.00	65.00	0.00	33.00	80.40	0.00	6.00	11.00	4.00	77.00	28.00
75.00	28.00	1.00	14.00	18.50	1.00	10.00	--	--	184.00	--
77.00	54.00	1.00	17.00	1.20	0.00	7.00	--	--	2.00	--
78.00	45.00	0.00	12.00	5.50	1.00	4.00	--	--	177.00	--
79.00	41.00	0.00	7.00	7.60	1.00	4.00	3.00	3.00	2.00	251.00
80.00	38.00	0.00	16.00	23.30	1.00	9.00	--	--	126.00	--
81.00	71.00	0.00	25.00	35.90	1.00	5.00	6.00	3.00	8.00	284.00
82.00	80.00	1.00	17.00	12.70	0.00	1.00	1.00	2.00	4.00	86.00
83.00	46.00	1.00	11.00	10.30	1.00	4.00	--	--	318.00	--
84.00	56.00	0.00	28.00	63.90	0.00	13.00	10.00	3.00	63.00	80.00
85.00	73.00	0.00	18.00	44.40	1.00	6.00	7.00	2.00	176.00	198.00
87.00	34.00	1.00	12.00	12.40	1.00	6.00	--	--	110.00	--
88.00	81.00	0.00	17.00	23.60	1.00	9.00	9.00	5.00	108.00	104.00
89.00	22.00	1.00	10.00	9.50	1.00	2.00	--	--	31.00	--
90.00	53.00	0.00	32.00	60.90	1.00	10.00	7.00	4.00	159.00	38.00

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)
91.00	62.00	0.00	22.00	58.90	1.00	9.00	--	--	268.00	--
96.00	63.00	1.00	18.00	4.80	0.00	3.00	2.00	3.00	76.00	27.00
99.00	37.00	0.00	13.00	16.50	1.00	5.00	--	--	287.00	--
100.00	50.00	1.00	26.00	38.20	0.00	4.00	4.00	3.00	253.00	171.00
101.00	35.00	1.00	9.00	4.70	1.00	5.00	--	--	231.00	--
102.00	26.00	0.00	23.00	45.70	1.00	4.00	2.00	2.00	28.00	198.00
103.00	52.00	0.00	16.00	12.20	1.00	2.00	1.00	3.00	--	69.00
104.00	38.00	0.00	3.00	2.70	0.00	2.00	--	--	1.00	--
105.00	39.00	0.00	34.00	87.50	0.00	11.00	8.00	2.00	25.00	23.00
107.00	45.00	1.00	12.00	6.60	1.00	8.00	--	--	261.00	--
108.00	44.00	1.00	6.00	0.90	0.00	3.00	--	--	48.00	--
110.00	48.00	1.00	26.00	35.20	0.00	7.00	12.00	5.00	199.00	153.00
111.00	23.00	0.00	9.00	4.30	1.00	7.00	2.00	5.00	6.00	245.00
112.00	45.00	1.00	24.00	22.40	1.00	1.00	10.00	4.00	2.00	165.00
113.00	70.00	0.00	27.00	47.70	0.00	4.00	5.00	3.00	14.00	205.00
114.00	65.00	1.00	24.00	65.70	1.00	9.00	8.00	4.00	49.00	98.00
115.00	47.00	0.00	10.00	1.50	0.00	2.00	--	--	1.00	--
116.00	61.00	0.00	13.00	10.60	0.00	1.00	--	--	3.00	--
117.00	48.00	0.00	13.00	8.50	1.00	3.00	3.00	4.00	9.00	88.00
118.00	59.00	0.00	23.00	39.90	1.00	9.00	12.00	2.00	252.00	204.00
119.00	67.00	1.00	18.00	14.40	0.00	2.00	--	--	2.00	--
121.00	86.00	0.00	22.00	42.20	1.00	7.00	4.00	5.00	203.00	88.00
123.00	60.00	0.00	16.00	14.60	0.00	7.00	6.00	3.00	2.00	207.00
124.00	33.00	0.00	14.00	27.40	1.00	7.00	6.00	2.00	97.00	149.00
126.00	68.00	0.00	27.00	46.00	0.00	5.00	--	--	3.00	--
127.00	100.00	0.00	25.00	64.60	1.00	12.00	--	--	316.00	--
128.00	80.00	1.00	30.00	56.90	1.00	7.00	--	--	51.00	--
129.00	31.00	0.00	19.00	44.00	0.00	12.00	--	--	63.00	--
131.00	65.00	0.00	21.00	55.30	1.00	8.00	--	--	165.00	--
133.00	35.00	0.00	14.00	7.20	1.00	--	--	--	35.00	--
135.00	41.00	1.00	16.00	14.30	1.00	2.00	--	--	221.00	--
137.00	69.00	1.00	19.00	32.00	1.00	4.00	--	--	218.00	--
139.00	54.00	1.00	23.00	55.80	1.00	8.00	--	--	72.00	--
140.00	74.00	0.00	21.00	41.60	1.00	8.00	--	--	51.00	--

Patient No.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	SOFA score admission	SOFA score follow-up	Follow-up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow-up (mg/l)
141.00	67.00	0.00	32.00	78.00	1.00	10.00	2.00	11.00	143.00	80.00
143.00	52.00	0.00	10.00	17.40	1.00	2.00	--	--	39.00	--
146.00	38.00	0.00	18.00	24.20	1.00	6.00	5.00	6.00	73.00	22.00
147.00	70.00	0.00	20.00	35.50	1.00	7.00	3.00	2.00	203.00	216.00
148.00	71.00	0.00	25.00	53.30	0.00	5.00	--	--	17.00	--
149.00	74.00	0.00	15.00	--	1.00	5.00	--	--	94.00	--
150.00	62.00	0.00	21.00	38.90	0.00	11.00	--	--	295.00	--
151.00	36.00	0.00	8.00	8.60	1.00	0.00	--	--	229.00	--
152.00	47.00	0.00	11.00	7.60	0.00	3.00	--	--	26.00	--
153.00	77.00	1.00	27.00	46.00	0.00	4.00	--	--	45.00	--
154.00	49.00	0.00	26.00	4.40	0.00	4.00	--	--	39.00	--
155.00	43.00	0.00	25.00	3.80	0.00	8.00	--	--	52.00	--
156.00	41.00	0.00	33.00	78.60	0.00	12.00	--	--	316.00	--
157.00	61.00	0.00	31.00	81.40	1.00	14.00	--	--	294.00	--
158.00	56.00	0.00	11.00	22.30	1.00	3.00	--	--	163.00	--
159.00	60.00	1.00	19.00	11.30	1.00	4.00	--	--	137.00	--
163.00	77.00	1.00	26.00	56.70	1.00	10.00	--	--	269.00	--
164.00	62.00	1.00	14.00	9.70	0.00	10.00	--	--	291.00	--
165.00	35.00	0.00	5.00	0.20	0.00	1.00	--	--	0.60	--
166.00	63.00	0.00	13.00	13.40	1.00	8.00	--	--	75.00	--
167.00	56.00	0.00	32.00	76.00	0.00	8.00	--	--	74.00	--
168.00	52.00	1.00	26.00	56.90	0.00	10.00	--	--	131.00	--
169.00	39.00	1.00	13.00	24.60	1.00	14.00	--	--	273.00	--
171.00	67.00	0.00	27.00	60.50	0.00	14.00	--	--	203.00	--

Patient No.	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)	Plasma retinol admission (umol/l)	Plasma retinol follow-up (umol/l)
12.00	24.00	23.00	46.00	45.00	0.61	0.43	1.70	1.73	0.88	1.53
13.00	27.00	22.00	55.00	47.00	2.80	1.95	4.27	2.08	1.30	0.31
14.00	12.00	10.00	45.00	37.00	1.55	0.82	2.19	1.28	0.42	0.42
15.00	23.00	16.00	63.00	54.00	1.52	--	2.75	--	0.18	1.50
16.00	28.00	--	72.00	--	0.75	--	3.76	--	1.31	--
18.00	10.00	10.00	31.00	30.00	0.80	2.75	0.55	1.47	0.39	1.18
19.00	12.00	14.00	68.00	38.00	0.72	1.77	3.06	2.09	1.11	1.69
21.00	10.00	--	25.00	--	1.60	--	2.60	--	1.45	--
22.00	27.00	15.00	71.00	40.00	1.10	--	3.00	--	1.44	0.75
23.00	10.00	13.00	39.00	48.00	0.50	0.80	1.10	1.60	0.24	0.51
24.00	35.00	--	74.00	--	0.80	--	4.90	--	1.80	--
27.00	23.00	17.00	54.00	46.00	1.80	0.80	3.90	2.50	1.18	0.70
29.00	9.00	10.00	25.00	39.00	0.80	1.10	0.90	1.80	0.22	1.48
30.00	16.00	11.00	44.00	42.00	1.20	0.60	3.80	3.20	4.20	2.92
31.00	22.00	--	46.00	--	0.60	--	2.50	--	1.22	--
32.00	17.00	--	39.00	--	1.10	--	2.00	--	0.09	--
33.00	17.00	10.00	41.00	35.00	0.80	1.00	2.10	1.70	0.35	0.46
34.00	30.00	15.00	56.00	39.00	2.01	1.31	1.25	1.94	1.27	0.77
35.00	9.00	13.00	27.00	36.00	0.70	1.28	1.27	1.74	0.21	0.35
36.00	14.00	14.00	35.00	39.00	0.68	1.02	0.53	1.94	0.15	0.75
37.00	12.00	12.00	45.00	39.00	0.38	0.65	0.84	1.35	0.85	1.49
38.00	14.00	--	56.00	--	1.66	--	2.40	--	0.50	--
40.00	20.00	18.00	39.00	41.00	--	2.10	--	2.00	0.90	0.52
41.00	19.00	--	33.00	--	0.64	--	1.51	--	1.62	--
42.00	17.00	18.00	32.00	37.00	0.50	0.80	2.10	2.40	0.84	0.57
44.00	10.00	16.00	37.00	43.00	0.44	1.18	0.98	3.44	0.60	2.71
45.00	14.00	14.00	41.00	45.00	0.69	1.18	2.41	2.07	1.04	2.12
46.00	11.00	--	32.00	--	1.19	--	1.88	--	0.17	--
47.00	24.00	24.00	58.00	53.00	0.80	0.70	2.20	1.80	0.61	0.49
48.00	10.00	9.00	57.00	47.00	0.70	0.80	0.90	0.90	0.02	0.03
50.00	23.00	15.00	31.00	42.00	1.20	1.10	1.50	1.50	1.62	0.74

Patient No.	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)	Plasma retinol admission (umol/l)	Plasma retinol follow-up (umol/l)
51.00	14.00	19.00	43.00	47.00	0.50	0.60	1.20	1.60	0.02	0.28
52.00	9.00	9.00	43.00	44.00	0.70	0.90	1.00	1.40	0.58	0.56
53.00	9.00	9.00	34.00	33.00	2.50	2.60	1.70	1.90	0.26	0.48
55.00	13.00	9.00	41.00	35.00	0.30	0.20	1.10	0.60	0.17	0.61
56.00	16.00	--	33.00	--	1.07	--	1.42	--	0.91	--
57.00	10.00	--	37.00	--	0.55	--	0.40	--	0.22	--
58.00	19.00	12.00	39.00	39.00	0.95	0.39	2.12	1.13	1.14	0.83
59.00	36.00	--	66.00	--	5.05	--	4.23	--	2.86	--
60.00	26.00	--	75.00	--	2.53	--	4.56	--	2.14	--
61.00	26.00	--	65.00	--	2.13	--	4.60	--	1.82	--
62.00	12.00	14.00	38.00	43.00	--	0.93	--	2.10	0.57	0.35
63.00	14.00	--	27.00	--	1.34	--	1.49	--	0.87	--
64.00	18.00	--	64.00	--	1.47	--	1.68	--	1.47	--
67.00	20.00	16.00	35.00	36.00	0.87	1.40	1.21	2.47	0.22	1.10
68.00	11.00	11.00	37.00	36.00	1.68	1.48	1.44	1.93	0.57	0.78
69.00	14.00	16.00	36.00	41.00	0.53	1.63	0.71	0.96	0.26	0.35
71.00	38.00	--	75.00	--	2.10	--	3.20	--	1.69	--
72.00	32.00	15.00	58.00	56.00	3.40	1.50	3.30	3.10	1.00	2.12
73.00	31.00	--	62.00	--	1.80	--	3.30	--	1.22	--
74.00	15.00	13.00	48.00	42.00	0.90	1.00	1.30	3.80	0.66	1.16
75.00	11.00	--	37.00	--	2.80	--	2.00	--	0.75	--
77.00	20.00	--	47.00	--	0.50	--	2.10	--	1.09	--
78.00	27.00	--	61.00	--	1.70	--	4.20	--	0.82	--
79.00	24.00	21.00	44.00	44.00	--	1.00	--	1.80	1.28	0.59
80.00	14.00	--	58.00	--	0.90	--	1.20	--	0.48	--
81.00	10.00	21.00	23.00	44.00	0.60	1.90	1.10	2.20	0.70	0.67
82.00	29.00	29.00	60.00	62.00	0.70	0.40	4.00	2.60	2.84	0.94
83.00	21.00	--	48.00	--	0.80	--	3.10	--	0.31	--
84.00	9.00	9.00	29.00	32.00	0.90	2.20	0.90	1.10	0.39	0.84
85.00	11.00	9.00	26.00	27.00	0.30	0.50	1.20	1.20	0.17	0.12
87.00	18.00	--	40.00	--	0.70	--	2.60	--	0.67	--
88.00	12.00	11.00	51.00	51.00	0.80	0.60	1.70	1.70	0.61	1.35
89.00	16.00	--	33.00	--	1.50	--	2.70	--	0.73	--
90.00	11.00	29.00	41.00	38.00	1.60	1.80	1.60	1.90	0.68	2.19

Patient No.	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)	Plasma retinol admission (umol/l)	Plasma retinol follow-up (umol/l)
91.00	14.00	--	40.00	--	1.30	--	1.60	--	0.36	--
96.00	29.00	28.00	64.00	56.00	1.60	1.10	4.40	4.40	1.60	2.00
99.00	22.00	--	52.00	--	1.06	--	2.50	--	0.40	--
100.00	13.00	9.00	41.00	33.00	2.40	0.50	2.30	1.40	0.60	0.50
101.00	25.00	--	46.00	--	0.70	--	2.20	--	0.30	--
102.00	19.00	19.00	38.00	39.00	0.60	2.40	1.70	1.80	0.90	0.50
103.00	42.00	27.00	73.00	49.00	3.30	--	4.10	--	1.80	1.10
104.00	34.00	--	71.00	--	4.70	--	6.40	--	2.10	--
105.00	30.00	29.00	66.00	57.00	1.00	0.60	2.00	1.90	0.40	0.20
107.00	17.00	--	39.00	--	--	--	--	--	0.30	--
108.00	35.00	--	70.00	--	--	--	--	--	0.80	--
110.00	13.00	11.00	48.00	34.00	1.10	3.20	1.60	2.10	0.40	0.20
111.00	28.00	13.00	53.00	37.00	2.00	2.10	4.00	1.60	1.80	0.20
112.00	20.00	9.00	42.00	26.00	1.20	0.80	5.20	1.70	1.30	0.20
113.00	31.00	19.00	60.00	43.00	1.30	0.40	2.70	1.40	2.00	0.60
114.00	16.00	9.00	37.00	27.00	1.40	0.70	2.20	1.00	0.40	0.20
115.00	39.00	--	63.00	--	0.90	--	5.30	--	1.90	--
116.00	24.00	--	48.00	--	1.00	--	2.70	--	1.30	--
117.00	32.00	23.00	55.00	45.00	4.50	2.80	4.20	3.00	1.90	0.70
118.00	12.00	9.00	33.00	28.00	0.70	1.10	0.70	1.00	0.30	0.30
119.00	25.00	--	50.00	--	0.50	--	4.60	--	1.20	--
121.00	15.00	15.00	37.00	39.00	0.30	1.00	1.00	1.40	0.20	0.80
123.00	45.00	28.00	83.00	58.00	1.90	0.80	4.80	3.90	2.70	1.80
124.00	15.00	14.00	40.00	44.00	0.40	0.80	1.10	1.30	0.20	0.30
126.00	21.00	--	46.00	--	0.80	--	5.00	--	0.70	--
127.00	17.00	--	59.00	--	0.60	--	1.20	--	0.60	--
128.00	16.00	--	46.00	--	1.10	--	2.40	--	0.80	--
129.00	20.00	--	53.00	--	--	--	--	--	0.20	--
131.00	21.00	--	47.00	--	0.90	--	1.00	--	0.30	--
133.00	16.00	--	36.00	--	0.30	--	0.90	--	0.30	--
135.00	18.00	--	58.00	--	1.90	--	2.80	--	1.10	--
137.00	11.00	--	34.00	--	1.40	--	2.20	--	0.40	--
139.00	14.00	--	33.00	--	--	--	0.94	--	0.80	--
140.00	9.00	--	26.00	--	0.40	--	2.40	--	0.40	--

Patient No.	Albumin admission (g/l)	Albumin follow-up (g/l)	Total protein admission (g/l)	Total protein follow-up (g/l)	Triglycerides admission (mmol/l)	Triglycerides follow-up (mmol/l)	Cholesterol admission (mmol/l)	Cholesterol follow-up (mmol/l)	Plasma retinol admission (umol/l)	Plasma retinol follow-up (umol/l)
141.00	9.00	9.00	40.00	42.00	--	0.90	0.62	2.40	1.40	--
143.00	14.00	--	60.00	--	1.20	--	2.20	--	0.70	--
146.00	26.00	18.00	38.00	45.00	1.70	1.20	2.60	2.60	0.80	1.60
147.00	32.00	24.00	56.00	51.00	0.30	0.30	3.10	2.70	0.50	0.20
148.00	23.00	--	54.00	--	1.10	--	3.90	--	0.80	--
149.00	13.00	--	40.00	--	0.70	--	1.00	--	0.40	--
150.00	24.00	--	55.00	--	1.10	--	1.60	--	0.60	--
151.00	13.00	--	47.00	--	1.20	--	4.70	--	1.60	--
152.00	35.00	--	65.00	--	1.30	--	4.80	--	1.80	--
153.00	19.00	--	50.00	--	2.20	--	2.30	--	0.60	--
154.00	33.00	--	62.00	--	0.60	--	4.60	--	2.50	--
155.00	19.00	--	68.00	--	0.50	--	2.80	--	1.90	--
156.00	20.00	--	73.00	--	1.90	--	2.60	--	0.20	--
157.00	13.00	--	44.00	--	0.60	--	0.90	--	0.20	--
158.00	10.00	--	34.00	--	2.10	--	1.60	--	0.20	--
159.00	15.00	--	39.00	--	0.50	--	1.40	--	0.90	--
163.00	12.00	--	38.00	--	0.70	--	1.50	--	0.20	--
164.00	24.00	--	58.00	--	0.90	--	1.90	--	0.30	--
165.00	47.00	--	75.00	--	1.40	--	5.50	--	3.10	--
166.00	13.00	--	37.00	--	2.30	--	4.10	--	0.60	--
167.00	23.00	--	43.00	--	0.60	--	2.50	--	0.80	--
168.00	11.00	--	38.00	--	0.60	--	0.60	--	0.30	--
169.00	14.00	--	50.00	--	2.40	--	2.60	--	0.20	--
171.00	20.00	--	52.00	--	0.90	--	1.80	--	1.10	--

Patient No.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma lutein admission (ug/l)	Plasma lutein/ cholesterol admission (ug/mmol)	Plasma lutein follow-up (ug/l)	Plasma lutein/ cholesterol follow-up (ug/mmol)	Plasma lycopene admission (ug/l)	Plasma lycopene/ cholesterol admission (ug/mmol)	Plasma lycopene follow-up (ug/l)	Plasma lycopene/ cholesterol follow-up (ug/mmol)
12.00	14.36	8.45	12.70	7.34	30.10	17.71	37.40	21.62	28.90	17.00	27.30	15.78
13.00	15.35	3.59	11.98	5.76	4.60	1.08	4.80	2.31	9.50	2.22	14.70	7.07
14.00	16.26	7.42	10.49	8.20	9.10	4.16	5.10	3.98	15.60	7.12	6.90	5.39
15.00	14.48	5.27	26.88	--	7.40	2.69	8.30	--	35.30	12.84	33.70	--
16.00	15.68	4.17	--	--	21.40	5.69	--	--	23.30	6.20	--	--
18.00	6.27	11.40	14.47	9.84	3.60	6.55	12.40	8.44	4.60	8.36	8.20	5.58
19.00	17.70	5.78	15.40	7.37	33.50	10.95	23.50	11.24	102.50	33.50	49.60	23.73
21.00	16.03	6.17	--	--	4.60	1.77	--	--	4.10	1.58	--	--
22.00	13.74	4.58	16.90	--	22.20	7.40	11.80	--	17.70	5.90	13.90	--
23.00	7.93	7.21	18.48	11.55	13.90	12.64	30.30	18.94	21.80	19.82	44.60	27.88
24.00	26.95	5.50	--	--	113.20	23.10	--	--	43.10	8.80	--	--
27.00	24.42	6.26	21.91	8.76	38.30	9.82	21.90	8.76	22.00	5.64	16.00	6.40
29.00	10.91	12.12	16.56	9.20	8.00	8.89	12.60	7.00	6.20	6.89	17.30	9.61
30.00	33.79	8.89	23.73	7.42	41.70	10.97	24.50	7.66	84.40	22.21	57.10	17.84
31.00	14.80	5.92	--	--	53.40	21.36	--	--	100.60	40.24	--	--
32.00	10.30	5.15	--	--	18.00	9.00	--	--	4.70	2.35	--	--
33.00	11.83	5.63	13.34	7.85	23.90	11.38	17.30	10.18	29.40	14.00	31.70	18.65
34.00	19.65	15.72	23.17	11.94	32.00	25.60	33.10	17.06	47.00	37.60	176.10	90.77
35.00	10.16	8.00	16.07	9.24	21.50	16.93	28.50	16.38	64.50	50.79	74.30	42.70
36.00	6.44	12.15	14.56	7.51	8.50	16.04	18.30	9.43	13.40	25.28	21.70	11.19
37.00	6.25	7.44	10.86	8.04	141.90	168.93	21.10	15.63	290.60	345.95	25.70	19.04
38.00	20.99	8.75	--	--	7.50	3.13	--	--	12.10	5.04	--	--
40.00	17.38	--	21.31	10.66	28.30	--	21.90	10.95	70.60	--	43.90	21.95
41.00	10.05	6.66	--	--	20.40	13.51	--	--	37.40	24.77	--	--
42.00	12.37	5.89	14.77	6.15	53.50	25.48	51.90	21.63	65.90	31.38	47.90	19.96
44.00	8.31	8.48	35.61	10.35	30.70	31.33	67.90	19.74	22.40	22.86	61.00	17.73
45.00	15.25	6.33	19.08	9.22	9.90	4.11	6.60	3.19	16.30	6.76	12.50	6.04
46.00	9.70	5.16	--	--	9.80	5.21	--	--	16.20	8.62	--	--
47.00	14.47	6.58	12.24	6.80	39.80	18.09	26.40	14.67	38.30	17.41	24.20	13.44
48.00	8.06	8.96	7.13	7.92	14.00	15.56	10.70	11.89	11.40	12.67	7.10	7.89

