# Impaired splenic function and tuftsin deficiency in patients with intestinal failure on long term intravenous nutrition

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**Abstract**

***Background*—Reticuloendothelial system function is impaired in humans receiving lipid regimens.**

***Aims*—To evaluate the effects of long term administration of long chain triglyceride emulsions on reticuloendothelial system function.**

***Methods*—Splenic function and tuftsin activity were measured in 20 patients on intravenous nutrition for intestinal failure, 20 patients with Crohn's disease who were not receiving intravenous nutrition, and 50 healthy controls.**

***Results*—Pitted red cells counts in patients on intravenous nutrition (8.0%) were significantly higher ($p<0.001$) than in healthy controls (0.6%) and in patients with Crohn's disease (0.9%). No difference was found between healthy controls and patients with Crohn's disease. There was a correlation ($r=0.50$; $p<0.03$) between percentage of pitted red cells and duration of intravenous nutrition. Tuftsin activity was significantly reduced in the intravenous nutrition patient group (6%) compared with both disease controls (16.5%, $p<0.01$) and healthy volunteers (17.8%, $p<0.001$) . An inverse correlation between tuftsin activity and pitted red cell percentage was found in the patients on intravenous nutrition ($r_s = -0.44$, $p<0.05$). No relation was found in the patients on intravenous nutrition between pitted red cell percentage or tuftsin activity and type of disease, percentage of ideal body weight, residual length of small intestine, or administration (quantity and frequency) of lipid emulsion. Eight patients on intravenous nutrition had serious infections within the previous 12 months.**

***Conclusions*—Patients with a short bowel treated with long term intravenous nutrition have impaired splenic function, reduced tuftsin activity, and an increased risk of infection.**

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Keywords: splenic function; hyposplenism; tuftsin; home parenteral nutrition; short bowel syndrome

Although lymph nodes, bone marrow, lung, and pleural and peritoneal cavities are part of the reticuloendothelial system (RES), 85–95% of reticuloendothelial function occurs under normal circumstances in the liver and spleen.[1]

Splenic hypofunction occurs in a number of medical conditions, including some intestinal diseases[2] and, like splenectomy, may predispose to fatal infections by encapsulated bacteria.[3 4] The protective effect of the spleen may be mainly because of a tetrapeptide (Thr-Lys-Pro-Arg), tuftsin, released from the $CH_2$ domain of IgG. Tuftsin, located in the Fc portion of the immunoglobulin molecule, requires two enzymes for release: a leucocyte membrane enzyme (leukokininase) is believed to cleave the aminolateral, and a splenic endocarboxypeptidase cleaves the carboxyl terminal.[5 6] Tuftsin also stimulates the bactericidal activities of phagocytic cells[7]; its activity falls after splenectomy,[8 9] and is low in sickle cell anaemia,[10] AIDS,[11] and coeliac disease.[12]

After an earlier report of diminished bacterial defences following parenteral administration of a long chain triglyceride emulsion in mice,[13] RES function has also been found to be impaired in humans receiving various lipid regimens.[14 15] As mixed fuel systems that incorporate intravenous lipid emulsion are increasingly used in intravenous nutrition (IVN), we studied splenic function, by measuring pitted red cell numbers and tuftsin activity, in patients receiving long term home IVN because of intestinal failure, in whom an increased susceptibility to infections could be highly relevant.

## Patients and methods

Twenty patients (median age 41 years, range 21–62; four men, 16 women), who had been receiving IVN for at least six months (median 56.8 months, range 6–138) due to intestinal failure (see table 1), 20 unoperated patients with inactive Crohn's disease who were not

*Table 1 Patient characteristics*

| *Patient* | *Sex* | *Age* | *% Ideal body weight* | *Residual bowel length* | *Diagnosis* |
|---|---|---|---|---|---|
| 1 | F | 62 | 95 | 61 | Ulcerative colitis |
| 2 | F | 59 | 110 | 100 | Familial adenomatous polyposis |
| 3 | F | 46 | 85 | 80 | Radiation enteritis |
| 4 | F | 38 | 105 | 150