Patient No.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma lutein admission (ug/l)	Plasma lutein/ cholesterol admission (ug/mmol)	Plasma lutein follow-up (ug/l)	Plasma lutein/ cholesterol follow-up (ug/mmol)	Plasma lycopene admission (ug/l)	Plasma lycopene/ cholesterol admission (ug/mmol)	Plasma lycopene follow-up (ug/l)	Plasma lycopene/ cholesterol follow-up (ug/mmol)
50.00	9.98	6.65	15.88	10.59	9.40	6.27	11.20	7.47	6.90	4.60	10.10	6.73
51.00	8.40	7.00	12.09	7.56	8.40	7.00	21.30	13.31	8.60	7.17	38.40	24.00
52.00	7.83	7.83	8.64	6.17	4.20	4.20	5.90	4.21	6.90	6.90	8.00	5.71
53.00	16.34	9.61	19.99	10.52	2.10	1.24	4.20	2.21	3.20	1.88	3.20	1.68
55.00	8.12	7.38	7.12	11.87	16.00	14.55	7.30	12.17	26.80	24.36	17.10	28.50
56.00	11.20	7.89	--	--	34.90	24.58	--	--	54.20	38.17	--	--
57.00	3.55	8.88	--	--	4.40	11.00	--	--	5.10	12.75	--	--
58.00	11.37	5.36	5.95	5.27	36.60	17.26	17.30	15.31	95.80	45.19	37.50	33.19
59.00	33.30	7.87	--	--	65.10	15.39	--	--	116.60	27.57	--	--
60.00	30.79	6.75	--	--	33.90	7.43	--	--	164.80	36.14	--	--
61.00	31.14	6.77	--	--	43.90	9.54	--	--	58.80	12.78	--	--
62.00	14.40	--	17.15	8.17	20.00	--	18.10	8.62	40.70	--	67.00	31.90
63.00	9.63	6.46	--	--	23.70	15.91	--	--	45.50	30.54	--	--
64.00	19.42	11.56	--	--	41.00	24.40	--	--	35.40	21.07	--	--
67.00	12.43	10.27	25.83	10.46	21.60	17.85	42.60	17.25	13.00	10.74	36.80	14.90
68.00	10.01	6.95	18.30	9.48	18.00	12.50	22.80	11.81	1.20	0.83	6.80	3.52
69.00	5.31	7.48	11.39	11.86	4.70	6.62	11.50	11.98	3.70	5.21	16.60	17.29
71.00	21.98	6.87	--	--	69.60	21.75	--	--	93.20	29.13	--	--
72.00	16.40	4.97	33.61	10.84	44.60	13.52	12.40	4.00	54.70	16.58	61.70	19.90
73.00	19.99	6.06	--	--	48.40	14.67	--	--	15.80	4.79	--	--
74.00	11.23	8.64	12.79	3.37	2.10	1.62	--	--	0.80	0.62	2.20	0.58
75.00	20.40	10.20	--	--	22.80	11.40	--	--	15.40	7.70	--	--
77.00	13.23	6.30	--	--	27.70	13.19	--	--	59.80	28.48	--	--
78.00	24.55	5.85	--	--	77.80	18.52	--	--	147.30	35.07	--	--
79.00	17.25	--	21.55	11.97	66.90	--	57.00	31.67	170.70	--	116.70	64.83
80.00	11.19	9.33	--	--	8.30	6.92	--	--	20.40	17.00	--	--
81.00	6.08	5.53	16.42	7.46	18.10	16.45	30.00	13.64	24.50	22.27	37.20	16.91
82.00	33.45	8.36	24.02	9.24	141.50	35.38	107.40	41.31	162.80	40.70	100.80	38.77
83.00	17.97	5.80	--	--	28.90	9.32	--	--	43.50	14.03	--	--
84.00	5.33	5.92	8.20	7.45	2.50	2.78	3.70	3.36	1.90	2.11	1.50	1.36
85.00	8.06	6.72	8.94	7.45	17.50	14.58	20.40	17.00	7.30	6.08	7.20	6.00
87.00	16.63	6.40	--	--	63.30	24.35	--	--	78.30	30.12	--	--
88.00	12.70	7.47	17.83	10.49	9.40	5.53	8.10	4.76	20.80	12.24	18.40	10.82

Patient No.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma lutein admission (ug/l)	Plasma lutein/ cholesterol admission (ug/mmol)	Plasma lutein follow-up (ug/l)	Plasma lutein/ cholesterol follow-up (ug/mmol)	Plasma lycopene admission (ug/l)	Plasma lycopene/ cholesterol admission (ug/mmol)	Plasma lycopene follow-up (ug/l)	Plasma lycopene/ cholesterol follow-up (ug/mmol)
89.00	19.22	7.12	--	--	80.30	29.74	--	--	133.20	49.33	--	--
90.00	15.93	9.96	17.29	9.10	18.80	11.75	19.30	10.16	63.40	39.63	83.50	43.95
91.00	15.56	9.73	--	--	44.10	27.56	--	--	28.40	17.75	--	--
96.00	27.00	6.14	27.00	6.14	84.00	19.09	76.00	17.27	130.00	29.55	143.00	32.50
99.00	15.00	6.00	--	--	21.00	8.40	--	--	71.00	28.40	--	--
100.00	19.00	8.26	12.00	8.57	9.00	3.91	9.00	6.43	9.00	3.91	9.00	6.43
101.00	15.00	6.82	--	--	31.00	14.09	--	--	45.00	20.45	--	--
102.00	11.00	6.47	14.00	7.78	35.00	20.59	33.00	18.33	60.00	35.29	51.00	28.33
103.00	41.00	10.00	29.00	--	29.00	7.07	18.00	--	142.00	34.63	120.00	--
104.00	36.00	5.63	--	--	90.00	14.06	--	--	193.00	30.16	--	--
105.00	13.00	6.50	10.00	5.26	50.00	25.00	32.00	16.84	44.00	22.00	21.00	11.05
107.00	16.00	--	--	--	12.00	--	--	--	27.00	--	--	--
108.00	23.00	--	--	--	68.00	--	--	--	234.00	--	--	--
110.00	17.00	10.63	27.00	12.86	25.00	15.63	27.00	12.86	9.00	5.63	9.00	4.29
111.00	26.00	6.50	18.00	11.25	106.00	26.50	29.00	18.13	273.00	68.25	65.00	40.63
112.00	28.00	5.38	12.00	7.06	246.00	47.31	64.00	37.65	32.00	6.15	39.00	22.94
113.00	17.00	6.30	9.00	6.43	38.00	14.07	14.00	10.00	57.00	21.11	20.00	14.29
114.00	15.00	6.82	10.00	10.00	29.00	13.18	9.00	9.00	90.00	40.91	36.00	36.00
115.00	24.00	4.53	--	--	97.00	18.30	--	--	188.00	35.47	--	--
116.00	12.00	4.44	--	--	22.00	8.15	--	--	96.00	35.56	--	--
117.00	36.00	8.57	29.00	9.67	54.00	12.86	26.00	8.67	139.00	33.10	109.00	36.33
118.00	7.00	10.00	9.00	9.00	9.00	12.86	9.00	9.00	9.00	12.86	9.00	9.00
119.00	32.00	6.96	--	--	168.00	36.52	--	--	176.00	38.26	--	--
121.00	8.00	8.00	15.00	10.71	9.00	9.00	17.00	12.14	9.00	9.00	9.00	6.43
123.00	27.00	5.63	24.00	6.15	68.00	14.17	48.00	12.31	60.00	12.50	46.00	11.79
124.00	7.00	6.36	11.00	8.46	9.00	8.18	9.00	6.92	16.00	14.55	22.00	16.92
126.00	20.00	4.00	--	--	36.00	7.20	--	--	110.00	22.00	--	--
127.00	16.00	13.33	--	--	88.00	73.33	--	--	13.00	10.83	--	--
128.00	17.00	7.08	--	--	11.00	4.58	--	--	17.00	7.08	--	--
129.00	12.00	--	--	--	9.00	--	--	--	11.00	--	--	--
131.00	7.00	7.00	--	--	9.00	9.00	--	--	24.00	24.00	--	--
133.00	6.00	6.67	--	--	11.00	12.22	--	--	32.00	35.56	--	--
135.00	25.00	8.93	--	--	9.00	3.21	--	--	9.00	3.21	--	--

Patient No.	Plasma α-tocopherol admission (umol/l)	Plasma α-tocopherol/ cholesterol admission (umol/mmol)	Plasma α-tocopherol follow-up (umol/l)	Plasma α-tocopherol/ cholesterol follow-up (umol/mmol)	Plasma lutein admission (ug/l)	Plasma lutein/ cholesterol admission (ug/mmol)	Plasma lutein follow-up (ug/l)	Plasma lutein/ cholesterol follow-up (ug/mmol)	Plasma lycopene admission (ug/l)	Plasma lycopene/ cholesterol admission (ug/mmol)	Plasma lycopene follow-up (ug/l)	Plasma lycopene/ cholesterol follow-up (ug/mmol)
137.00	20.00	9.09	--	--	37.00	16.82	--	--	103.00	46.82	--	--
139.00	6.00	6.38	--	--	9.00	9.57	--	--	16.00	17.02	--	--
140.00	5.00	2.08	--	--	9.00	3.75	--	--	9.00	3.75	--	--
141.00	6.00	9.68	22.00	9.17	9.00	14.52	11.00	4.58	12.00	19.35	24.00	10.00
143.00	22.00	10.00	--	--	9.00	4.09	--	--	55.00	25.00	--	--
146.00	12.00	4.62	22.00	8.46	9.00	3.46	9.00	3.46	126.00	48.46	56.00	21.54
147.00	12.00	3.87	11.00	4.07	47.00	15.16	36.00	13.33	56.00	18.06	48.00	17.78
148.00	17.00	4.36	--	--	16.00	4.10	--	--	46.00	11.79	--	--
149.00	6.00	6.00	--	--	9.00	9.00	--	--	9.00	9.00	--	--
150.00	12.00	7.50	--	--	12.00	7.50	--	--	15.00	9.38	--	--
151.00	22.00	4.68	--	--	67.00	14.26	--	--	294.00	62.55	--	--
152.00	21.00	4.38	--	--	69.00	14.38	--	--	260.00	54.17	--	--
153.00	21.00	9.13	--	--	15.00	6.52	--	--	48.00	20.87	--	--
154.00	23.00	5.00	--	--	70.00	15.22	--	--	78.00	16.96	--	--
155.00	11.00	3.93	--	--	15.00	5.36	--	--	101.00	36.07	--	--
156.00	9.00	3.46	--	--	9.00	3.46	--	--	14.00	5.38	--	--
157.00	6.00	6.67	--	--	9.00	10.00	--	--	9.00	10.00	--	--
158.00	9.00	5.63	--	--	9.00	5.63	--	--	9.00	5.63	--	--
159.00	9.00	6.43	--	--	30.00	21.43	--	--	9.00	6.43	--	--
163.00	12.00	8.00	--	--	19.00	12.67	--	--	11.00	7.33	--	--
164.00	12.00	6.32	--	--	32.00	16.84	--	--	9.00	4.74	--	--
165.00	32.00	5.82	--	--	92.00	16.73	--	--	318.00	57.82	--	--
166.00	29.00	7.07	--	--	8.00	1.95	--	--	10.00	2.44	--	--
167.00	10.00	4.00	--	--	19.00	7.60	--	--	19.00	7.60	--	--
168.00	6.00	10.00	--	--	12.00	20.00	--	--	15.00	25.00	--	--
169.00	12.00	4.62	--	--	9.00	3.46	--	--	3.00	1.15	--	--
171.00	16.00	8.89	--	--	24.00	13.33	--	--	29.00	16.11	--	--

Patient No.	Plasma α-carotene admission (ug/l)	Plasma α-carotene/ cholesterol admission (ug/mmol)	Plasma α-carotene follow-up (ug/l)	Plasma α-carotene/ cholesterol follow-up (ug/mmol)	Plasma β-carotene admission (ug/l)	Plasma β-carotene/ cholesterol admission (ug/mmol)	Plasma β-carotene follow-up (ug/l)	Plasma β-carotene/ cholesterol follow-up (ug/mmol)
12.00	21.20	12.47	20.00	11.56	58.00	34.12	62.70	36.24
13.00	9.00	2.11	1.40	0.67	0.50	0.12	10.90	5.24
14.00	4.60	2.10	--	--	8.90	4.06	0.60	0.47
15.00	1.80	0.65	--	--	3.90	1.42	0.40	--
16.00	4.20	1.12	--	--	20.40	5.43	--	--
18.00	9.00	16.36	1.20	0.82	0.30	0.55	2.80	1.90
19.00	16.60	5.42	8.90	4.26	117.90	38.53	60.30	28.85
21.00	1.60	0.62	--	--	10.20	3.92	--	--
22.00	11.30	3.77	6.90	--	39.00	13.00	39.00	--
23.00	9.00	8.18	15.20	9.50	28.30	25.73	115.40	72.13
24.00	12.00	2.45	--	--	115.60	23.59	--	--
27.00	9.20	2.36	6.50	2.60	67.60	17.33	183.50	73.40
29.00	0.90	1.00	1.60	0.89	4.40	4.89	38.90	21.61
30.00	20.30	5.34	13.30	4.16	40.10	10.55	25.30	7.91
31.00	13.10	5.24	--	--	66.90	26.76	--	--
32.00	3.00	1.50	--	--	9.30	4.65	--	--
33.00	12.60	6.00	12.90	7.59	39.00	18.57	57.80	34.00
34.00	1.40	1.12	6.90	3.56	0.60	0.48	69.50	35.82
35.00	8.30	6.54	11.20	6.44	34.30	27.01	54.40	31.26
36.00	3.80	7.17	4.80	2.47	14.40	27.17	30.40	15.67
37.00	29.10	34.64	6.70	4.96	200.70	238.93	83.20	61.63
38.00	12.60	5.25	--	--	110.90	46.21	--	--
40.00	8.00	--	3.10	1.55	31.70	--	11.10	5.55
41.00	17.10	11.32	--	--	45.20	29.93	--	--
42.00	11.80	5.62	14.60	6.08	57.10	27.19	76.40	31.83
44.00	1.70	1.73	6.40	1.86	14.70	15.00	198.90	57.82
45.00	2.40	1.00	1.70	0.82	15.60	6.47	50.20	24.25
46.00	0.80	0.43	--	--	2.60	1.38	--	--
47.00	11.50	5.23	8.30	4.61	31.20	14.18	22.50	12.50
48.00	9.00	10.00	1.80	2.00	5.70	6.33	2.60	2.89
50.00	1.60	1.07	1.70	1.13	25.90	17.27	23.30	15.53
51.00	1.50	1.25	5.70	3.56	23.90	19.92	46.70	29.19

Patient No.	Plasma α-carotene admission (ug/l)	Plasma α-carotene/ cholesterol admission (ug/mmol)	Plasma α-carotene follow-up (ug/l)	Plasma α-carotene/ cholesterol follow-up (ug/mmol)	Plasma β-carotene admission (ug/l)	Plasma β-carotene/ cholesterol admission (ug/mmol)	Plasma β-carotene follow-up (ug/l)	Plasma β-carotene/ cholesterol follow-up (ug/mmol)
52.00	0.70	0.70	0.70	0.50	0.60	0.60	0.40	0.29
53.00	9.00	5.29	0.70	0.37	0.90	0.53	1.50	0.79
55.00	11.70	10.64	6.20	10.33	18.30	16.64	9.30	15.50
56.00	9.30	6.55	--	--	29.70	20.92	--	--
57.00	0.90	2.25	--	--	2.80	7.00	--	--
58.00	15.00	7.08	5.70	5.04	73.60	34.72	24.50	21.68
59.00	6.60	1.56	--	--	10.70	2.53	--	--
60.00	21.80	4.78	--	--	85.00	18.64	--	--
61.00	5.60	1.22	--	--	23.30	5.07	--	--
62.00	15.60	--	22.40	10.67	53.10	--	85.00	40.48
63.00	6.20	4.16	--	--	20.90	14.03	--	--
64.00	14.90	8.87	--	--	65.60	39.05	--	--
67.00	4.70	3.88	11.00	4.45	22.00	18.18	126.00	51.01
68.00	1.30	0.90	6.50	3.37	0.30	0.21	4.10	2.12
69.00	0.60	0.85	0.80	0.83	2.00	2.82	10.60	11.04
71.00	5.50	1.72	--	--	16.00	5.00	--	--
72.00	2.90	0.88	7.70	2.48	0.50	0.15	187.20	60.39
73.00	9.00	2.73	--	--	51.50	15.61	--	--
74.00	0.50	0.38	9.00	2.37	0.30	0.23	0.50	0.13
75.00	4.80	2.40	--	--	18.20	9.10	--	--
77.00	6.80	3.24	--	--	37.90	18.05	--	--
78.00	20.30	4.83	--	--	109.60	26.10	--	--
79.00	8.90	--	5.90	3.28	41.90	--	22.10	12.28
80.00	2.30	1.92	--	--	10.60	8.83	--	--
81.00	4.30	3.91	8.00	3.64	14.60	13.27	21.60	9.82
82.00	75.00	18.75	49.00	18.85	401.80	100.45	248.20	95.46
83.00	15.90	5.13	--	--	91.60	29.55	--	--
84.00	9.00	10.00	9.00	8.18	0.70	0.78	14.40	13.09
85.00	6.10	5.08	7.00	5.83	9.00	7.50	13.40	11.17
87.00	27.60	10.62	--	--	141.30	54.35	--	--
88.00	5.80	3.41	6.10	3.59	5.30	3.12	43.00	25.29
89.00	15.50	5.74	--	--	62.60	23.19	--	--
90.00	2.70	1.69	3.50	1.84	10.10	6.31	64.70	34.05

Patient No.	Plasma α-carotene admission (ug/l)	Plasma α-carotene/ cholesterol admission (ug/mmol)	Plasma α-carotene follow-up (ug/l)	Plasma α-carotene/ cholesterol follow-up (ug/mmol)	Plasma β-carotene admission (ug/l)	Plasma β-carotene/ cholesterol admission (ug/mmol)	Plasma β-carotene follow-up (ug/l)	Plasma β-carotene/ cholesterol follow-up (ug/mmol)
91.00	9.30	5.81	--	--	12.50	7.81	--	--
96.00	12.00	2.73	13.00	2.95	31.00	7.05	33.00	7.50
99.00	9.00	3.60	--	--	9.00	3.60	--	--
100.00	9.00	3.91	9.00	6.43	9.00	3.91	9.00	6.43
101.00	11.00	5.00	--	--	46.00	20.91	--	--
102.00	9.00	5.29	9.00	5.00	44.00	25.88	56.00	31.11
103.00	173.00	42.20	143.00	--	167.00	40.73	218.00	--
104.00	9.00	1.41	--	--	32.00	5.00	--	--
105.00	16.00	8.00	10.00	5.26	52.00	26.00	32.00	16.84
107.00	9.00	--	--	--	311.00	--	--	--
108.00	14.00	--	--	--	48.00	--	--	--
110.00	9.00	5.63	9.00	4.29	11.00	6.88	16.00	7.62
111.00	9.00	2.25	9.00	5.63	50.00	12.50	50.00	31.25
112.00	36.00	6.92	15.00	8.82	522.00	100.38	164.00	96.47
113.00	9.00	3.33	9.00	6.43	25.00	9.26	9.00	6.43
114.00	10.00	4.55	9.00	9.00	53.00	24.09	18.00	18.00
115.00	10.00	1.89	--	--	51.00	9.62	--	--
116.00	9.00	3.33	--	--	9.00	3.33	--	--
117.00	9.00	2.14	9.00	3.00	16.00	3.81	15.00	5.00
118.00	9.00	12.86	9.00	9.00	9.00	12.86	9.00	9.00
119.00	19.00	4.13	--	--	140.00	30.43	--	--
121.00	9.00	9.00	9.00	6.43	9.00	9.00	27.00	19.29
123.00	11.00	2.29	10.00	2.56	24.00	5.00	73.00	18.72
124.00	9.00	8.18	9.00	6.92	9.00	8.18	22.00	16.92
126.00	9.00	1.80	--	--	14.00	2.80	--	--
127.00	9.00	7.50	--	--	101.00	84.17	--	--
128.00	10.00	4.17	--	--	39.00	16.25	--	--
129.00	9.00	--	--	--	9.00	--	--	--
131.00	9.00	9.00	--	--	16.00	16.00	--	--
133.00	9.00	10.00	--	--	9.00	10.00	--	--
135.00	9.00	3.21	--	--	176.00	62.86	--	--
137.00	16.00	7.27	--	--	48.00	21.82	--	--
139.00	9.00	9.57	--	--	9.00	9.57	--	--

Patient No.	Plasma α-carotene admission (ug/l)	Plasma α-carotene/ cholesterol admission (ug/mmol)	Plasma α-carotene follow-up (ug/l)	Plasma α-carotene/ cholesterol follow-up (ug/mmol)	Plasma β-carotene admission (ug/l)	Plasma β-carotene/ cholesterol admission (ug/mmol)	Plasma β-carotene follow-up (ug/l)	Plasma β-carotene/ cholesterol follow-up (ug/mmol)
140.00	9.00	3.75	--	--	9.00	3.75	--	--
141.00	9.00	14.52	9.00	3.75	9.00	14.52	13.00	5.42
143.00	44.00	20.00	--	--	518.00	235.45	--	--
146.00	9.00	3.46	9.00	3.46	9.00	3.46	131.00	50.38
147.00	9.00	2.90	9.00	3.33	23.00	7.42	24.00	8.89
148.00	9.00	2.31	--	--	11.00	2.82	--	--
149.00	9.00	9.00	--	--	9.00	9.00	--	--
150.00	9.00	5.63	--	--	9.00	5.63	--	--
151.00	38.00	8.09	--	--	224.00	47.66	--	--
152.00	34.00	7.08	--	--	185.00	38.54	--	--
153.00	--	--	--	--	71.00	30.87	--	--
154.00	13.00	2.83	--	--	10.00	2.17	--	--
155.00	5.00	1.79	--	--	92.00	32.86	--	--
156.00	9.00	3.46	--	--	9.00	3.46	--	--
157.00	9.00	10.00	--	--	9.00	10.00	--	--
158.00	9.00	5.63	--	--	9.00	5.63	--	--
159.00	9.00	6.43	--	--	9.00	6.43	--	--
163.00	6.00	4.00	--	--	31.00	20.67	--	--
164.00	4.00	2.11	--	--	8.00	4.21	--	--
165.00	20.00	3.64	--	--	115.00	20.91	--	--
166.00	0.70	0.17	--	--	2.00	0.49	--	--
167.00	17.00	6.80	--	--	20.00	8.00	--	--
168.00	3.00	5.00	--	--	7.00	11.67	--	--
169.00	3.00	1.15	--	--	6.00	2.31	--	--
171.00	3.00	1.67	--	--	81.00	45.00	--	--

Patient No.	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	Total MDA follow-up (umol/l)	Free MDA follow-up (umol/l)	Free/ Total MDA follow-up	MDA/ Total Protein admission (umol/g)	MDA/ Total Protein follow-up (umol/g)
12.00	0.65	0.14	0.22	0.51	0.12	0.24	0.01	0.01
13.00	0.91	0.16	0.18	0.54	0.17	0.31	0.02	0.01
14.00	0.60	0.58	0.97	0.64	0.16	0.25	0.01	0.02
15.00	0.96	0.17	0.18	0.76	0.14	0.18	0.02	0.01
16.00	1.02	0.20	0.20	--	--	--	0.01	--
18.00	0.80	0.19	0.24	0.49	0.17	0.35	0.03	0.02
19.00	0.87	0.26	0.30	1.18	0.40	0.34	0.01	0.03
21.00	2.34	0.62	0.26	--	--	--	0.09	--
22.00	1.03	0.38	0.37	--	--	--	0.01	--
23.00	0.22	0.19	0.86	0.40	0.11	0.28	0.01	0.01
24.00	1.37	0.37	0.27	--	--	--	0.02	--
27.00	1.12	0.22	0.20	0.75	0.25	0.33	0.02	0.02
29.00	0.53	0.37	0.70	1.11	0.32	0.29	0.02	0.03
30.00	0.93	0.97	1.04	0.76	0.56	0.74	0.02	0.02
31.00	0.55	0.21	0.38	--	--	--	0.01	--
32.00	0.77	0.23	0.30	--	--	--	0.02	--
33.00	0.63	0.31	0.49	1.08	0.61	0.56	0.02	0.03
34.00	0.82	0.14	0.17	0.45	0.13	0.29	0.01	0.01
35.00	14.27	5.96	0.42	20.40	9.41	0.46	0.53	0.57
36.00	0.78	0.20	0.26	1.70	0.33	0.19	0.02	0.04
37.00	0.35	0.05	0.14	0.38	0.10	0.26	0.01	0.01
38.00	0.52	0.04	0.08	--	--	--	0.01	--
40.00	0.31	0.06	0.19	0.45	0.03	0.07	0.01	0.01
41.00	0.48	0.05	0.10	--	--	--	0.01	--
42.00	--	--	--	--	--	--	--	--
44.00	--	--	--	--	--	--	--	--
45.00	0.42	0.06	0.14	0.47	0.08	0.17	0.01	0.01
46.00	0.32	0.03	0.09	--	--	--	0.01	--
47.00	0.56	0.07	0.13	0.54	0.13	0.24	0.01	0.01
48.00	0.34	0.04	0.12	0.51	0.14	0.27	0.01	0.01
50.00	0.40	0.15	0.38	0.51	0.09	0.18	0.01	0.01
51.00	0.27	0.10	0.37	0.54	0.13	0.24	0.01	0.01
52.00	0.50	0.18	0.36	0.51	0.21	0.41	0.01	0.01

Patient No.	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	Total MDA follow-up (umol/l)	Free MDA follow-up (umol/l)	Free/ Total MDA follow-up	MDA/ Total Protein admission (umol/g)	MDA/ Total Protein follow-up (umol/g)
53.00	0.39	0.10	0.26	0.42	0.09	0.21	0.01	0.01
55.00	0.22	0.10	0.45	0.32	0.13	0.41	0.01	0.01
56.00	0.25	--	--	--	--	--	0.01	--
57.00	0.29	0.15	0.52	--	--	--	0.01	--
58.00	0.67	0.09	0.13	0.44	0.10	0.23	0.02	0.01
59.00	2.20	0.28	0.13	--	--	--	0.03	--
60.00	0.75	0.07	0.09	--	--	--	0.01	--
61.00	1.37	1.37	1.00	--	--	--	0.02	--
62.00	0.69	0.05	0.07	0.71	0.71	1.00	0.02	0.02
63.00	0.62	0.06	0.10	--	--	--	0.02	--
64.00	0.60	0.02	0.03	--	--	--	0.01	--
67.00	0.83	0.26	0.31	0.61	0.08	0.13	0.02	0.02
68.00	0.37	0.16	0.43	0.76	0.14	0.18	0.01	0.02
69.00	--	--	--	--	--	--	--	--
71.00	0.60	0.04	0.07	--	--	--	0.01	--
72.00	--	--	--	--	--	--	--	--
73.00	1.34	0.05	0.04	--	--	--	0.02	--
74.00	--	--	--	--	--	--	--	--
75.00	1.19	0.18	0.15	--	--	--	0.03	--
77.00	1.03	0.08	0.08	--	--	--	0.02	--
78.00	--	--	--	--	--	--	--	--
79.00	--	--	--	--	--	--	--	--
80.00	--	--	--	--	--	--	--	--
81.00	0.41	0.08	0.20	0.63	0.07	0.11	0.02	0.01
82.00	--	--	--	--	--	--	--	--
83.00	0.89	0.04	0.04	--	--	--	0.02	--
84.00	0.78	0.13	0.17	0.94	0.15	0.16	0.03	0.03
85.00	--	--	--	--	--	--	--	--
87.00	0.24	0.02	0.08	--	--	--	0.01	--
88.00	0.42	0.08	0.19	0.55	0.19	0.35	0.01	0.01
89.00	--	--	--	--	--	--	--	--
90.00	--	--	--	--	--	--	--	--
91.00	--	--	--	--	--	--	--	--
96.00	--	--	--	--	--	--	--	--

Patient No.	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	Total MDA follow-up (umol/l)	Free MDA follow-up (umol/l)	Free/ Total MDA follow-up	MDA/ Total Protein admission (umol/g)	MDA/ Total Protein follow-up (umol/g)
99.00	0.73	0.05	0.07	--	--	--	0.01	--
100.00	0.68	0.19	0.28	0.45	0.12	0.27	0.02	0.01
101.00	0.32	0.07	0.22	--	--	--	0.01	--
102.00	--	--	--	--	--	--	--	--
103.00	--	--	--	--	--	--	--	--
104.00	--	--	--	--	--	--	--	--
105.00	0.71	0.15	0.21	0.48	0.17	0.35	0.01	0.01
107.00	0.77	0.08	0.10	--	--	--	0.02	--
108.00	0.48	0.10	0.21	--	--	--	0.01	--
110.00	0.48	0.09	0.19	0.84	0.12	0.14	0.01	0.02
111.00	--	--	--	--	--	--	--	--
112.00	1.31	0.06	0.05	2.62	1.40	0.53	0.03	0.10
113.00	1.56	0.35	0.22	0.47	0.05	0.11	0.03	0.01
114.00	0.73	0.12	0.16	0.51	0.16	0.31	0.02	0.02
115.00	1.06	0.05	0.05	--	--	--	0.02	--
116.00	0.81	0.06	0.07	--	--	--	0.02	--
117.00	1.61	0.06	0.04	0.46	0.06	0.13	0.03	0.01
118.00	0.61	0.05	0.08	0.66	0.17	0.26	0.02	0.02
119.00	1.40	0.06	0.04	--	--	--	0.03	--
121.00	0.57	0.08	0.14	0.47	0.06	0.13	0.02	0.01
123.00	0.87	0.04	0.05	0.81	0.10	0.12	0.01	0.01
124.00	0.35	0.08	0.23	0.50	0.06	0.12	0.01	0.01
126.00	0.55	0.06	0.11	--	--	--	0.01	--
127.00	0.79	0.11	0.14	--	--	--	0.01	--
128.00	0.94	0.10	0.11	--	--	--	0.02	--
129.00	1.84	0.14	0.08	--	--	--	0.03	--
131.00	0.44	0.09	0.20	--	--	--	0.01	--
133.00	0.61	0.08	0.13	--	--	--	0.02	--
135.00	0.56	0.06	0.11	--	--	--	0.01	--
137.00	0.45	0.04	0.08	--	--	--	0.01	--
139.00	0.63	0.04	0.06	--	--	--	0.02	--
140.00	0.62	0.08	0.13	--	--	--	0.02	--
141.00	0.66	0.12	0.18	0.40	0.11	0.28	0.02	0.01
143.00	0.59	0.08	0.14	--	--	--	0.01	--

Patient No.	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	Total MDA follow-up (umol/l)	Free MDA follow-up (umol/l)	Free/ Total MDA follow-up	MDA/ Total Protein admission (umol/g)	MDA/ Total Protein follow-up (umol/g)
146.00	0.70	0.07	0.10	0.47	0.11	0.23	0.02	0.01
147.00	0.47	0.05	0.11	0.63	0.05	0.08	0.01	0.01
148.00	--	--	--	--	--	--	--	--
149.00	0.52	0.07	0.13	--	--	--	0.01	--
150.00	0.41	0.06	0.15	--	--	--	0.01	--
151.00	0.48	0.06	0.13	--	--	--	0.01	--
152.00	1.65	0.07	0.04	--	--	--	0.03	--
153.00	0.68	0.10	0.15	--	--	--	0.01	--
154.00	0.85	0.09	0.11	--	--	--	0.01	--
155.00	1.08	0.19	0.18	--	--	--	0.02	--
156.00	0.69	0.13	0.19	--	--	--	0.01	--
157.00	0.38	0.22	0.58	--	--	--	0.01	--
158.00	1.00	0.08	0.08	--	--	--	0.03	--
159.00	0.62	0.08	0.13	--	--	--	0.02	--
163.00	0.72	0.08	0.11	--	--	--	0.02	--
164.00	0.56	0.05	0.09	--	--	--	0.01	--
165.00	1.24	0.05	0.04	--	--	--	0.02	--
166.00	0.92	0.09	0.10	--	--	--	0.02	--
167.00	0.42	0.08	0.19	--	--	--	0.01	--
168.00	--	--	--	--	--	--	--	--
169.00	--	--	--	--	--	--	--	--
171.00	--	--	--	--	--	--	--	--

12.12 Appendix 12 Chapter 8 data

Characteristics and biochemical measurements of patients (Chapter 8)

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital death	Hospital stay (days)
12.00	61.00	0.00	29.00	76.60	0.00	0.00	0.00	0.00	7.40	0.00	13.40
13.00	43.00	1.00	12.00	11.70	1.00	0.00	2.00	0.00	48.10	1.00	50.10
14.00	71.00	1.00	18.00	28.90	1.00	0.00	0.00	1.00	12.90	1.00	12.90
15.00	53.00	1.00	19.00	32.20	0.00	0.00	1.00	0.00	4.80	0.00	10.80
16.00	61.00	0.00	23.00	28.50	0.00	0.00	--	0.00	1.00	0.00	10.00
17.00	18.00	0.00	12.00	20.20	0.00	0.00	3.00	1.00	6.20	1.00	6.20
18.00	67.00	0.00	18.00	44.40	1.00	0.00	0.00	1.00	59.50	1.00	59.50
19.00	50.00	0.00	21.00	38.90	0.00	0.00	3.00	1.00	46.30	1.00	46.30
21.00	61.00	0.00	34.00	76.80	1.00	0.00	--	0.00	2.00	0.00	53.00
22.00	52.00	0.00	13.00	9.90	0.00	95.00	101.00	0.00	3.70	0.00	77.00
23.00	71.00	0.00	23.00	42.50	1.00	9.00	10.00	1.00	20.90	1.00	20.90
24.00	77.00	1.00	21.00	35.00	0.00	0.00	--	0.00	2.00	0.00	6.00
27.00	76.00	1.00	23.00	46.00	0.00	1.00	3.00	0.00	21.70	0.00	21.70
29.00	70.00	0.00	16.00	20.20	1.00	4.00	10.00	0.00	5.10	0.00	85.00
30.00	73.00	1.00	25.00	53.30	1.00	0.00	2.00	1.00	15.50	1.00	15.50
31.00	62.00	0.00	19.00	27.10	1.00	0.00	--	0.00	0.50	0.00	25.00
32.00	55.00	1.00	15.00	21.00	1.00	0.00	--	0.00	2.20	0.00	22.20
33.00	80.00	1.00	31.00	84.20	1.00	0.00	0.00	1.00	12.00	1.00	12.00
34.00	53.00	0.00	26.00	68.60	1.00	6.00	7.00	0.00	29.40	0.00	85.00
35.00	20.00	0.00	18.00	39.70	1.00	0.00	1.00	1.00	44.00	1.00	44.00
36.00	43.00	1.00	38.00	92.60	0.00	5.00	10.00	0.00	25.50	0.00	27.50
37.00	60.00	0.00	27.00	63.10	0.00	0.00	0.00	0.00	21.80	0.00	29.00
38.00	74.00	0.00	20.00	23.00	1.00	0.00	--	0.00	1.00	0.00	150.00
40.00	47.00	0.00	22.00	28.50	1.00	0.00	2.00	0.00	1.90	0.00	19.90
41.00	76.00	1.00	29.00	79.90	1.00	0.00	--	0.00	1.40	0.00	21.40
42.00	79.00	0.00	21.00	30.90	1.00	0.00	3.00	0.00	3.20	0.00	27.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital death	Hospital stay (days)
44.00	81.00	0.00	34.00	86.30	0.00	0.00	0.00	1.00	16.10	1.00	16.10
45.00	74.00	0.00	33.00	75.20	0.00	0.00	0.00	1.00	16.00	1.00	16.00
46.00	41.00	1.00	8.00	15.60	1.00	0.00	--	0.00	0.80	0.00	18.80
47.00	76.00	0.00	29.00	77.20	1.00	0.00	0.00	1.00	34.60	1.00	36.80
48.00	67.00	0.00	31.00	68.10	1.00	1,000.00	1,007.00	1.00	18.40	1.00	18.40
50.00	60.00	0.00	19.00	48.00	1.00	0.00	0.00	0.00	6.20	0.00	124.20
51.00	46.00	1.00	24.00	49.70	0.00	1,000.00	1,006.00	1.00	4.60	1.00	4.60
52.00	61.00	0.00	33.00	78.60	0.00	7.00	10.00	1.00	7.70	1.00	7.70
53.00	67.00	1.00	18.00	21.20	0.00	0.00	0.00	1.00	11.10	1.00	11.10
55.00	80.00	1.00	31.00	73.30	0.00	0.00	3.00	1.00	8.20	1.00	8.20
56.00	40.00	1.00	6.00	10.20	1.00	0.00	--	0.00	0.40	0.00	0.40
57.00	60.00	0.00	27.00	60.20	1.00	0.00	--	1.00	1.80	1.00	1.80
58.00	41.00	0.00	21.00	17.80	1.00	0.00	0.00	0.00	19.70	0.00	36.70
59.00	68.00	0.00	20.00	32.30	0.00	0.00	--	0.00	0.80	0.00	12.00
60.00	57.00	0.00	24.00	67.00	0.00	0.00	--	0.00	6.50	0.00	59.50
61.00	76.00	1.00	18.00	14.40	0.00	0.00	--	0.00	0.70	0.00	22.70
62.00	74.00	0.00	20.00	19.90	1.00	0.00	0.00	0.00	0.90	0.00	253.00
63.00	62.00	0.00	18.00	44.40	1.00	1,000.00	--	0.00	1.00	0.00	508.00
64.00	66.00	0.00	34.00	81.00	0.00	0.00	--	0.00	22.40	0.00	46.00
65.00	38.00	1.00	17.00	26.20	0.00	0.00	--	0.00	18.90	0.00	37.00
67.00	68.00	1.00	31.00	80.30	0.00	1,000.00	0.00	0.00	5.00	1.00	8.00
68.00	74.00	0.00	24.00	49.70	0.00	0.00	7.00	0.00	10.70	0.00	27.70
69.00	61.00	0.00	22.00	54.90	1.00	1,000.00	1,004.00	0.00	76.40	1.00	122.40
71.00	25.00	0.00	17.00	1.20	0.00	0.00	--	0.00	2.00	0.00	3.50
72.00	51.00	0.00	21.00	12.40	0.00	1.00	1.00	0.00	15.50	0.00	40.00
73.00	69.00	0.00	12.00	20.20	0.00	0.00	--	0.00	5.80	0.00	23.00
74.00	65.00	0.00	33.00	80.40	0.00	0.00	7.00	0.00	19.60	0.00	40.00
75.00	28.00	1.00	14.00	18.50	1.00	0.00	--	0.00	0.60	0.00	58.00
77.00	54.00	1.00	17.00	1.20	0.00	0.00	--	0.00	0.80	0.00	12.00
78.00	45.00	0.00	12.00	5.50	1.00	0.00	--	0.00	0.90	0.00	12.00
79.00	41.00	0.00	7.00	7.60	1.00	0.00	0.00	0.00	9.30	0.00	19.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital death	Hospital stay (days)
80.00	38.00	0.00	16.00	23.30	1.00	1,000.00	--	0.00	0.80	0.00	41.00
81.00	71.00	0.00	25.00	35.90	1.00	0.00	1.00	0.00	8.30	0.00	20.00
82.00	80.00	1.00	17.00	12.70	0.00	0.00	1.00	0.00	10.30	0.00	29.00
83.00	46.00	1.00	11.00	10.30	1.00	0.00	--	0.00	2.90	0.00	10.00
84.00	56.00	0.00	28.00	63.90	0.00	1,000.00	1,006.00	0.00	15.40	0.00	35.00
85.00	73.00	0.00	18.00	44.40	1.00	0.00	0.00	0.00	1.40	0.00	160.00
87.00	34.00	1.00	12.00	12.40	1.00	0.00	--	0.00	2.30	0.00	12.00
88.00	81.00	0.00	17.00	23.60	1.00	1,000.00	1,004.00	1.00	9.30	1.00	10.00
89.00	22.00	1.00	10.00	9.50	1.00	0.00	--	0.00	0.20	0.00	1.00
90.00	53.00	0.00	32.00	60.90	1.00	0.00	2.00	0.00	3.00	0.00	36.00
91.00	62.00	0.00	22.00	58.90	1.00	0.00	--	0.00	1.70	0.00	14.00
94.00	32.00	1.00	25.00	53.30	0.00	0.00	--	0.00	17.30	0.00	24.00
96.00	63.00	1.00	18.00	4.80	0.00	0.00	0.00	0.00	1.90	0.00	15.00
99.00	37.00	0.00	13.00	16.50	1.00	0.00	--	0.00	2.10	0.00	11.00
100.00	50.00	1.00	26.00	38.20	0.00	0.00	12.00	0.00	2.60	0.00	119.00
101.00	35.00	1.00	9.00	4.70	1.00	0.00	--	0.00	0.80	0.00	6.00
102.00	26.00	0.00	23.00	45.70	1.00	0.00	0.00	0.00	2.30	0.00	77.00
103.00	52.00	0.00	16.00	12.20	1.00	0.00	0.00	0.00	2.00	0.00	48.00
104.00	38.00	0.00	3.00	2.70	0.00	0.00	--	0.00	0.30	0.00	5.00
105.00	39.00	0.00	34.00	87.50	0.00	1,000.00	1,001.00	0.00	3.00	0.00	58.00
107.00	45.00	1.00	12.00	6.60	1.00	0.00	--	0.00	3.10	0.00	48.00
108.00	44.00	1.00	6.00	0.90	0.00	0.00	--	0.00	0.80	0.00	5.00
110.00	48.00	1.00	26.00	35.20	0.00	0.00	8.00	1.00	4.90	1.00	6.00
111.00	23.00	0.00	9.00	4.30	1.00	0.00	7.00	0.00	9.20	0.00	48.00
112.00	45.00	1.00	24.00	22.40	1.00	0.00	0.00	1.00	10.70	1.00	12.00
113.00	70.00	0.00	27.00	47.70	0.00	0.00	2.00	1.00	6.50	1.00	7.00
114.00	65.00	1.00	24.00	65.70	1.00	0.00	3.00	0.00	32.80	1.00	61.00
115.00	47.00	0.00	10.00	1.50	0.00	0.00	--	0.00	0.60	0.00	14.00
116.00	61.00	0.00	13.00	10.60	0.00	0.00	--	0.00	2.40	0.00	37.00
117.00	48.00	0.00	13.00	8.50	1.00	0.00	2.00	0.00	4.20	0.00	18.00
118.00	59.00	0.00	23.00	39.90	1.00	0.00	1.00	0.00	12.10	0.00	24.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital death	Hospital stay (days)
119.00	67.00	1.00	18.00	14.40	0.00	0.00	--	0.00	1.30	0.00	9.00
121.00	86.00	0.00	22.00	42.20	1.00	0.00	3.00	0.00	6.60	0.00	20.00
123.00	60.00	0.00	16.00	14.60	0.00	1,000.00	1,004.00	0.00	3.10	0.00	23.00
124.00	33.00	0.00	14.00	27.40	1.00	1.00	4.00	0.00	4.70	0.00	10.00
126.00	68.00	0.00	27.00	46.00	0.00	1,000.00	--	0.00	1.60	0.00	240.00
127.00	100.00	0.00	25.00	64.60	1.00	0.00	--	0.00	3.50	0.00	60.00
128.00	80.00	1.00	30.00	56.90	1.00	0.00	--	0.00	2.90	0.00	144.00
129.00	31.00	0.00	19.00	44.00	0.00	0.00	--	0.00	2.90	0.00	54.00
131.00	65.00	0.00	21.00	55.30	1.00	0.00	--	0.00	6.70	1.00	24.00
133.00	35.00	0.00	14.00	7.20	1.00	0.00	--	0.00	0.90	0.00	9.00
135.00	41.00	1.00	16.00	14.30	1.00	0.00	--	0.00	0.60	0.00	17.00
137.00	69.00	1.00	19.00	32.00	1.00	0.00	--	0.00	0.80	0.00	7.00
139.00	54.00	1.00	23.00	55.80	1.00	0.00	--	0.00	2.60	0.00	69.00
140.00	74.00	0.00	21.00	41.60	1.00	0.00	--	0.00	2.10	1.00	11.00
141.00	67.00	0.00	32.00	78.00	1.00	0.00	--	0.00	19.60	0.00	50.00
143.00	52.00	0.00	10.00	17.40	1.00	0.00	--	1.00	9.80	1.00	11.00
146.00	38.00	0.00	18.00	24.20	1.00	6.00	21.00	0.00	10.30	0.00	20.00
147.00	70.00	0.00	20.00	35.50	1.00	0.00	0.00	0.00	1.50	0.00	29.00
148.00	71.00	0.00	25.00	53.30	0.00	0.00	--	0.00	0.80	1.00	5.00
149.00	74.00	0.00	15.00	--	1.00	0.00	--	1.00	13.80	1.00	15.00
150.00	62.00	0.00	21.00	38.90	0.00	0.00	--	0.00	35.20	0.00	66.00
151.00	36.00	0.00	8.00	8.60	1.00	0.00	--	0.00	1.20	0.00	62.00
152.00	47.00	0.00	11.00	7.60	0.00	0.00	--	0.00	2.00	0.00	10.00
153.00	77.00	1.00	27.00	46.00	0.00	8.00	--	0.00	3.10	0.00	14.00
154.00	49.00	0.00	26.00	4.40	0.00	0.00	--	0.00	0.90	0.00	8.00
155.00	43.00	0.00	25.00	3.80	0.00	0.00	--	0.00	0.80	0.00	7.00
156.00	41.00	0.00	33.00	78.60	0.00	0.00	--	1.00	1.80	1.00	3.00
157.00	61.00	0.00	31.00	81.40	1.00	0.00	--	0.00	11.40	0.00	53.00
158.00	56.00	0.00	11.00	22.30	1.00	1.00	--	0.00	9.00	0.00	74.00
159.00	60.00	1.00	19.00	11.30	1.00	0.00	--	0.00	1.50	0.00	22.00
163.00	77.00	1.00	26.00	56.70	1.00	0.00	--	0.00	1.40	0.00	48.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted Mortality (%)	Medical/Surgical (0/1)	Pro-ICU Pabrinex (doses)	Pro + ICU Pabrinex (doses)	ICU Death	ICU stay (days)	Hospital death	Hospital stay (days)
164.00	62.00	1.00	14.00	9.70	0.00	0.00	--	0.00	7.00	0.00	16.00
165.00	35.00	0.00	5.00	0.20	0.00	0.00	--	0.00	1.00	0.00	6.00
166.00	63.00	0.00	13.00	13.40	1.00	0.00	--	0.00	0.60	0.00	13.00
167.00	56.00	0.00	32.00	76.00	0.00	1.00	--	0.00	2.10	1.00	4.00
168.00	52.00	1.00	26.00	56.90	0.00	3.00	--	0.00	34.10	0.00	57.00
169.00	39.00	1.00	13.00	24.60	1.00	66.00	--	0.00	24.60	0.00	55.00
171.00	67.00	0.00	27.00	60.50	0.00	0.00	--	0.00	2.30	0.00	10.00

Patient no.	Ventilation (days)	SOFA score admission	SOFA score follow up	Follow up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow up (mg/l)	Albumin admission (g/l)	Albumin follow up (g/l)	Whole blood TDP admission (ng/g Hb)	Whole blood TDP follow up (ng/g Hb)	Plasma FAD admission (nmol/l)
12.00	6.00	7.00	7.00	2.00	126.00	156.00	24.00	23.00	928.00	886.00	77.20
13.00	49.00	5.00	9.00	3.00	22.00	261.00	27.00	22.00	789.00	1,450.00	111.70
14.00	14.00	8.00	9.00	2.00	220.00	124.00	12.00	10.00	1,173.00	1,050.00	81.20
15.00	5.00	8.00	2.00	5.00	257.00	73.00	23.00	16.00	1,435.00	796.00	375.90
16.00	1.00	6.00	--	--	122.00	--	28.00	--	994.00	--	75.00
17.00	6.00	2.00	0.00	4.00	3.00	55.00	33.00	26.00	693.00	593.00	289.60
18.00	60.00	10.00	10.00	4.00	152.00	64.00	10.00	10.00	402.00	374.00	29.80
19.00	47.00	10.00	9.00	4.00	143.00	101.00	12.00	14.00	1,094.00	2,445.00	133.40
21.00	2.00	8.00	--	--	23.00	--	10.00	--	617.00	--	195.90
22.00	4.00	2.00	3.00	4.00	20.00	67.00	27.00	15.00	2,341.00	2,112.00	58.70
23.00	22.00	8.00	4.00	7.00	125.00	216.00	10.00	13.00	978.00	1,036.00	87.00
24.00	3.00	4.00	--	--	81.00	--	35.00	--	1,317.00	--	70.30
27.00	21.60	5.00	4.00	3.00	103.00	133.00	23.00	17.00	705.00	1,530.00	170.10
29.00	4.00	4.00	4.00	4.00	59.00	20.00	9.00	10.00	1,652.00	1,906.00	22.80
30.00	13.00	11.00	7.00	3.00	26.00	179.00	16.00	11.00	891.00	5,491.00	826.50
31.00	1.00	11.00	--	--	50.00	--	22.00	--	744.00	--	42.40
32.00	0.00	6.00	--	--	434.00	--	17.00	--	778.00	--	58.80
33.00	13.00	7.00	8.00	5.00	259.00	173.00	17.00	10.00	510.00	583.00	41.30
34.00	1.00	6.00	7.00	4.00	404.00	336.00	30.00	15.00	3,650.00	3,498.00	658.40
35.00	44.00	10.00	7.00	3.00	119.00	303.00	9.00	13.00	483.00	752.00	36.70
36.00	24.00	13.00	10.00	4.00	132.00	70.00	14.00	14.00	2,672.00	3,000.00	1,915.70
37.00	16.00	10.00	9.00	4.00	81.00	35.00	12.00	12.00	1,270.00	915.00	321.50
38.00	2.00	4.00	--	--	127.00	--	14.00	--	626.00	--	41.90
40.00	3.00	9.00	4.00	3.00	15.00	234.00	20.00	18.00	330.00	1,043.00	67.40
41.00	2.00	8.00	--	--	184.00	--	19.00	--	768.00	--	37.60
42.00	3.00	4.00	6.00	4.00	8.00	149.00	17.00	18.00	574.00	1,241.00	29.90
44.00	16.10	7.00	3.00	8.00	151.00	55.00	10.00	16.00	506.00	892.00	546.20

Patient no.	Ventilation (days)	SOFA score admission	SOFA score follow up	Follow up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow up (mg/l)	Albumin admission (g/l)	Albumin follow up (g/l)	Whole blood TDP admission (ng/g Hb)	Whole blood TDP follow up (ng/g Hb)	Plasma FAD admission (nmol/l)
45.00	16.00	11.00	5.00	5.00	258.00	164.00	14.00	14.00	1,379.00	860.00	37.00
46.00	1.00	1.00	--	--	250.00	--	11.00	--	804.00	--	31.10
47.00	33.00	7.00	7.00	2.00	64.00	72.00	24.00	24.00	1,002.00	1,131.00	1,179.40
48.00	15.00	6.00	11.00	4.00	119.00	123.00	10.00	9.00	2,127.00	3,085.00	529.60
50.00	5.00	7.00	4.00	6.00	180.00	141.00	23.00	15.00	828.00	860.00	52.40
51.00	5.00	14.00	16.00	3.00	56.00	58.00	14.00	19.00	1,406.00	1,664.00	53.70
52.00	8.00	11.00	11.00	3.00	565.00	257.00	9.00	9.00	3,524.00	4,591.00	67.20
53.00	12.00	11.00	10.00	3.00	148.00	131.00	9.00	9.00	196.00	207.00	61.80
55.00	9.00	8.00	8.00	4.00	107.00	71.00	13.00	9.00	676.00	3,011.00	30.40
56.00	1.00	6.00	--	--	37.00	--	16.00	--	518.00	--	28.60
57.00	3.00	10.00	--	--	181.00	--	10.00	--	560.00	--	10.40
58.00	19.00	4.00	12.00	2.00	6.00	36.00	19.00	12.00	506.00	502.00	35.60
59.00	2.00	5.00	--	--	32.00	--	36.00	--	1,130.00	--	69.30
60.00	5.00	3.00	--	--	166.00	--	26.00	--	923.00	--	52.70
61.00	0.00	5.00	--	--	380.00	--	26.00	--	1,037.00	--	58.70
62.00	2.00	1.00	2.00	3.00	88.00	180.00	12.00	14.00	800.00	598.00	28.70
63.00	2.00	2.00	--	--	117.00	--	14.00	--	2,092.00	--	18.80
64.00	21.00	13.00	--	--	311.00	--	18.00	--	556.00	--	31.00
65.00	18.00	9.00	--	--	163.00	--	23.00	--	1,226.00	--	41.20
67.00	5.00	4.00	4.00	5.00	438.00	52.00	20.00	16.00	763.00	1,456.00	24.10
68.00	11.00	14.00	12.00	5.00	67.00	139.00	11.00	11.00	459.00	1,515.00	19.60
69.00	75.00	10.00	13.00	4.00	179.00	356.00	14.00	16.00	544.00	2,446.00	10.30
71.00	1.00	3.00	--	--	2.00	--	38.00	--	792.00	--	42.20
72.00	15.00	11.00	7.00	12.00	40.00	89.00	32.00	15.00	1,677.00	2,300.00	78.20
73.00	7.00	3.00	--	--	2.00	--	31.00	--	--	--	38.20
74.00	18.00	6.00	11.00	4.00	77.00	28.00	15.00	13.00	631.00	2,192.00	--
75.00	1.00	10.00	--	--	184.00	--	11.00	--	727.00	--	28.70
77.00	2.00	7.00	--	--	2.00	--	20.00	--	667.00	--	33.20
78.00	2.00	4.00	--	--	177.00	--	27.00	--	598.00	--	53.00
79.00	8.00	4.00	3.00	3.00	2.00	251.00	24.00	21.00	500.00	689.00	77.60
80.00	2.00	9.00	--	--	126.00	--	14.00	--	2,300.00	--	54.50

Patient no.	Ventilation (days)	SOFA score admission	SOFA score follow up	Follow up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow up (mg/l)	Albumin admission (g/l)	Albumin follow up (g/l)	Whole blood TDP admission (ng/g Hb)	Whole blood TDP follow up (ng/g Hb)	Plasma FAD admission (nmol/l)
81.00	9.00	5.00	6.00	3.00	8.00	284.00	10.00	21.00	617.00	1,973.00	43.60
82.00	8.00	1.00	1.00	2.00	4.00	86.00	29.00	29.00	739.00	1,961.00	19.20
83.00	0.00	4.00	--	--	318.00	--	21.00	--	511.00	--	42.20
84.00	14.00	13.00	10.00	3.00	63.00	80.00	9.00	9.00	1,652.00	2,661.00	27.40
85.00	2.00	6.00	7.00	2.00	176.00	198.00	11.00	9.00	350.00	359.00	25.30
87.00	2.00	6.00	--	--	110.00	--	18.00	--	700.00	--	39.30
88.00	10.00	9.00	9.00	5.00	108.00	104.00	12.00	11.00	1,176.00	2,306.00	29.50
89.00	1.00	2.00	--	--	31.00	--	16.00	--	579.00	--	41.90
90.00	3.00	10.00	7.00	4.00	159.00	38.00	11.00	29.00	724.00	2,220.00	20.90
91.00	1.00	9.00	--	--	268.00	--	14.00	--	687.00	--	--
94.00	17.00	18.00	--	--	147.00	--	14.00	--	810.00	--	61.40
96.00	2.00	3.00	2.00	3.00	76.00	27.00	29.00	28.00	894.00	691.00	46.40
99.00	2.00	5.00	--	--	287.00	--	22.00	--	1,983.00	--	33.40
100.00	0.00	4.00	4.00	3.00	253.00	171.00	13.00	9.00	2,287.00	2,459.00	20.30
101.00	0.00	5.00	--	--	231.00	--	25.00	--	385.00	--	24.30
102.00	2.00	4.00	2.00	2.00	28.00	198.00	19.00	19.00	415.00	508.00	26.20
103.00	2.00	2.00	1.00	3.00	--	69.00	42.00	27.00	910.00	1,538.00	53.10
104.00	1.00	2.00	--	--	1.00	--	34.00	--	545.00	--	73.40
105.00	3.00	11.00	8.00	2.00	25.00	23.00	30.00	29.00	1,271.00	2,232.00	25.50
107.00	0.00	8.00	--	--	261.00	--	17.00	--	827.00	--	45.30
108.00	0.00	3.00	--	--	48.00	--	35.00	--	653.00	--	44.90
110.00	6.00	7.00	12.00	5.00	199.00	153.00	13.00	11.00	918.00	3,164.00	18.20
111.00	7.00	7.00	2.00	5.00	6.00	245.00	28.00	13.00	664.00	1,220.00	42.70
112.00	12.00	1.00	10.00	4.00	2.00	165.00	20.00	9.00	923.00	459.00	64.70
113.00	7.00	4.00	5.00	3.00	14.00	205.00	31.00	19.00	576.00	1,123.00	51.40
114.00	34.00	9.00	8.00	4.00	49.00	98.00	16.00	9.00	288.00	2,218.00	30.40
115.00	0.00	2.00	--	--	1.00	--	39.00	--	610.00	--	67.80
116.00	3.00	1.00	--	--	3.00	--	24.00	--	391.00	--	34.90
117.00	4.00	3.00	3.00	4.00	9.00	88.00	32.00	23.00	713.00	804.00	64.40
118.00	12.00	9.00	12.00	2.00	252.00	204.00	12.00	9.00	1,367.00	333.00	16.60
119.00	1.00	2.00	--	--	2.00	--	25.00	--	635.00	--	68.20

Patient no.	Ventilation (days)	SOFA score admission	SOFA score follow up	Follow up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow up (mg/l)	Albumin admission (g/l)	Albumin follow up (g/l)	Whole blood TDP admission (ng/g Hb)	Whole blood TDP follow up (ng/g Hb)	Plasma FAD admission (nmol/l)
121.00	6.00	7.00	4.00	5.00	203.00	88.00	15.00	15.00	482.00	1,776.00	13.70
123.00	2.00	7.00	6.00	3.00	2.00	207.00	45.00	28.00	1,729.00	1,627.00	85.80
124.00	4.00	7.00	6.00	2.00	97.00	149.00	15.00	14.00	1,884.00	2,241.00	18.00
126.00	1.00	5.00	--	--	3.00	--	21.00	--	1,248.00	--	158.60
127.00	0.00	12.00	--	--	316.00	--	17.00	--	494.00	--	17.40
128.00	2.00	7.00	--	--	51.00	--	16.00	--	685.00	--	34.60
129.00	3.00	12.00	--	--	63.00	--	20.00	--	1,779.00	--	37.80
131.00	6.00	8.00	--	--	165.00	--	21.00	--	531.00	--	19.90
133.00	2.00	0.00	--	--	35.00	--	16.00	--	1,627.00	--	47.70
135.00	2.00	2.00	--	--	221.00	--	18.00	--	1,302.00	--	40.50
137.00	1.00	4.00	--	--	218.00	--	11.00	--	610.00	--	43.70
139.00	2.00	8.00	--	--	72.00	--	14.00	--	773.00	--	48.30
140.00	0.00	8.00	--	--	51.00	--	9.00	--	502.00	--	99.80
141.00	20.00	10.00	2.00	11.00	143.00	80.00	9.00	9.00	610.00	979.00	40.10
143.00	45.00	2.00	--	--	39.00	--	14.00	--	1,140.00	--	61.50
146.00	10.00	6.00	5.00	6.00	73.00	22.00	26.00	18.00	2,413.00	2,604.00	58.00
147.00	0.00	7.00	3.00	2.00	203.00	216.00	32.00	24.00	545.00	598.00	57.60
148.00	2.00	5.00	--	--	17.00	--	23.00	--	423.00	--	78.40
149.00	11.00	5.00	--	--	94.00	--	13.00	--	364.00	--	26.10
150.00	26.00	11.00	--	--	295.00	--	24.00	--	653.00	--	41.80
151.00	2.00	0.00	--	--	229.00	--	13.00	--	257.00	--	41.80
152.00	2.00	3.00	--	--	26.00	--	35.00	--	595.00	--	100.40
153.00	0.00	4.00	--	--	45.00	--	19.00	--	608.00	--	52.80
154.00	2.00	4.00	--	--	39.00	--	33.00	--	2,552.00	--	84.50
155.00	2.00	8.00	--	--	52.00	--	19.00	--	1,788.00	--	45.30
156.00	3.00	12.00	--	--	316.00	--	20.00	--	300.00	--	48.00
157.00	9.00	14.00	--	--	294.00	--	13.00	--	1,005.00	--	21.40
158.00	2.00	3.00	--	--	163.00	--	10.00	--	1,656.00	--	45.80
159.00	2.00	4.00	--	--	137.00	--	15.00	--	992.00	--	43.00
163.00	1.00	10.00	--	--	269.00	--	12.00	--	520.00	--	34.60
164.00	5.00	10.00	--	--	291.00	--	24.00	--	854.00	--	45.80

Patient no.	Ventilation (days)	SOFA score admission	SOFA score follow up	Follow up sample (days)	C-reactive protein admission (mg/l)	C-reactive protein follow up (mg/l)	Albumin admission (g/l)	Albumin follow up (g/l)	Whole blood TDP admission (ng/g Hb)	Whole blood TDP follow up (ng/g Hb)	Plasma FAD admission (nmol/l)
165.00	2.00	1.00	--	--	0.60	--	47.00	--	853.00	--	87.60
166.00	2.00	8.00	--	--	75.00	--	13.00	--	598.00	--	66.90
167.00	3.00	8.00	--	--	74.00	--	23.00	--	1,926.00	--	58.90
168.00	35.00	10.00	--	--	131.00	--	11.00	--	2,061.00	--	39.40
169.00	25.00	14.00	--	--	273.00	--	14.00	--	2,574.00	--	75.80
171.00	0.00	14.00	--	--	203.00	--	20.00	--	808.00	--	49.50

Patient no.	Plasma FAD follow up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow up (nmol/l)	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow up (nmol/l)	Red cell FAD admission (nmol/g Hb)	Red cell FAD follow up (nmol/g Hb)	Red cell FMN admission (nmol/g Hb)	Red cell FMN follow up (nmol/g Hb)	Red cell riboflavin admission (nmol/ g Hb)	Red cell riboflavin follow up (nmol/ g Hb)
12.00	79.90	--	--	32.70	39.50	1.76	1.70	0.14	0.14	0.05	0.05
13.00	63.50	--	--	42.70	103.90	1.53	1.54	0.16	0.14	0.06	0.05
14.00	56.80	--	--	170.30	300.00	1.82	1.91	0.14	0.12	0.09	0.08
15.00	145.60	--	--	--	--	1.46	1.42	--	--	0.03	--
16.00	--	--	--	77.20	--	1.05	--	0.08	--	0.03	--
17.00	73.70	--	--	--	--	1.33	1.24	0.08	0.08	0.04	0.02
18.00	62.20	--	--	7.80	3.00	1.13	1.08	0.07	0.06	0.01	0.01
19.00	1,510.30	--	--	--	--	1.69	1.90	--	--	0.04	0.12
21.00	--	--	--	53.00	--	1.42	--	0.09	--	0.03	--
22.00	55.00	18.50	4.70	279.00	172.10	2.09	2.00	0.22	0.21	0.22	0.14
23.00	157.00	--	--	--	--	1.63	1.67	0.09	0.09	0.02	0.03
24.00	--	--	--	62.70	--	2.29	--	--	--	0.05	--
27.00	307.10	--	--	--	--	1.63	1.88	--	--	0.10	0.10
29.00	33.20	--	--	198.70	66.20	1.57	1.86	0.11	0.13	0.07	0.06
30.00	1,697.50	--	--	--	--	1.97	2.02	--	--	0.14	0.27
31.00	--	--	--	13.50	--	1.30	--	0.08	--	0.03	--
32.00	--	--	--	18.20	--	1.38	--	0.09	--	0.03	--
33.00	25.30	--	--	--	--	1.20	1.26	0.08	0.08	0.01	0.01
34.00	908.30	--	--	--	--	1.86	2.04	0.13	0.18	0.19	0.10
35.00	42.50	--	--	--	--	1.35	1.68	0.09	0.10	0.01	0.01
36.00	911.30	--	--	--	--	2.17	2.03	0.14	0.13	0.17	0.06
37.00	414.40	--	--	--	--	1.58	1.74	0.10	0.11	0.07	0.08
38.00	--	--	--	45.60	--	1.70	--	0.11	--	0.06	--
40.00	51.70	--	--	--	--	1.41	1.33	0.09	0.09	0.01	0.01
41.00	--	--	--	--	--	1.20	--	0.06	--	0.04	--
42.00	28.90	--	--	43.00	40.10	1.29	--	0.08	--	0.02	--
44.00	346.50	--	--	--	--	2.04	2.15	0.17	0.17	0.07	0.06

Patient no.	Plasma FAD follow up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow up (nmol/l)	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow up (nmol/l)	Red cell FAD admission (nmol/g Hb)	Red cell FAD follow up (nmol/g Hb)	Red cell FMN admission (nmol/g Hb)	Red cell FMN follow up (nmol/g Hb)	Red cell riboflavin admission (nmol/ g Hb)	Red cell riboflavin follow up (nmol/ g Hb)
45.00	881.50	--	--	--	--	2.21	2.19	0.14	0.15	0.02	0.06
46.00	--	--	--	31.90	--	1.21	--	0.08	--	0.01	--
47.00	1,224.70	--	--	--	--	1.85	2.15	0.15	0.16	0.09	0.17
48.00	5,478.50	--	--	--	--	2.14	2.55	0.16	0.18	0.11	0.62
50.00	58.20	34.20	6.90	44.30	29.10	1.49	1.46	0.10	0.10	0.04	0.02
51.00	89.60	11.50	7.10	106.50	330.60	1.80	1.89	0.16	0.15	0.11	0.17
52.00	100.40	--	--	748.10	910.80	2.30	2.30	0.11	0.10	0.17	0.24
53.00	56.30	--	--	--	--	1.04	0.96	0.05	0.05	0.01	0.00
55.00	43.80	--	3.60	4.10	237.70	1.22	2.00	0.08	0.14	--	0.12
56.00	--	4.90	--	16.60	--	1.45	--	0.07	--	0.14	--
57.00	--	--	--	52.50	--	1.33	--	0.13	--	0.04	--
58.00	22.70	7.70	5.20	15.40	12.40	1.16	1.14	0.07	0.07	0.02	0.02
59.00	--	--	--	53.70	--	1.22	--	0.07	--	0.01	--
60.00	--	--	--	11.80	--	1.17	--	0.05	--	0.00	--
61.00	--	--	--	38.00	--	1.21	--	0.06	--	0.01	--
62.00	32.80	5.40	4.30	6.60	9.60	1.20	1.09	0.06	0.05	0.01	0.01
63.00	--	--	--	48.00	--	1.80	--	0.26	--	0.09	--
64.00	--	--	--	10.00	--	1.06	--	0.06	--	0.01	--
65.00	--	--	--	57.30	--	1.91	--	0.36	--	0.03	--
67.00	26.70	--	--	34.00	39.80	1.72	1.68	0.19	0.19	0.08	0.07
68.00	38.90	--	8.20	5.90	577.10	1.54	2.30	0.11	0.17	0.02	0.20
69.00	36.00	--	10.70	571.90	616.80	1.34	2.26	0.12	0.22	0.03	0.25
71.00	--	--	--	8.60	--	0.97	--	0.05	--	0.01	--
72.00	86.30	6.10	12.10	55.70	559.90	1.78	2.40	0.09	0.13	0.03	0.21
73.00	--	--	--	40.70	--	1.38	--	0.14	--	0.05	--
74.00	115.40	--	6.80	--	1,166.10	1.44	1.96	0.13	0.17	0.04	0.28
75.00	--	--	--	53.90	--	2.10	--	0.48	--	0.09	--
77.00	--	--	--	24.80	--	1.81	--	0.13	--	0.03	--
78.00	--	--	--	139.00	--	1.93	--	0.17	--	0.06	--
79.00	51.10	12.40	6.90	18.40	17.40	1.24	1.21	0.07	0.07	0.01	0.01

Patient no.	Plasma FAD follow up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow up (nmol/l)	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow up (nmol/l)	Red cell FAD admission (nmol/g Hb)	Red cell FAD follow up (nmol/g Hb)	Red cell FMN admission (nmol/g Hb)	Red cell FMN follow up (nmol/g Hb)	Red cell riboflavin admission (nmol/ g Hb)	Red cell riboflavin follow up (nmol/ g Hb)
80.00	--	--	--	140.80	--	2.23	--	0.28	--	0.14	--
81.00	68.70	--	--	7.10	33.00	1.56	1.66	0.09	0.09	0.01	0.03
82.00	57.30	2.80	4.60	3.00	14.50	1.31	1.68	0.08	0.11	0.01	0.02
83.00	--	--	--	7.80	--	1.49	--	0.08	--	0.01	--
84.00	26.70	21.90	19.30	62.00	356.00	1.75	2.51	0.14	0.20	0.04	0.17
85.00	34.80	3.60	4.10	6.70	8.00	1.30	1.33	0.09	0.10	0.05	0.03
87.00	--	24.40	--	65.60	--	1.62	--	0.12	--	0.03	--
88.00	43.90	--	--	43.30	401.00	1.35	2.21	0.07	0.14	0.03	0.15
89.00	--	--	--	6.80	--	1.17	--	0.08	--	0.01	--
90.00	39.70	--	--	11.70	74.30	2.06	1.88	0.11	0.11	0.03	0.06
91.00	--	--	--	--	--	1.25	--	0.08	--	0.04	--
94.00	--	--	--	14.30	--	1.87	--	0.14	--	0.02	--
96.00	55.00	--	--	20.20	11.20	2.12	2.00	0.15	0.14	0.06	0.06
99.00	--	--	--	43.40	--	1.48	--	0.09	--	0.01	--
100.00	12.80	--	--	337.50	172.10	2.56	2.50	0.33	0.25	0.21	0.12
101.00	--	--	--	11.90	--	--	--	--	--	--	--
102.00	26.20	28.10	23.10	27.00	36.80	1.31	1.27	0.08	0.07	0.02	0.02
103.00	35.00	--	--	43.10	26.30	1.59	1.59	0.16	0.16	0.07	0.07
104.00	--	28.40	--	33.80	--	1.31	--	0.06	--	0.02	--
105.00	19.10	29.30	--	133.80	114.20	1.80	1.70	0.19	0.19	0.19	0.13
107.00	--	--	--	29.60	--	1.67	--	0.17	--	0.06	--
108.00	--	28.70	--	11.80	--	1.23	--	0.05	--	0.01	--
110.00	71.80	--	16.70	--	516.30	1.13	2.63	0.06	0.17	0.01	0.16
111.00	25.50	--	--	24.30	20.90	1.37	1.80	0.06	0.11	0.01	0.02
112.00	18.70	19.40	--	28.60	54.00	1.15	1.33	0.12	0.14	0.03	0.05
113.00	24.10	30.70	--	68.60	62.20	1.31	1.37	0.06	0.06	0.01	0.01
114.00	12.40	--	34.10	--	66.20	1.14	1.40	0.06	0.08	0.01	0.02
115.00	--	17.30	--	7.30	--	1.02	--	0.05	--	0.01	--
116.00	--	6.80	--	13.60	--	1.29	--	--	--	0.01	--
117.00	36.00	19.60	--	39.90	44.00	1.53	1.58	--	--	0.02	0.03

Patient no.	Plasma FAD follow up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow up (nmol/l)	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow up (nmol/l)	Red cell FAD admission (nmol/g Hb)	Red cell FAD follow up (nmol/g Hb)	Red cell FMN admission (nmol/g Hb)	Red cell FMN follow up (nmol/g Hb)	Red cell riboflavin admission (nmol/ g Hb)	Red cell riboflavin follow up (nmol/ g Hb)
118.00	22.10	--	--	193.10	29.00	1.85	1.48	--	--	0.04	0.02
119.00	--	16.60	--	24.00	--	1.33	--	0.08	--	0.02	--
121.00	30.50	--	--	14.00	26.20	1.54	1.79	0.14	0.16	0.03	0.05
123.00	50.50	7.70	--	115.00	69.40	1.77	1.88	--	--	0.05	0.06
124.00	39.40	11.60	--	55.80	97.90	1.56	1.78	--	--	0.02	0.01
126.00	--	75.60	--	458.00	--	1.23	--	--	--	0.04	--
127.00	--	--	--	374.20	--	2.58	--	--	--	0.23	--
128.00	--	--	--	14.30	--	1.75	--	0.09	--	0.03	--
129.00	--	--	--	188.00	--	1.88	--	--	--	0.04	--
131.00	--	--	--	69.70	--	1.68	--	--	--	0.04	--
133.00	--	7.20	--	40.20	--	1.55	--	--	--	0.02	--
135.00	--	--	--	81.50	--	1.91	--	--	--	0.03	--
137.00	--	--	--	4.50	--	1.84	--	0.10	--	0.12	--
139.00	--	--	--	48.60	--	2.02	--	0.11	--	0.03	--
140.00	--	--	--	21.90	--	2.13	--	0.13	--	0.09	--
141.00	56.20	--	--	9.20	199.10	1.53	2.11	0.08	0.14	0.02	0.08
143.00	--	--	--	34.60	--	1.84	--	0.12	--	0.08	--
146.00	85.50	--	--	196.30	288.50	2.13	2.22	0.13	0.13	0.07	0.07
147.00	59.90	--	--	6.50	7.50	1.23	1.17	0.06	0.06	0.01	0.02
148.00	--	--	--	26.20	--	1.54	--	0.09	--	0.01	--
149.00	--	--	--	27.60	--	1.36	--	0.08	--	0.04	--
150.00	--	--	--	5.50	--	1.42	--	0.07	--	0.01	--
151.00	--	--	--	5.50	--	1.24	--	0.06	--	0.01	--
152.00	--	--	--	18.60	--	1.29	--	0.07	--	0.03	--
153.00	--	--	--	23.00	--	1.37	--	0.07	--	0.00	--
154.00	--	--	--	90.50	--	1.82	--	0.15	--	0.11	--
155.00	--	--	--	26.50	--	1.44	--	0.09	--	0.03	--
156.00	--	--	--	7.80	--	1.22	--	0.06	--	0.01	--
157.00	--	--	--	228.60	--	1.37	--	0.08	--	0.02	--
158.00	--	--	--	62.20	--	1.58	--	0.12	--	0.09	--

Patient no.	Plasma FAD follow up (nmol/l)	Plasma FMN admission (nmol/l)	Plasma FMN follow up (nmol/l)	Plasma riboflavin admission (nmol/l)	Plasma riboflavin follow up (nmol/l)	Red cell FAD admission (nmol/g Hb)	Red cell FAD follow up (nmol/g Hb)	Red cell FMN admission (nmol/g Hb)	Red cell FMN follow up (nmol/g Hb)	Red cell riboflavin admission (nmol/ g Hb)	Red cell riboflavin follow up (nmol/ g Hb)
159.00	--	--	--	21.10	--	1.43	--	0.07	--	0.01	--
163.00	--	--	--	9.40	--	1.15	--	0.05	--	0.00	--
164.00	--	--	--	10.60	--	1.47	--	0.08	--	0.01	--
165.00	--	--	--	19.80	--	1.27	--	0.06	--	0.01	--
166.00	--	--	--	29.20	--	1.47	--	0.09	--	0.05	--
167.00	--	--	--	7.30	--	1.39	--	0.09	--	0.02	--
168.00	--	--	--	307.40	--	1.82	--	0.11	--	0.05	--
169.00	--	--	--	85.60	--	1.77	--	0.14	--	0.07	--
171.00	--	--	--	20.30	--	1.77	--	0.13	--	0.06	--

Patient no.	White cell FAD admission (pmol/ 10 (6) cells)	White cell FAD follow up (pmol/ 10 (6) cells)	White cell FMN admission (pmol/ 10 (6) cells)	White cell FMN follow up (pmol/ 10 (6) cells)	White cell riboflavin admission (pmol/ 10 (6) cells)	White cell riboflavin follow up (pmol/ 10 (6) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow up (nmol/l)
12.00	11.77	10.93	0.55	0.52	0.41	0.43	16.90	12.10
13.00	11.59	13.64	0.53	0.67	0.33	0.55	49.20	17.60
14.00	11.54	11.18	0.43	0.41	0.62	0.64	5.10	3.10
15.00	13.99	11.58	--	--	0.68	0.52	48.20	37.40
16.00	11.53	--	0.53	--	0.35	--	67.80	--
17.00	12.30	12.78	0.81	0.94	0.34	0.46	98.00	30.00
18.00	12.77	10.47	0.49	0.38	0.59	0.39	8.50	6.60
19.00	13.00	14.92	--	--	0.54	0.89	13.10	59.10
21.00	13.48	--	0.94	--	0.54	--	332.60	--
22.00	12.59	--	0.64	--	0.69	--	197.50	71.50
23.00	13.28	12.20	0.91	0.69	0.69	0.59	15.80	9.30
24.00	13.59	--	--	--	0.64	--	39.40	--
27.00	15.98	13.36	--	--	0.97	0.86	21.70	30.30
29.00	13.25	12.27	0.99	0.78	0.66	0.52	26.70	19.80
30.00	12.81	13.76	--	--	0.84	1.00	12.60	15.10
31.00	13.50	--	0.93	--	0.59	--	17.20	--
32.00	9.92	--	0.71	--	0.47	--	3.80	--
33.00	15.23	16.25	0.62	0.60	0.55	0.53	6.80	2.30
34.00	15.76	15.82	0.59	0.56	0.83	0.86	69.10	19.70
35.00	16.89	17.11	0.55	0.79	0.59	0.79	6.90	11.60
36.00	17.29	13.87	0.82	0.64	1.28	0.84	33.10	7.60
37.00	12.97	12.86	0.82	0.76	0.54	0.58	141.40	27.00
38.00	--	--	--	--	--	--	6.00	--
40.00	12.88	14.80	0.84	0.83	0.42	0.68	13.90	30.30
41.00	14.46	--	0.80	--	0.51	--	9.00	--
42.00	12.23	--	0.39	--	0.42	--	21.40	45.90
44.00	11.22	15.13	0.52	0.56	0.82	0.81	37.10	26.20

Patient no.	White cell FAD admission (pmol/ 10 (6) cells)	White cell FAD follow up (pmol/ 10 (6) cells)	White cell FMN admission (pmol/ 10 (6) cells)	White cell FMN follow up (pmol/ 10 (6) cells)	White cell riboflavin admission (pmol/ 10 (6) cells)	White cell riboflavin follow up (pmol/ 10 (6) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow up (nmol/l)
45.00	12.41	12.33	0.68	0.59	0.52	0.68	154.40	7.20
46.00	9.52	--	0.56	--	0.60	--	4.80	--
47.00	13.08	13.17	0.52	0.58	0.81	0.78	295.70	357.20
48.00	--	12.45	--	0.69	--	0.90	9.40	16.90
50.00	13.82	13.30	0.63	0.70	0.83	0.66	7.90	5.00
51.00	16.31	15.85	0.74	0.90	0.93	1.22	28.80	45.50
52.00	14.41	13.55	0.71	0.67	1.32	1.13	38.10	70.10
53.00	13.19	13.42	0.74	0.79	0.48	0.54	1.70	1.50
55.00	--	12.09	--	0.53	--	0.59	1.20	22.00
56.00	12.73	--	0.40	--	0.61	--	4.00	--
57.00	9.24	--	0.46	--	0.47	--	10.30	--
58.00	--	--	--	--	--	--	37.40	28.10
59.00	8.32	--	0.53	--	0.33	--	151.80	--
60.00	13.01	--	0.76	--	0.42	--	13.20	--
61.00	--	--	--	--	--	--	10.90	--
62.00	15.78	11.46	0.78	0.55	0.47	0.38	6.40	6.00
63.00	12.94	--	0.84	--	0.78	--	15.90	--
64.00	11.29	--	0.86	--	0.75	--	34.40	--
65.00	12.65	--	0.65	--	0.89	--	63.10	--
67.00	14.52	--	0.77	--	1.01	--	2.60	9.30
68.00	11.43	--	0.52	--	3.27	--	9.20	13.10
69.00	18.25	--	1.51	--	0.67	--	17.60	121.70
71.00	13.37	--	0.76	--	0.48	--	89.80	--
72.00	14.37	14.22	0.53	0.71	0.61	0.91	169.60	20.60
73.00	--	--	--	--	--	--	27.30	--
74.00	12.38	13.51	0.65	0.67	0.56	0.93	45.60	42.50
75.00	14.16	--	0.93	--	1.04	--	20.70	--
77.00	11.23	--	1.02	--	0.31	--	43.80	--
78.00	12.69	--	0.92	--	0.66	--	30.60	--
79.00	15.04	15.05	0.81	0.76	0.62	0.67	65.20	26.70
80.00	15.30	--	1.03	--	1.10	--	21.10	--

Patient no.	White cell FAD admission (pmol/ 10 (6) cells)	White cell FAD follow up (pmol/ 10 (6) cells)	White cell FMN admission (pmol/ 10 (6) cells)	White cell FMN follow up (pmol/ 10 (6) cells)	White cell riboflavin admission (pmol/ 10 (6) cells)	White cell riboflavin follow up (pmol/ 10 (6) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow up (nmol/l)
81.00	12.45	14.36	0.37	0.44	0.33	0.74	43.30	174.80
82.00	15.78	16.22	0.75	0.88	0.53	0.61	40.80	84.40
83.00	13.31	--	0.98	--	0.49	--	8.60	--
84.00	13.59	11.15	0.37	0.28	0.77	0.89	22.00	27.00
85.00	15.81	13.64	0.72	0.90	0.71	0.69	10.40	10.20
87.00	13.10	--	1.10	--	0.73	--	9.60	--
88.00	8.21	11.95	0.37	0.30	0.28	0.69	17.90	24.30
89.00	20.45	--	1.73	--	1.02	--	6.80	--
90.00	--	--	--	--	--	--	2.20	45.20
91.00	--	--	--	--	--	--	14.10	--
94.00	--	--	--	--	--	--	23.00	--
96.00	--	--	--	--	--	--	9.00	9.00
99.00	--	--	--	--	--	--	111.00	--
100.00	16.74	29.25	0.43	0.55	1.36	2.07	49.00	44.00
101.00	--	--	--	--	--	--	9.00	--
102.00	--	--	--	--	--	--	33.00	54.00
103.00	14.01	11.20	0.60	0.37	0.33	0.45	34.00	84.00
104.00	--	--	--	--	--	--	28.00	--
105.00	12.74	13.24	0.50	0.61	0.61	0.56	50.00	101.00
107.00	14.19	--	0.46	--	0.77	--	9.00	--
108.00	12.17	--	0.40	--	0.42	--	9.00	--
110.00	11.30	11.57	0.48	0.51	0.36	0.67	9.00	63.00
111.00	12.89	13.63	0.38	0.51	0.45	0.60	126.00	93.00
112.00	12.53	--	0.41	--	0.62	--	16.00	9.00
113.00	--	--	--	--	--	--	25.00	24.00
114.00	7.89	10.52	0.27	0.34	0.23	0.55	19.00	9.00
115.00	11.85	--	0.39	--	0.37	--	9.00	--
116.00	90.15	--	--	--	--	--	31.00	--
117.00	11.62	8.34	--	--	--	--	60.00	155.00
118.00	8.75	5.85	--	--	--	--	20.00	10.00
119.00	--	--	--	--	--	--	18.00	--

Patient no.	White cell FAD admission (pmol/ 10 (6) cells)	White cell FAD follow up (pmol/ 10 (6) cells)	White cell FMN admission (pmol/ 10 (6) cells)	White cell FMN follow up (pmol/ 10 (6) cells)	White cell riboflavin admission (pmol/ 10 (6) cells)	White cell riboflavin follow up (pmol/ 10 (6) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow up (nmol/l)
121.00	--	--	--	--	--	--	12.00	28.00
123.00	6.58	8.47	--	--	--	--	306.00	74.00
124.00	8.40	8.25	--	--	--	--	38.00	37.00
126.00	--	--	--	--	--	--	127.00	--
127.00	--	--	--	--	--	--	9.00	--
128.00	11.66	--	0.40	--	0.53	--	9.00	--
129.00	--	--	--	--	--	--	22.00	--
131.00	--	--	--	--	--	--	15.00	--
133.00	--	--	--	--	--	--	155.00	--
135.00	8.46	--	--	--	--	--	9.00	--
137.00	--	--	--	--	--	--	15.00	--
139.00	--	--	--	--	--	--	21.00	--
140.00	--	--	--	--	--	--	27.00	--
141.00	--	--	--	--	--	--	11.00	9.00
143.00	--	--	--	--	--	--	9.00	--
146.00	--	--	--	--	--	--	131.00	33.00
147.00	--	--	--	--	--	--	29.00	32.00
148.00	--	--	--	--	--	--	30.00	--
149.00	--	--	--	--	--	--	13.00	--
150.00	--	--	--	--	--	--	13.00	--
151.00	--	--	--	--	--	--	10.00	--
152.00	--	--	--	--	--	--	15.00	--
153.00	--	--	--	--	--	--	16.00	--
154.00	--	--	--	--	--	--	251.00	--
155.00	--	--	--	--	--	--	29.00	--
156.00	--	--	--	--	--	--	21.00	--
157.00	--	--	--	--	--	--	132.00	--
158.00	--	--	--	--	--	--	25.00	--
159.00	--	--	--	--	--	--	35.00	--
163.00	--	--	--	--	--	--	22.00	--
164.00	--	--	--	--	--	--	7.00	--

Patient no.	White cell FAD admission (pmol/ 10 (6) cells)	White cell FAD follow up (pmol/ 10 (6) cells)	White cell FMN admission (pmol/ 10 (6) cells)	White cell FMN follow up (pmol/ 10 (6) cells)	White cell riboflavin admission (pmol/ 10 (6) cells)	White cell riboflavin follow up (pmol/ 10 (6) cells)	Plasma PLP admission (nmol/l)	Plasma PLP follow up (nmol/l)
165.00	--	--	--	--	--	--	62.00	--
166.00	--	--	--	--	--	--	12.00	--
167.00	--	--	--	--	--	--	45.00	--
168.00	--	--	--	--	--	--	32.00	--
169.00	--	--	--	--	--	--	39.00	--
171.00	--	--	--	--	--	--	22.00	--

Patient no.	Plasma PL admission (nmol/l)	Plasma PL follow up (nmol/l)	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red cell PL follow up (pmol/g Hb)
12.00	3.00	4.70	348.39	23.50	59.14	79.31
13.00	10.00	6.60	345.35	41.10	70.93	209.78
14.00	9.80	12.00	624.69	34.30	129.63	126.74
15.00	359.20	14.30	2,282.98	11.20	12,431.91	102.35
16.00	1,111.60	--	7,870.37	--	23,635.80	--
17.00	9.00	--	331.03	14.00	101.15	84.34
18.00	12.00	--	216.30	14.50	54.35	65.59
19.00	3.80	41.10	334.18	138.70	45.57	894.38
21.00	27.60	--	563.10	--	279.76	--
22.00	246.30	87.60	25,583.33	334.40	53,479.76	2,716.46
23.00	4.70	3.40	396.30	15.50	101.23	54.43
24.00	6.90	--	241.98	--	103.70	--
27.00	2.30	12.20	116.28	47.70	39.53	442.53
29.00	75.10	18.40	2,274.16	139.80	4,038.20	918.52
30.00	14.80	42.60	173.91	107.20	90.22	823.60
31.00	3.10	--	158.89	--	36.67	--
32.00	4.10	--	317.24	--	40.23	--
33.00	0.90	3.10	176.25	16.80	18.75	53.66
34.00	89.80	42.10	3,922.58	167.40	3,627.96	1,798.72
35.00	1.60	7.50	131.03	13.30	35.63	83.33
36.00	155.10	20.10	2,983.13	70.80	7,756.63	403.61
37.00	2.90	5.70	205.26	21.60	50.00	71.43
38.00	0.60	--	310.26	--	32.05	--
40.00	2.70	10.90	155.42	29.80	18.07	116.28
41.00	9.50	--	198.77	--	30.86	--
42.00	4.70	16.30	240.45	--	41.57	--

Patient no.	Plasma PL admission (nmol/l)	Plasma PL follow up (nmol/l)	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red cell PL follow up (pmol/g Hb)
44.00	5.50	10.40	720.99	38.00	97.53	94.12
45.00	2.50	6.00	325.93	17.00	105.56	--
46.00	2.40	--	211.63	--	26.74	--
47.00	18.30	30.30	316.88	36.50	64.94	54.93
48.00	19.20	752.70	1,013.92	1,496.10	241.77	28,867.65
50.00	0.50	2.10	313.92	21.30	--	--
51.00	285.50	634.50	11,113.89	2,636.20	13,498.61	25,896.15
52.00	109.70	259.20	7,519.74	216.40	3,788.16	1,432.43
53.00	1.30	2.00	168.67	12.00	28.92	23.81
55.00	0.90	109.90	191.80	411.70	--	5,027.14
56.00	1.00	--	260.98	--	23.17	--
57.00	8.90	--	208.33	--	15.48	--
58.00	6.50	3.90	241.05	19.80	58.95	49.06
59.00	1,106.40	--	8,840.00	--	22,254.44	--
60.00	5.30	--	138.71	--	21.51	--
61.00	6.10	--	181.25	--	27.50	--
62.00	8.10	6.10	176.74	6.80	--	--
63.00	7.10	--	321.35	--	51.69	--
64.00	2.20	--	139.74	--	24.36	--
65.00	12.60	--	642.50	--	166.25	--
67.00	1.30	6.60	111.39	4.70	17.72	15.22
68.00	0.90	250.60	213.98	497.30	26.88	8,679.57
69.00	2.40	134.50	265.88	181.60	24.71	1,624.39
71.00	3.60	--	103.57	103.57	14.29	--
72.00	864.70	1.20	13,716.88	27.00	12,640.26	76.19
73.00	9.00	7.00	150.00	--	57.14	--
74.00	9.00	114.60	254.55	262.90	--	--
75.00	0.70	--	153.85	--	6.41	--
77.00	5.00	--	263.46	--	44.23	--
78.00	1.70	--	279.07	--	44.19	--
79.00	1.80	4.10	157.14	10.70	25.51	12.24

Patient no.	Plasma PL admission (nmol/l)	Plasma PL follow up (nmol/l)	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red cell PL follow up (pmol/g Hb)
80.00	9.50	--	798.80	--	90.36	--
81.00	--	--	307.69	66.00	39.74	317.86
82.00	--	19.90	171.26	76.00	27.59	713.16
83.00	2.90	--	104.55	--	31.82	--
84.00	--	--	1,598.80	314.70	585.54	4,074.68
85.00	6.10	4.70	201.20	21.50	--	30.34
87.00	2.00	--	177.89	--	26.32	--
88.00	117.20	28.60	2,785.37	188.90	2,850.00	1,067.53
89.00	2.60	--	204.85	--	41.75	--
90.00	9.00	46.00	241.46	150.40	39.02	2,013.79
91.00	3.00	--	209.00	--	28.00	--
94.00	9.00	--	243.68	--	--	--
96.00	9.00	9.00	245.83	9.10	16.67	29.87
99.00	258.00	--	2,581.63	--	4,807.14	--
100.00	912.00	55.00	10,787.95	330.00	38,222.89	3,465.91
101.00	9.00	--	--	--	--	--
102.00	9.00	--	177.89	11.90	35.79	30.21
103.00	15.00	--	149.44	42.60	38.20	301.18
104.00	10.00	--	219.15	--	60.64	--
105.00	45.00	160.00	808.79	154.40	263.74	2,152.81
107.00	9.00	--	232.98	--	--	--
108.00	9.00	--	147.47	--	75.76	--
110.00	9.00	1,057.00	186.21	145.40	--	2,950.00
111.00	9.00	15.00	300.00	98.40	38.82	331.25
112.00	9.00	9.00	160.67	19.10	21.35	37.65
113.00	9.00	22.00	265.22	75.40	40.22	333.01
114.00	8.00	122.00	196.77	166.40	--	1,959.79
115.00	9.00	--	127.00	--	23.00	--
116.00	12.00	--	383.87	--	107.53	--
117.00	9.00	25.00	222.68	66.50	--	258.82
118.00	30.00	9.00	594.38	18.30	312.36	--

Patient no.	Plasma PL admission (nmol/l)	Plasma PL follow up (nmol/l)	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red cell PL follow up (pmol/g Hb)
119.00	9.00	--	127.55	--	21.43	--
121.00	9.00	--	209.30	83.40	29.07	440.23
123.00	519.00	--	11,912.94	409.70	6,764.71	1,747.92
124.00	11.00	19.00	846.15	94.80	--	--
126.00	1,346.00	--	21,893.41	--	35,813.19	--
127.00	9.00	--	342.68	--	--	--
128.00	9.00	--	285.23	--	23.86	--
129.00	744.00	--	23,853.33	--	48,515.56	--
131.00	9.00	--	348.31	--	--	--
133.00	84.00	--	1,989.00	--	2,460.00	--
135.00	9.00	--	454.88	--	--	--
137.00	4.00	--	244.59	--	66.22	--
139.00	--	--	305.41	--	39.19	--
140.00	23.00	--	195.71	--	17.14	--
141.00	19.00	19.00	31.71	16.20	20.73	74.29
143.00	2.00	--	380.28	--	46.48	--
146.00	78.00	21.00	5,550.67	186.90	3,320.00	452.94
147.00	4.00	3.00	310.81	20.40	50.00	46.84
148.00	3.00	--	502.67	--	77.33	--
149.00	1.00	--	179.73	--	55.41	--
150.00	9.00	--	167.53	--	36.36	--
151.00	9.00	--	234.21	--	51.32	--
152.00	4.00	--	166.25	--	47.50	--
153.00	2.00	--	193.06	--	51.39	--
154.00	332.00	--	27,441.10	--	9,686.30	--
155.00	4.00	--	405.13	--	58.97	--
156.00	9.00	--	289.33	--	12.00	--
157.00	308.00	--	21,889.74	--	9,160.26	--
158.00	151.00	--	4,984.62	--	6,673.08	--
159.00	4.00	--	266.23	--	44.16	--
163.00	18.00	--	158.02	--	48.15	--

Patient no.	Plasma PL admission (nmol/l)	Plasma PL follow up (nmol/l)	Red cell PLP admission (pmol/g Hb)	Red cell PLP follow up (pmol/g Hb)	Red cell PL admission (pmol/g Hb)	Red cell PL follow up (pmol/g Hb)
164.00	14.00	--	180.25	--	43.21	--
165.00	21.00	--	310.98	--	89.02	--
166.00	14.00	--	387.80	--	50.00	--
167.00	256.00	--	3,000.00	--	2,523.81	--
168.00	301.00	--	4,359.26	--	7,925.93	--
169.00	301.00	--	1,150.60	--	787.95	--
171.00	177.00	--	347.56	--	59.76	--

Patient no.	White cell PLP admission (pmol/ 10 (6) cells)	White cell PLP follow up (pmol/ 10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow up (pmol/ 10 (6) cells)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/ 10 (9) cells)	Plasma ascorbic acid follow up (umol/l)	White cell ascorbic acid follow up (umol/ 10 (9) cells)
12.00	1.98	1.97	0.75	0.77	--	--	21.00	--
13.00	2.67	2.55	0.79	1.31	24.00	1.90	2.00	2.64
14.00	2.30	2.09	1.15	1.45	0.90	2.45	3.00	3.12
15.00	4.06	2.69	6.88	0.68	16.00	2.93	0.90	2.10
16.00	8.24	--	5.35	--	1.00	1.39	--	--
17.00	3.29	2.48	0.48	0.43	1.00	--	0.90	--
18.00	2.34	2.08	0.70	0.73	4.00	1.91	3.00	1.45
19.00	2.22	5.18	0.43	4.34	37.00	1.76	15.00	2.17
21.00	3.01	--	1.30	--	2.00	0.69	--	--
22.00	6.16	--	10.29	--	136.00	2.57	25.00	--
23.00	3.13	2.15	0.66	0.42	20.00	3.02	10.00	1.11
24.00	2.52	--	1.03	--	21.00	1.81	--	--
27.00	2.19	2.35	0.73	1.71	13.00	3.39	28.00	1.41
29.00	4.38	3.57	6.08	3.58	--	--	17.00	3.76
30.00	2.42	4.09	1.22	7.17	11.00	1.56	--	--
31.00	2.31	--	0.32	--	3.00	1.91	--	--
32.00	1.30	--	0.24	--	3.00	1.32	--	--
33.00	1.39	1.95	0.34	0.98	8.00	1.70	5.00	2.10
34.00	5.18	5.88	5.29	3.52	59.00	2.91	25.00	2.69
35.00	1.93	2.79	0.38	0.69	0.90	2.63	4.00	2.50
36.00	2.46	2.12	9.79	3.07	59.00	3.81	16.00	3.53
37.00	1.74	1.90	0.56	0.73	--	--	6.00	2.04
38.00	--	--	--	--	--	--	--	--
40.00	3.47	1.94	0.35	0.56	20.00	1.93	11.00	2.20
41.00	2.18	--	0.34	--	19.00	3.30	--	--
42.00	2.64	--	0.38	--	0.90	1.82	4.00	--

Patient no.	White cell PLP admission (pmol/ 10 (6) cells)	White cell PLP follow up (pmol/ 10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow up (pmol/ 10 (6) cells)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/ 10 (9) cells)	Plasma ascorbic acid follow up (umol/l)	White cell ascorbic acid follow up (umol/ 10 (9) cells)
44.00	1.85	2.07	0.60	1.04	--	--	9.00	1.29
45.00	2.05	1.88	0.72	0.48	1.00	0.75	2.00	0.84
46.00	1.75	--	0.34	--	--	--	--	--
47.00	2.12	2.14	0.54	0.55	0.90	0.60	0.90	0.67
48.00	--	3.81	--	9.22	--	--	74.00	--
50.00	1.50	1.62	0.52	0.60	50.00	2.35	3.00	2.43
51.00	6.15	4.85	4.66	7.28	11.00	3.06	7.00	4.86
52.00	1.97	1.54	6.15	5.95	102.00	--	60.00	--
53.00	1.44	1.48	0.26	0.37	0.90	--	1.00	--
55.00	--	4.67	--	6.65	--	--	--	--
56.00	1.64	--	0.17	--	4.00	--	--	--
57.00	0.37	--	0.07	--	--	--	--	--
58.00	--	--	--	--	--	--	--	--
59.00	7.21	--	4.39	--	--	--	--	--
60.00	1.68	--	0.18	--	12.00	--	--	--
61.00	--	--	--	--	--	--	--	--
62.00	1.94	1.38	0.27	0.17	0.90	--	--	--
63.00	2.79	--	0.56	--	0.90	--	--	--
64.00	1.35	--	0.31	--	4.00	--	--	--
65.00	1.46	--	1.04	--	14.00	--	--	--
67.00	1.58	--	0.44	--	11.00	--	9.00	--
68.00	1.32	--	--	--	--	--	--	--
69.00	1.76	--	--	--	--	--	--	--
71.00	2.49	--	0.30	--	6.00	--	--	--
72.00	3.98	1.81	8.06	1.48	79.00	--	--	--
73.00	--	--	--	--	0.90	--	--	--
74.00	2.26	7.24	0.28	6.46	2.00	--	69.00	--
75.00	2.07	--	0.37	--	0.90	--	--	--
77.00	2.57	--	0.24	--	--	--	--	--
78.00	2.05	--	0.38	--	--	--	--	--

Patient no.	White cell PLP admission (pmol/ 10 (6) cells)	White cell PLP follow up (pmol/ 10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow up (pmol/ 10 (6) cells)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/ 10 (9) cells)	Plasma ascorbic acid follow up (umol/l)	White cell ascorbic acid follow up (umol/ 10 (9) cells)
79.00	3.56	1.86	0.53	0.54	0.90	--	2.00	--
80.00	2.68	--	1.09	--	2.00	1.47	--	--
81.00	2.83	2.77	0.37	1.70	10.00	1.54	6.00	1.46
82.00	2.58	5.13	0.43	4.73	0.90	--	22.00	--
83.00	1.65	--	0.17	--	1.00	1.60	--	--
84.00	3.35	3.26	3.00	5.22	1.00	1.57	16.00	1.42
85.00	1.64	1.84	0.34	0.40	1.00	--	0.90	--
87.00	1.36	--	0.20	--	3.00	--	--	--
88.00	3.83	2.68	4.02	3.93	--	--	20.00	--
89.00	2.05	--	0.43	--	12.00	1.94	--	--
90.00	--	--	--	--	--	--	6.00	--
91.00	--	--	--	--	--	--	--	--
94.00	1.02	--	--	--	--	--	--	--
96.00	--	--	--	--	--	--	13.00	--
99.00	6.10	--	7.13	--	29.00	1.79	--	--
100.00	3.63	5.70	7.78	10.32	2.00	1.95	24.00	5.80
101.00	--	--	--	--	17.00	2.12	--	--
102.00	--	--	--	--	9.00	--	5.00	--
103.00	2.64	2.14	0.46	1.37	35.00	--	18.00	--
104.00	--	--	--	--	0.90	--	--	--
105.00	2.14	4.80	0.97	4.41	5.00	8.77	0.90	1.90
107.00	1.89	--	0.53	--	16.00	--	--	--
108.00	2.32	--	0.50	--	3.00	1.42	--	--
110.00	0.89	3.48	0.18	5.35	5.00	1.50	17.00	2.54
111.00	3.20	3.43	0.49	1.18	2.00	1.99	3.00	4.08
112.00	2.47	--	0.53	--	--	2.27	1.00	--
113.00	--	--	--	--	2.00	--	2.00	--
114.00	1.43	3.34	0.33	6.95	0.90	0.46	2.00	1.02
115.00	2.18	--	0.35	--	17.00	0.99	--	--
116.00	--	--	--	--	0.90	0.74	--	--

Patient no.	White cell PLP admission (pmol/ 10 (6) cells)	White cell PLP follow up (pmol/ 10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow up (pmol/ 10 (6) cells)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/ 10 (9) cells)	Plasma ascorbic acid follow up (umol/l)	White cell ascorbic acid follow up (umol/ 10 (9) cells)
117.00	--	--	--	--	2.00	--	2.00	--
118.00	--	--	--	--	0.90	--	0.90	--
119.00	--	--	--	--	11.00	--	--	--
121.00	--	--	--	--	2.00	--	7.00	--
123.00	--	--	--	--	2.00	--	20.00	--
124.00	--	--	--	--	--	--	7.00	--
126.00	--	--	--	--	10.00	--	--	--
127.00	--	--	--	--	--	--	--	--
128.00	1.63	--	0.35	--	2.00	--	--	--
129.00	--	--	--	--	--	--	--	--
131.00	--	--	--	--	--	--	--	--
133.00	--	--	--	--	17.00	--	--	--
135.00	--	--	--	--	5.00	2.04	--	--
137.00	--	--	--	--	4.00	--	--	--
139.00	--	--	--	--	11.00	--	--	--
140.00	--	--	--	--	1.00	--	--	--
141.00	--	--	--	--	0.90	--	0.90	--
143.00	--	--	--	--	3.00	--	--	--
146.00	--	--	--	--	--	--	8.00	--
147.00	--	--	--	--	12.00	--	10.00	--
148.00	--	--	--	--	--	--	--	--
149.00	--	--	--	--	--	--	--	--
150.00	--	--	--	--	5.00	--	--	--
151.00	--	--	--	--	--	--	--	--
152.00	--	--	--	--	--	--	--	--
153.00	--	--	--	--	--	--	--	--
154.00	--	--	--	--	58.00	--	--	--
155.00	--	--	--	--	28.00	--	--	--
156.00	--	--	--	--	--	--	--	--
157.00	--	--	--	--	29.00	--	--	--

Patient no.	White cell PLP admission (pmol/ 10 (6) cells)	White cell PLP follow up (pmol/ 10 (6) cells)	White cell PL admission (pmol/ 10 (6) cells)	White cell PL follow up (pmol/ 10 (6) cells)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/ 10 (9) cells)	Plasma ascorbic acid follow up (umol/l)	White cell ascorbic acid follow up (umol/ 10 (9) cells)
158.00	--	--	--	--	--	--	--	--
159.00	--	--	--	--	--	--	--	--
163.00	--	--	--	--	--	--	--	--
164.00	--	--	--	--	0.90	--	--	--
165.00	--	--	--	--	--	--	--	--
166.00	--	--	--	--	--	--	--	--
167.00	--	--	--	--	--	--	--	--
168.00	--	--	--	--	--	--	--	--
169.00	--	--	--	--	--	--	--	--
171.00	--	--	--	--	--	--	--	--

12.13 Appendix 13 Chapter 9 data

Appendix 13. Characteristics and biochemical measurements of patients (Chapter 9)

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	ICU stay (days)	Hospital death	Hospital stay (days)	Ventilation (days)	SOFA score admission
12.00	61.00	0.00	29.00	76.60	0.00	7.40	0.00	13.40	6.00	7.00
13.00	43.00	1.00	12.00	11.70	1.00	48.10	1.00	50.10	49.00	5.00
14.00	71.00	1.00	18.00	28.90	1.00	12.90	1.00	12.90	14.00	8.00
15.00	53.00	1.00	19.00	32.20	0.00	4.80	0.00	10.80	5.00	8.00
16.00	61.00	0.00	23.00	28.50	0.00	1.00	0.00	10.00	1.00	6.00
17.00	18.00	0.00	12.00	20.20	0.00	6.20	1.00	6.20	6.00	2.00
18.00	67.00	0.00	18.00	44.40	1.00	59.50	1.00	59.50	60.00	10.00
19.00	50.00	0.00	21.00	38.90	0.00	46.30	1.00	46.30	47.00	10.00
21.00	61.00	0.00	34.00	76.80	1.00	2.00	0.00	53.00	2.00	8.00
22.00	52.00	0.00	13.00	9.90	0.00	3.70	0.00	77.00	4.00	2.00
23.00	71.00	0.00	23.00	42.50	1.00	20.90	1.00	20.90	22.00	8.00
24.00	77.00	1.00	21.00	35.00	0.00	2.00	0.00	6.00	3.00	4.00
27.00	76.00	1.00	23.00	46.00	0.00	21.70	0.00	21.70	21.60	5.00
29.00	70.00	0.00	16.00	20.20	1.00	5.10	0.00	85.00	4.00	4.00
30.00	73.00	1.00	25.00	53.30	1.00	15.50	1.00	15.50	13.00	11.00
31.00	62.00	0.00	19.00	27.10	1.00	0.50	0.00	25.00	1.00	11.00
32.00	55.00	1.00	15.00	21.00	1.00	2.20	0.00	22.20	0.00	6.00
33.00	80.00	1.00	31.00	84.20	1.00	12.00	1.00	12.00	13.00	7.00
34.00	53.00	0.00	26.00	68.60	1.00	29.40	0.00	85.00	1.00	6.00
35.00	20.00	0.00	18.00	39.70	1.00	44.00	1.00	44.00	44.00	10.00
36.00	43.00	1.00	38.00	92.60	0.00	25.50	0.00	27.50	24.00	13.00
37.00	60.00	0.00	27.00	63.10	0.00	21.80	0.00	29.00	16.00	10.00
38.00	74.00	0.00	20.00	23.00	1.00	1.00	0.00	150.00	2.00	4.00
40.00	47.00	0.00	22.00	28.50	1.00	1.90	0.00	19.90	3.00	9.00
41.00	76.00	1.00	29.00	79.90	1.00	1.40	0.00	21.40	2.00	8.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	ICU stay (days)	Hospital death	Hospital stay (days)	Ventilation (days)	SOFA score admission
42.00	79.00	0.00	21.00	30.90	1.00	3.20	0.00	27.00	3.00	4.00
44.00	81.00	0.00	34.00	86.30	0.00	16.10	1.00	16.10	16.10	7.00
45.00	74.00	0.00	33.00	75.20	0.00	16.00	1.00	16.00	16.00	11.00
46.00	41.00	1.00	8.00	15.60	1.00	0.80	0.00	18.80	1.00	1.00
47.00	76.00	0.00	29.00	77.20	1.00	34.60	1.00	36.80	33.00	7.00
48.00	67.00	0.00	31.00	68.10	1.00	18.40	1.00	18.40	15.00	6.00
50.00	60.00	0.00	19.00	48.00	1.00	6.20	0.00	124.20	5.00	7.00
51.00	46.00	1.00	24.00	49.70	0.00	4.60	1.00	4.60	5.00	14.00
52.00	61.00	0.00	33.00	78.60	0.00	7.70	1.00	7.70	8.00	11.00
53.00	67.00	1.00	18.00	21.20	0.00	11.10	1.00	11.10	12.00	11.00
55.00	80.00	1.00	31.00	73.30	0.00	8.20	1.00	8.20	9.00	8.00
56.00	40.00	1.00	6.00	10.20	1.00	0.40	0.00	0.40	1.00	6.00
57.00	60.00	0.00	27.00	60.20	1.00	1.80	1.00	1.80	3.00	10.00
58.00	41.00	0.00	21.00	17.80	1.00	19.70	0.00	36.70	19.00	4.00
59.00	68.00	0.00	20.00	32.30	0.00	0.80	0.00	12.00	2.00	5.00
60.00	57.00	0.00	24.00	67.00	0.00	6.50	0.00	59.50	5.00	3.00
61.00	76.00	1.00	18.00	14.40	0.00	0.70	0.00	22.70	0.00	5.00
62.00	74.00	0.00	20.00	19.90	1.00	0.90	0.00	253.00	2.00	1.00
63.00	62.00	0.00	18.00	44.40	1.00	1.00	0.00	508.00	2.00	2.00
64.00	66.00	0.00	34.00	81.00	0.00	22.40	0.00	46.00	21.00	13.00
65.00	38.00	1.00	17.00	26.20	0.00	18.90	0.00	37.00	18.00	9.00
67.00	68.00	1.00	31.00	80.30	0.00	5.00	1.00	8.00	5.00	4.00
68.00	74.00	0.00	24.00	49.70	0.00	10.70	0.00	27.70	11.00	14.00
69.00	61.00	0.00	22.00	54.90	1.00	76.40	1.00	122.40	75.00	10.00
71.00	25.00	0.00	17.00	1.20	0.00	2.00	0.00	3.50	1.00	3.00
72.00	51.00	0.00	21.00	12.40	0.00	15.50	0.00	40.00	15.00	11.00
73.00	69.00	0.00	12.00	20.20	0.00	5.80	0.00	23.00	7.00	3.00
74.00	65.00	0.00	33.00	80.40	0.00	19.60	0.00	40.00	18.00	6.00
75.00	28.00	1.00	14.00	18.50	1.00	0.60	0.00	58.00	1.00	10.00
77.00	54.00	1.00	17.00	1.20	0.00	0.80	0.00	12.00	2.00	7.00
78.00	45.00	0.00	12.00	5.50	1.00	0.90	0.00	12.00	2.00	4.00
79.00	41.00	0.00	7.00	7.60	1.00	9.30	0.00	19.00	8.00	4.00
80.00	38.00	0.00	16.00	23.30	1.00	0.80	0.00	41.00	2.00	9.00
81.00	71.00	0.00	25.00	35.90	1.00	8.30	0.00	20.00	9.00	5.00
82.00	80.00	1.00	17.00	12.70	0.00	10.30	0.00	29.00	8.00	1.00
83.00	46.00	1.00	11.00	10.30	1.00	2.90	0.00	10.00	0.00	4.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	ICU stay (days)	Hospital death	Hospital stay (days)	Ventilation (days)	SOFA score admission
84.00	56.00	0.00	28.00	63.90	0.00	15.40	0.00	35.00	14.00	13.00
85.00	73.00	0.00	18.00	44.40	1.00	1.40	0.00	160.00	2.00	6.00
87.00	34.00	1.00	12.00	12.40	1.00	2.30	0.00	12.00	2.00	6.00
88.00	81.00	0.00	17.00	23.60	1.00	9.30	1.00	10.00	10.00	9.00
89.00	22.00	1.00	10.00	9.50	1.00	0.20	0.00	1.00	1.00	2.00
90.00	53.00	0.00	32.00	60.90	1.00	3.00	0.00	36.00	3.00	10.00
91.00	62.00	0.00	22.00	58.90	1.00	1.70	0.00	14.00	1.00	9.00
94.00	32.00	1.00	25.00	53.30	0.00	17.30	0.00	24.00	17.00	18.00
96.00	63.00	1.00	18.00	4.80	0.00	1.90	0.00	15.00	2.00	3.00
99.00	37.00	0.00	13.00	16.50	1.00	2.10	0.00	11.00	2.00	5.00
100.00	50.00	1.00	26.00	38.20	0.00	2.60	0.00	119.00	0.00	4.00
101.00	35.00	1.00	9.00	4.70	1.00	0.80	0.00	6.00	0.00	5.00
102.00	26.00	0.00	23.00	45.70	1.00	2.30	0.00	77.00	2.00	4.00
103.00	52.00	0.00	16.00	12.20	1.00	2.00	0.00	48.00	2.00	2.00
104.00	38.00	0.00	3.00	2.70	0.00	0.30	0.00	5.00	1.00	2.00
105.00	39.00	0.00	34.00	87.50	0.00	3.00	0.00	58.00	3.00	11.00
107.00	45.00	1.00	12.00	6.60	1.00	3.10	0.00	48.00	0.00	8.00
108.00	44.00	1.00	6.00	0.90	0.00	0.80	0.00	5.00	0.00	3.00
110.00	48.00	1.00	26.00	35.20	0.00	4.90	1.00	6.00	6.00	7.00
111.00	23.00	0.00	9.00	4.30	1.00	9.20	0.00	48.00	7.00	7.00
112.00	45.00	1.00	24.00	22.40	1.00	10.70	1.00	12.00	12.00	1.00
113.00	70.00	0.00	27.00	47.70	0.00	6.50	1.00	7.00	7.00	4.00
114.00	65.00	1.00	24.00	65.70	1.00	32.80	1.00	61.00	34.00	9.00
115.00	47.00	0.00	10.00	1.50	0.00	0.60	0.00	14.00	0.00	2.00
116.00	61.00	0.00	13.00	10.60	0.00	2.40	0.00	37.00	3.00	1.00
117.00	48.00	0.00	13.00	8.50	1.00	4.20	0.00	18.00	4.00	3.00
118.00	59.00	0.00	23.00	39.90	1.00	12.10	0.00	24.00	12.00	9.00
119.00	67.00	1.00	18.00	14.40	0.00	1.30	0.00	9.00	1.00	2.00
121.00	86.00	0.00	22.00	42.20	1.00	6.60	0.00	20.00	6.00	7.00
123.00	60.00	0.00	16.00	14.60	0.00	3.10	0.00	23.00	2.00	7.00
124.00	33.00	0.00	14.00	27.40	1.00	4.70	0.00	10.00	4.00	7.00
126.00	68.00	0.00	27.00	46.00	0.00	1.60	0.00	240.00	1.00	5.00
127.00	100.00	0.00	25.00	64.60	1.00	3.50	0.00	60.00	0.00	12.00
128.00	80.00	1.00	30.00	56.90	1.00	2.90	0.00	144.00	2.00	7.00
129.00	31.00	0.00	19.00	44.00	0.00	2.90	0.00	54.00	3.00	12.00
131.00	65.00	0.00	21.00	55.30	1.00	6.70	1.00	24.00	6.00	8.00

Patient no.	Age (yrs)	Male/ Female (0/1)	APACHE II	Predicted mortality (%)	Medical/ Surgical (0/1)	ICU stay (days)	Hospital death	Hospital stay (days)	Ventilation (days)	SOFA score admission
133.00	35.00	0.00	14.00	7.20	1.00	0.90	0.00	9.00	2.00	0.00
135.00	41.00	1.00	16.00	14.30	1.00	0.60	0.00	17.00	2.00	2.00
137.00	69.00	1.00	19.00	32.00	1.00	0.80	0.00	7.00	1.00	4.00
139.00	54.00	1.00	23.00	55.80	1.00	2.60	0.00	69.00	2.00	8.00
140.00	74.00	0.00	21.00	41.60	1.00	2.10	1.00	11.00	0.00	8.00
141.00	67.00	0.00	32.00	78.00	1.00	19.60	0.00	50.00	20.00	10.00
143.00	52.00	0.00	10.00	17.40	1.00	9.80	1.00	11.00	45.00	2.00
146.00	38.00	0.00	18.00	24.20	1.00	10.30	0.00	20.00	10.00	6.00
147.00	70.00	0.00	20.00	35.50	1.00	1.50	0.00	29.00	0.00	7.00
148.00	71.00	0.00	25.00	53.30	0.00	0.80	1.00	5.00	2.00	5.00
149.00	74.00	0.00	15.00	--	1.00	13.80	1.00	15.00	11.00	5.00
150.00	62.00	0.00	21.00	38.90	0.00	35.20	0.00	66.00	26.00	11.00
151.00	36.00	0.00	8.00	8.60	1.00	1.20	0.00	62.00	2.00	0.00
152.00	47.00	0.00	11.00	7.60	0.00	2.00	0.00	10.00	2.00	3.00
153.00	77.00	1.00	27.00	46.00	0.00	3.10	0.00	14.00	0.00	4.00
154.00	49.00	0.00	26.00	4.40	0.00	0.90	0.00	8.00	2.00	4.00
155.00	43.00	0.00	25.00	3.80	0.00	0.80	0.00	7.00	2.00	8.00
156.00	41.00	0.00	33.00	78.60	0.00	1.80	1.00	3.00	3.00	12.00
157.00	61.00	0.00	31.00	81.40	1.00	11.40	0.00	53.00	9.00	14.00
158.00	56.00	0.00	11.00	22.30	1.00	9.00	0.00	74.00	2.00	3.00
159.00	60.00	1.00	19.00	11.30	1.00	1.50	0.00	22.00	2.00	4.00
163.00	77.00	1.00	26.00	56.70	1.00	1.40	0.00	48.00	1.00	10.00
164.00	62.00	1.00	14.00	9.70	0.00	7.00	0.00	16.00	5.00	10.00
165.00	35.00	0.00	5.00	0.20	0.00	1.00	0.00	6.00	2.00	1.00
166.00	63.00	0.00	13.00	13.40	1.00	0.60	0.00	13.00	2.00	8.00
167.00	56.00	0.00	32.00	76.00	0.00	2.10	1.00	4.00	3.00	8.00
168.00	52.00	1.00	26.00	56.90	0.00	34.10	0.00	57.00	35.00	10.00
169.00	39.00	1.00	13.00	24.60	1.00	24.60	0.00	55.00	25.00	14.00
171.00	67.00	0.00	27.00	60.50	0.00	2.30	0.00	10.00	0.00	14.00

Patient no.	C-reactive protein admission (mg/l)	Albumin admission (g/l)	Total protein admission (g/l)	Whole blood TDP admission (ng/gHb)	Red cell FAD admission (nmol/gHb)	Red cell PLP admission (pmol/gHb)	Triglycerides admission (mmol/l)	Cholesterol admission (mmol/l)	Plasma retinol admission (umol/l)	Plasma α-tocopherol admission (umol/l)
12.00	126.00	24.00	46.00	928.00	1.76	348.39	0.61	1.70	0.88	14.36
13.00	22.00	27.00	55.00	789.00	1.53	345.35	2.80	4.27	1.30	15.35
14.00	220.00	12.00	45.00	1,173.00	1.82	624.69	1.55	2.19	0.42	16.26
15.00	257.00	23.00	63.00	1,435.00	1.46	2,282.98	1.52	2.75	0.18	14.48
16.00	122.00	28.00	72.00	994.00	1.05	7,870.37	0.75	3.76	1.31	15.68
17.00	3.00	33.00	73.00	693.00	1.33	331.03	0.71	2.83	1.90	17.00
18.00	152.00	10.00	31.00	402.00	1.13	216.30	0.80	0.55	0.39	6.27
19.00	143.00	12.00	68.00	1,094.00	1.69	334.18	0.72	3.06	1.11	17.70
21.00	23.00	10.00	25.00	617.00	1.42	563.10	1.60	2.60	1.45	16.03
22.00	20.00	27.00	71.00	2,341.00	2.09	25,583.33	1.10	3.00	1.44	13.74
23.00	125.00	10.00	39.00	978.00	1.63	396.30	0.50	1.10	0.24	7.93
24.00	81.00	35.00	74.00	1,317.00	2.29	241.98	0.80	4.90	1.80	26.95
27.00	103.00	23.00	54.00	705.00	1.63	116.28	1.80	3.90	1.18	24.42
29.00	59.00	9.00	25.00	1,652.00	1.57	2,274.16	0.80	0.90	0.22	10.91
30.00	26.00	16.00	44.00	891.00	1.97	173.91	1.20	3.80	4.20	33.79
31.00	50.00	22.00	46.00	744.00	1.30	158.89	0.60	2.50	1.22	14.80
32.00	434.00	17.00	39.00	778.00	1.38	317.24	1.10	2.00	0.09	10.30
33.00	259.00	17.00	41.00	510.00	1.20	176.25	0.80	2.10	0.35	11.83
34.00	404.00	30.00	56.00	3,650.00	1.86	3,922.58	2.01	1.25	1.27	19.65
35.00	119.00	9.00	27.00	483.00	1.35	131.03	0.70	1.27	0.21	10.16
36.00	132.00	14.00	35.00	2,672.00	2.17	2,983.13	0.68	0.53	0.15	6.44
37.00	81.00	12.00	45.00	1,270.00	1.58	205.26	0.38	0.84	0.85	6.25
38.00	127.00	14.00	56.00	626.00	1.70	310.26	1.66	2.40	0.50	20.99
40.00	15.00	20.00	39.00	330.00	1.41	155.42	--	--	0.90	17.38
41.00	184.00	19.00	33.00	768.00	1.20	198.77	0.64	1.51	1.62	10.05
42.00	8.00	17.00	32.00	574.00	1.29	240.45	0.50	2.10	0.84	12.37
44.00	151.00	10.00	37.00	506.00	2.04	720.99	0.44	0.98	0.60	8.31
45.00	258.00	14.00	41.00	1,379.00	2.21	325.93	0.69	2.41	1.04	15.25
46.00	250.00	11.00	32.00	804.00	1.21	211.63	1.19	1.88	0.17	9.70
47.00	64.00	24.00	58.00	1,002.00	1.85	316.88	0.80	2.20	0.61	14.47
48.00	119.00	10.00	57.00	2,127.00	2.14	1,013.92	0.70	0.90	0.02	8.06

Patient no.	C-reactive protein admission (mg/l)	Albumin admission (g/l)	Total protein admission (g/l)	Whole blood TDP admission (ng/gHb)	Red cell FAD admission (nmol/gHb)	Red cell PLP admission (pmol/gHb)	Triglycerides admission (mmol/l)	Cholesterol admission (mmol/l)	Plasma retinol admission (umol/l)	Plasma α-tocopherol admission (umol/l)
50.00	180.00	23.00	31.00	828.00	1.49	313.92	1.20	1.50	1.62	9.98
51.00	56.00	14.00	43.00	1,406.00	1.80	11,113.89	0.50	1.20	0.02	8.40
52.00	565.00	9.00	43.00	3,524.00	2.30	7,519.74	0.70	1.00	0.58	7.83
53.00	148.00	9.00	34.00	196.00	1.04	168.67	2.50	1.70	0.26	16.34
55.00	107.00	13.00	41.00	676.00	1.22	191.80	0.30	1.10	0.17	8.12
56.00	37.00	16.00	33.00	518.00	1.45	260.98	1.07	1.42	0.91	11.20
57.00	181.00	10.00	37.00	560.00	1.33	208.33	0.55	0.40	0.22	3.55
58.00	6.00	19.00	39.00	506.00	1.16	241.05	0.95	2.12	1.14	11.37
59.00	32.00	36.00	66.00	1,130.00	1.22	8,840.00	5.05	4.23	2.86	33.30
60.00	166.00	26.00	75.00	923.00	1.17	138.71	2.53	4.56	2.14	30.79
61.00	380.00	26.00	65.00	1,037.00	1.21	181.25	2.13	4.60	1.82	31.14
62.00	88.00	12.00	38.00	800.00	1.20	176.74	--	--	0.57	14.40
63.00	117.00	14.00	27.00	2,092.00	1.80	321.35	1.34	1.49	0.87	9.63
64.00	311.00	18.00	64.00	556.00	1.06	139.74	1.47	1.68	1.47	19.42
65.00	163.00	23.00	54.00	1,226.00	1.91	642.50	1.12	1.36	0.23	10.05
67.00	438.00	20.00	35.00	763.00	1.72	111.39	0.87	1.21	0.22	12.43
68.00	67.00	11.00	37.00	459.00	1.54	213.98	1.68	1.44	0.57	10.01
69.00	179.00	14.00	36.00	544.00	1.34	265.88	0.53	0.71	0.26	5.31
71.00	2.00	38.00	75.00	792.00	0.97	103.57	2.10	3.20	1.69	21.98
72.00	40.00	32.00	58.00	1,677.00	1.78	13,716.88	3.40	3.30	1.00	16.40
73.00	2.00	31.00	62.00	--	1.38	150.00	1.80	3.30	1.22	19.99
74.00	77.00	15.00	48.00	631.00	1.44	254.55	0.90	1.30	0.66	11.23
75.00	184.00	11.00	37.00	727.00	2.10	153.85	2.80	2.00	0.75	20.40
77.00	2.00	20.00	47.00	667.00	1.81	263.46	0.50	2.10	1.09	13.23
78.00	177.00	27.00	61.00	598.00	1.93	279.07	1.70	4.20	0.82	24.55
79.00	2.00	24.00	44.00	500.00	1.24	157.14	--	--	1.28	17.25
80.00	126.00	14.00	58.00	2,300.00	2.23	798.80	0.90	1.20	0.48	11.19
81.00	8.00	10.00	23.00	617.00	1.56	307.69	0.60	1.10	0.70	6.08
82.00	4.00	29.00	60.00	739.00	1.31	171.26	0.70	4.00	2.84	33.45
83.00	318.00	21.00	48.00	511.00	1.49	104.55	0.80	3.10	0.31	17.97
84.00	63.00	9.00	29.00	1,652.00	1.75	1,598.80	0.90	0.90	0.39	5.33
85.00	176.00	11.00	26.00	350.00	1.30	201.20	0.30	1.20	0.17	8.06
87.00	110.00	18.00	40.00	700.00	1.62	177.89	0.70	2.60	0.67	16.63
88.00	108.00	12.00	51.00	1,176.00	1.35	2,785.37	0.80	1.70	0.61	12.70

Patient no.	C-reactive protein admission (mg/l)	Albumin admission (g/l)	Total protein admission (g/l)	Whole blood TDP admission (ng/gHb)	Red cell FAD admission (nmol/gHb)	Red cell PLP admission (pmol/gHb)	Triglycerides admission (mmol/l)	Cholesterol admission (mmol/l)	Plasma retinol admission (umol/l)	Plasma α-tocopherol admission (umol/l)
89.00	31.00	16.00	33.00	579.00	1.17	204.85	1.50	2.70	0.73	19.22
90.00	159.00	11.00	41.00	724.00	2.06	241.46	1.60	1.60	0.68	15.93
91.00	268.00	14.00	40.00	687.00	1.25	209.00	1.30	1.60	0.36	15.56
94.00	147.00	14.00	47.00	810.00	1.87	243.68	2.60	1.90	0.20	14.00
96.00	76.00	29.00	64.00	894.00	2.12	245.83	1.60	4.40	1.60	27.00
99.00	287.00	22.00	52.00	1,983.00	1.48	2,581.63	1.06	2.50	0.40	15.00
100.00	253.00	13.00	41.00	2,287.00	2.56	10,787.95	2.40	2.30	0.60	19.00
101.00	231.00	25.00	46.00	385.00	--	--	0.70	2.20	0.30	15.00
102.00	28.00	19.00	38.00	415.00	1.31	177.89	0.60	1.70	0.90	11.00
103.00	--	42.00	73.00	910.00	1.59	149.44	3.30	4.10	1.80	41.00
104.00	1.00	34.00	71.00	545.00	1.31	219.15	4.70	6.40	2.10	36.00
105.00	25.00	30.00	66.00	1,271.00	1.80	808.79	1.00	2.00	0.40	13.00
107.00	261.00	17.00	39.00	827.00	1.67	232.98	--	--	0.30	16.00
108.00	48.00	35.00	70.00	653.00	1.23	147.47	--	--	0.80	23.00
110.00	199.00	13.00	48.00	918.00	1.13	186.21	1.10	1.60	0.40	17.00
111.00	6.00	28.00	53.00	664.00	1.37	300.00	2.00	4.00	1.80	26.00
112.00	2.00	20.00	42.00	923.00	1.15	160.67	1.20	5.20	1.30	28.00
113.00	14.00	31.00	60.00	576.00	1.31	265.22	1.30	2.70	2.00	17.00
114.00	49.00	16.00	37.00	288.00	1.14	196.77	1.40	2.20	0.40	15.00
115.00	1.00	39.00	63.00	610.00	1.02	127.00	0.90	5.30	1.90	24.00
116.00	3.00	24.00	48.00	391.00	1.29	383.87	1.00	2.70	1.30	12.00
117.00	9.00	32.00	55.00	713.00	1.53	222.68	4.50	4.20	1.90	36.00
118.00	252.00	12.00	33.00	1,367.00	1.85	594.38	0.70	0.70	0.30	7.00
119.00	2.00	25.00	50.00	635.00	1.33	127.55	0.50	4.60	1.20	32.00
121.00	203.00	15.00	37.00	482.00	1.54	209.30	0.30	1.00	0.20	8.00
123.00	2.00	45.00	83.00	1,729.00	1.77	11,912.94	1.90	4.80	2.70	27.00
124.00	97.00	15.00	40.00	1,884.00	1.56	846.15	0.40	1.10	0.20	7.00
126.00	3.00	21.00	46.00	1,248.00	1.23	21,893.41	0.80	5.00	0.70	20.00
127.00	316.00	17.00	59.00	494.00	2.58	342.68	0.60	1.20	0.60	16.00
128.00	51.00	16.00	46.00	685.00	1.75	285.23	1.10	2.40	0.80	17.00
129.00	63.00	20.00	53.00	1,779.00	1.88	23,853.33	--	--	0.20	12.00
131.00	165.00	21.00	47.00	531.00	1.68	348.31	0.90	1.00	0.30	7.00
133.00	35.00	16.00	36.00	1,627.00	1.55	1,989.00	0.30	0.90	0.30	6.00
135.00	221.00	18.00	58.00	1,302.00	1.91	454.88	1.90	2.80	1.10	25.00

Patient no.	C-reactive protein admission (mg/l)	Albumin admission (g/l)	Total protein admission (g/l)	Whole blood TDP admission (ng/gHb)	Red cell FAD admission (nmol/gHb)	Red cell PLP admission (pmol/gHb)	Triglycerides admission (mmol/l)	Cholesterol admission (mmol/l)	Plasma retinol admission (umol/l)	Plasma α-tocopherol admission (umol/l)
137.00	218.00	11.00	34.00	610.00	1.84	244.59	1.40	2.20	0.40	20.00
139.00	72.00	14.00	33.00	773.00	2.02	305.41	--	0.94	0.80	6.00
140.00	51.00	9.00	26.00	502.00	2.13	195.71	0.40	2.40	0.40	5.00
141.00	143.00	9.00	40.00	610.00	1.53	31.71	--	0.62	1.40	6.00
143.00	39.00	14.00	60.00	1,140.00	1.84	380.28	1.20	2.20	0.70	22.00
146.00	73.00	26.00	38.00	2,413.00	2.13	5,550.67	1.70	2.60	0.80	12.00
147.00	203.00	32.00	56.00	545.00	1.23	310.81	0.30	3.10	0.50	12.00
148.00	17.00	23.00	54.00	423.00	1.54	502.67	1.10	3.90	0.80	17.00
149.00	94.00	13.00	40.00	364.00	1.36	179.73	0.70	1.00	0.40	6.00
150.00	295.00	24.00	55.00	653.00	1.42	167.53	1.10	1.60	0.60	12.00
151.00	229.00	13.00	47.00	257.00	1.24	234.21	1.20	4.70	1.60	22.00
152.00	26.00	35.00	65.00	595.00	1.29	166.25	1.30	4.80	1.80	21.00
153.00	45.00	19.00	50.00	608.00	1.37	193.06	2.20	2.30	0.60	21.00
154.00	39.00	33.00	62.00	2,552.00	1.82	27,441.10	0.60	4.60	2.50	23.00
155.00	52.00	19.00	68.00	1,788.00	1.44	405.13	0.50	2.80	1.90	11.00
156.00	316.00	20.00	73.00	300.00	1.22	289.33	1.90	2.60	0.20	9.00
157.00	294.00	13.00	44.00	1,005.00	1.37	21,889.74	0.60	0.90	0.20	6.00
158.00	163.00	10.00	34.00	1,656.00	1.58	4,984.62	2.10	1.60	0.20	9.00
159.00	137.00	15.00	39.00	992.00	1.43	266.23	0.50	1.40	0.90	9.00
163.00	269.00	12.00	38.00	520.00	1.15	158.02	0.70	1.50	0.20	12.00
164.00	291.00	24.00	58.00	854.00	1.47	180.25	0.90	1.90	0.30	12.00
165.00	0.60	47.00	75.00	853.00	1.27	310.98	1.40	5.50	3.10	32.00
166.00	75.00	13.00	37.00	598.00	1.47	387.80	2.30	4.10	0.60	29.00
167.00	74.00	23.00	43.00	1,926.00	1.39	3,000.00	0.60	2.50	0.80	10.00
168.00	131.00	11.00	38.00	2,061.00	1.82	4,359.26	0.60	0.60	0.30	6.00
169.00	273.00	14.00	50.00	2,574.00	1.77	1,150.60	2.40	2.60	0.20	12.00
171.00	203.00	20.00	52.00	808.00	1.77	347.56	0.90	1.80	1.10	16.00

Patient no.	Red cell α-tocopherol admission (pmol/gHb)	Plasma lutein admission (ug/l)	Plasma lycopene admission (ug/l)	Plasma α-carotene admission (ug/l)	Plasma β-carotene admission (ug/l)	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	MDA/ Total protein admission (umol/g)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/10(9) cells)
12.00	17.58	30.10	28.90	21.20	58.00	0.65	0.14	0.22	0.01	--	--
13.00	11.30	4.60	9.50	9.00	0.50	0.91	0.16	0.18	0.02	24.00	1.90
14.00	22.20	9.10	15.60	4.60	8.90	0.60	0.58	0.97	0.01	0.90	2.45
15.00	3.40	7.40	35.30	1.80	3.90	0.96	0.17	0.18	0.02	16.00	2.93
16.00	16.48	21.40	23.30	4.20	20.40	1.02	0.20	0.20	0.01	1.00	1.39
17.00	19.66	--	--	--	--	1.11	0.23	0.21	0.02	1.00	--
18.00	16.01	3.60	4.60	9.00	0.30	0.80	0.19	0.24	0.03	4.00	1.91
19.00	18.62	33.50	102.50	16.60	117.90	0.87	0.26	0.30	0.01	37.00	1.76
21.00	16.00	4.60	4.10	1.60	10.20	2.34	0.62	0.26	0.09	2.00	0.69
22.00	18.73	22.20	17.70	11.30	39.00	1.03	0.38	0.37	0.01	136.00	2.57
23.00	13.47	13.90	21.80	9.00	28.30	0.22	0.19	0.86	0.01	20.00	3.02
24.00	22.18	113.20	43.10	12.00	115.60	1.37	0.37	0.27	0.02	21.00	1.81
27.00	19.14	38.30	22.00	9.20	67.60	1.12	0.22	0.20	0.02	13.00	3.39
29.00	19.67	8.00	6.20	0.90	4.40	0.53	0.37	0.70	0.02	--	--
30.00	39.28	41.70	84.40	20.30	40.10	0.93	0.97	1.04	0.02	11.00	1.56
31.00	17.83	53.40	100.60	13.10	66.90	0.55	0.21	0.38	0.01	3.00	1.91
32.00	11.39	18.00	4.70	3.00	9.30	0.77	0.23	0.30	0.02	3.00	1.32
33.00	16.94	23.90	29.40	12.60	39.00	0.63	0.31	0.49	0.02	8.00	1.70
34.00	15.33	32.00	47.00	1.40	0.60	0.82	0.14	0.17	0.01	59.00	2.91
35.00	26.99	21.50	64.50	8.30	34.30	14.27	5.96	0.42	0.53	0.90	2.63
36.00	12.38	8.50	13.40	3.80	14.40	0.78	0.20	0.26	0.02	59.00	3.81
37.00	16.08	141.90	290.60	29.10	200.70	0.35	0.05	0.14	0.01	--	--
38.00	18.59	7.50	12.10	12.60	110.90	0.52	0.04	0.08	0.01	--	--
40.00	21.05	28.30	70.60	8.00	31.70	0.31	0.06	0.19	0.01	20.00	1.93
41.00	25.22	20.40	37.40	17.10	45.20	0.48	0.05	0.10	0.01	19.00	3.30
42.00	15.59	53.50	65.90	11.80	57.10	--	--	--	--	0.90	1.82
44.00	18.50	30.70	22.40	1.70	14.70	--	--	--	--	--	--
45.00	16.23	9.90	16.30	2.40	15.60	0.42	0.06	0.14	0.01	1.00	0.75
46.00	--	9.80	16.20	0.80	2.60	0.32	0.03	0.09	0.01	--	--
47.00	18.55	39.80	38.30	11.50	31.20	0.56	0.07	0.13	0.01	0.90	0.60

Patient no.	Red cell α-tocopherol admission (pmol/gHb)	Plasma lutein admission (ug/l)	Plasma lycopene admission (ug/l)	Plasma α-carotene admission (ug/l)	Plasma β-carotene admission (ug/l)	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	MDA/ Total protein admission (umol/g)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/10(9) cells)
48.00	24.40	14.00	11.40	9.00	5.70	0.34	0.04	0.12	0.01	--	--
50.00	18.10	9.40	6.90	1.60	25.90	0.40	0.15	0.38	0.01	50.00	2.35
51.00	--	8.40	8.60	1.50	23.90	0.27	0.10	0.37	0.01	11.00	3.06
52.00	16.99	4.20	6.90	0.70	0.60	0.50	0.18	0.36	0.01	102.00	--
53.00	20.19	2.10	3.20	9.00	0.90	0.39	0.10	0.26	0.01	0.90	--
55.00	26.96	16.00	26.80	11.70	18.30	0.22	0.10	0.45	0.01	--	--
56.00	17.05	34.90	54.20	9.30	29.70	0.25	--	--	0.01	4.00	--
57.00	21.03	4.40	5.10	0.90	2.80	0.29	0.15	0.52	0.01	--	--
58.00	17.17	36.60	95.80	15.00	73.60	0.67	0.09	0.13	0.02	--	--
59.00	21.69	65.10	116.60	6.60	10.70	2.20	0.28	0.13	0.03	--	--
60.00	21.47	33.90	164.80	21.80	85.00	0.75	0.07	0.09	0.01	12.00	--
61.00	27.94	43.90	58.80	5.60	23.30	1.37	1.37	1.00	0.02	--	--
62.00	18.07	20.00	40.70	15.60	53.10	0.69	0.05	0.07	0.02	0.90	--
63.00	13.00	23.70	45.50	6.20	20.90	0.62	0.06	0.10	0.02	0.90	--
64.00	20.23	41.00	35.40	14.90	65.60	0.60	0.02	0.03	0.01	4.00	--
65.00	20.07	--	--	--	--	0.63	0.08	0.13	0.01	14.00	--
67.00	29.75	21.60	13.00	4.70	22.00	0.83	0.26	0.31	0.02	11.00	--
68.00	19.01	18.00	1.20	1.30	0.30	0.37	0.16	0.43	0.01	--	--
69.00	16.86	4.70	3.70	0.60	2.00	--	--	--	--	--	--
71.00	17.68	69.60	93.20	5.50	16.00	0.60	0.04	0.07	0.01	6.00	--
72.00	9.77	44.60	54.70	2.90	0.50	--	--	--	--	79.00	--
73.00	16.66	48.40	15.80	9.00	51.50	1.34	0.05	0.04	0.02	0.90	--
74.00	13.46	2.10	0.80	0.50	0.30	--	--	--	--	2.00	--
75.00	17.85	22.80	15.40	4.80	18.20	1.19	0.18	0.15	0.03	0.90	--
77.00	26.49	27.70	59.80	6.80	37.90	1.03	0.08	0.08	0.02	--	--
78.00	--	77.80	147.30	20.30	109.60	--	--	--	--	--	--
79.00	25.28	66.90	170.70	8.90	41.90	--	--	--	--	0.90	--
80.00	--	8.30	20.40	2.30	10.60	--	--	--	--	2.00	1.47
81.00	21.71	18.10	24.50	4.30	14.60	0.41	0.08	0.20	0.02	10.00	1.54
82.00	21.44	141.50	162.80	75.00	401.80	--	--	--	--	0.90	--
83.00	12.79	28.90	43.50	15.90	91.60	0.89	0.04	0.04	0.02	1.00	1.60
84.00	16.02	2.50	1.90	9.00	0.70	0.78	0.13	0.17	0.03	1.00	1.57
85.00	22.33	17.50	7.30	6.10	9.00	--	--	--	--	1.00	--

Patient no.	Red cell α-tocopherol admission (pmol/gHb)	Plasma lutein admission (ug/l)	Plasma lycopene admission (ug/l)	Plasma α-carotene admission (ug/l)	Plasma β-carotene admission (ug/l)	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	MDA/ Total protein admission (umol/g)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/10(9) cells)
87.00	18.91	63.30	78.30	27.60	141.30	0.24	0.02	0.08	0.01	3.00	--
88.00	23.14	9.40	20.80	5.80	5.30	0.42	0.08	0.19	0.01	--	--
89.00	19.83	80.30	133.20	15.50	62.60	--	--	--	--	12.00	1.94
90.00	22.65	18.80	63.40	2.70	10.10	--	--	--	--	--	--
91.00	18.69	44.10	28.40	9.30	12.50	--	--	--	--	--	--
94.00	13.45	--	--	--	--	0.85	0.21	0.25	0.02	--	--
96.00	29.52	84.00	130.00	12.00	31.00	--	--	--	--	--	--
99.00	11.60	21.00	71.00	9.00	9.00	0.73	0.05	0.07	0.01	29.00	1.79
100.00	22.67	9.00	9.00	9.00	9.00	0.68	0.19	0.28	0.02	2.00	1.95
101.00	16.68	31.00	45.00	11.00	46.00	0.32	0.07	0.22	0.01	17.00	2.12
102.00	23.38	35.00	60.00	9.00	44.00	--	--	--	--	9.00	--
103.00	31.49	29.00	142.00	173.00	167.00	--	--	--	--	35.00	--
104.00	13.43	90.00	193.00	9.00	32.00	--	--	--	--	0.90	--
105.00	21.66	50.00	44.00	16.00	52.00	0.71	0.15	0.21	0.01	5.00	8.77
107.00	12.87	12.00	27.00	9.00	311.00	0.77	0.08	0.10	0.02	16.00	--
108.00	--	68.00	234.00	14.00	48.00	0.48	0.10	0.21	0.01	3.00	1.42
110.00	23.88	25.00	9.00	9.00	11.00	0.48	0.09	0.19	0.01	5.00	1.50
111.00	22.55	106.00	273.00	9.00	50.00	--	--	--	--	2.00	1.99
112.00	24.97	246.00	32.00	36.00	522.00	1.31	0.06	0.05	0.03	--	2.27
113.00	13.13	38.00	57.00	9.00	25.00	1.56	0.35	0.22	0.03	2.00	--
114.00	16.57	29.00	90.00	10.00	53.00	0.73	0.12	0.16	0.02	0.90	0.46
115.00	--	97.00	188.00	10.00	51.00	1.06	0.05	0.05	0.02	17.00	0.99
116.00	--	22.00	96.00	9.00	9.00	0.81	0.06	0.07	0.02	0.90	0.74
117.00	--	54.00	139.00	9.00	16.00	1.61	0.06	0.04	0.03	2.00	--
118.00	16.69	9.00	9.00	9.00	9.00	0.61	0.05	0.08	0.02	0.90	--
119.00	--	168.00	176.00	19.00	140.00	1.40	0.06	0.04	0.03	11.00	--
121.00	--	9.00	9.00	9.00	9.00	0.57	0.08	0.14	0.02	2.00	--
123.00	19.07	68.00	60.00	11.00	24.00	0.87	0.04	0.05	0.01	2.00	--
124.00	--	9.00	16.00	9.00	9.00	0.35	0.08	0.23	0.01	--	--
126.00	--	36.00	110.00	9.00	14.00	0.55	0.06	0.11	0.01	10.00	--
127.00	--	88.00	13.00	9.00	101.00	0.79	0.11	0.14	0.01	--	--
128.00	--	11.00	17.00	10.00	39.00	0.94	0.10	0.11	0.02	2.00	--
129.00	5.15	9.00	11.00	9.00	9.00	1.84	0.14	0.08	0.03	--	--

Patient no.	Red cell α-tocopherol admission (pmol/gHb)	Plasma lutein admission (ug/l)	Plasma lycopene admission (ug/l)	Plasma α-carotene admission (ug/l)	Plasma β-carotene admission (ug/l)	Total MDA admission (umol/l)	Free MDA admission (umol/l)	Free/ Total MDA admission	MDA/ Total protein admission (umol/g)	Plasma ascorbic acid admission (umol/l)	White cell ascorbic acid admission (umol/10(9) cells)
131.00	--	9.00	24.00	9.00	16.00	0.44	0.09	0.20	0.01	--	--
133.00	--	11.00	32.00	9.00	9.00	0.61	0.08	0.13	0.02	17.00	--
135.00	--	9.00	9.00	9.00	176.00	0.56	0.06	0.11	0.01	5.00	2.04
137.00	--	37.00	103.00	16.00	48.00	0.45	0.04	0.08	0.01	4.00	--
139.00	--	9.00	16.00	9.00	9.00	0.63	0.04	0.06	0.02	11.00	--
140.00	--	9.00	9.00	9.00	9.00	0.62	0.08	0.13	0.02	1.00	--
141.00	--	9.00	12.00	9.00	9.00	0.66	0.12	0.18	0.02	0.90	--
143.00	--	9.00	55.00	44.00	518.00	0.59	0.08	0.14	0.01	3.00	--
146.00	--	9.00	126.00	9.00	9.00	0.70	0.07	0.10	0.02	--	--
147.00	--	47.00	56.00	9.00	23.00	0.47	0.05	0.11	0.01	12.00	--
148.00	--	16.00	46.00	9.00	11.00	--	--	--	--	--	--
149.00	--	9.00	9.00	9.00	9.00	0.52	0.07	0.13	0.01	--	--
150.00	--	12.00	15.00	9.00	9.00	0.41	0.06	0.15	0.01	5.00	--
151.00	--	67.00	294.00	38.00	224.00	0.48	0.06	0.13	0.01	--	--
152.00	--	69.00	260.00	34.00	185.00	1.65	0.07	0.04	0.03	--	--
153.00	--	15.00	48.00	--	71.00	0.68	0.10	0.15	0.01	--	--
154.00	--	70.00	78.00	13.00	10.00	0.85	0.09	0.11	0.01	58.00	--
155.00	--	15.00	101.00	5.00	92.00	1.08	0.19	0.18	0.02	28.00	--
156.00	--	9.00	14.00	9.00	9.00	0.69	0.13	0.19	0.01	--	--
157.00	--	9.00	9.00	9.00	9.00	0.38	0.22	0.58	0.01	29.00	--
158.00	--	9.00	9.00	9.00	9.00	1.00	0.08	0.08	0.03	--	--
159.00	--	30.00	9.00	9.00	9.00	0.62	0.08	0.13	0.02	--	--
163.00	--	19.00	11.00	6.00	31.00	0.72	0.08	0.11	0.02	--	--
164.00	--	32.00	9.00	4.00	8.00	0.56	0.05	0.09	0.01	0.90	--
165.00	--	92.00	318.00	20.00	115.00	1.24	0.05	0.04	0.02	--	--
166.00	--	8.00	10.00	0.70	2.00	0.92	0.09	0.10	0.02	--	--
167.00	--	19.00	19.00	17.00	20.00	0.42	0.08	0.19	0.01	--	--
168.00	--	12.00	15.00	3.00	7.00	--	--	--	--	--	--
169.00	--	9.00	3.00	3.00	6.00	--	--	--	--	--	--
171.00	--	24.00	29.00	3.00	81.00	--	--	--	--	--	--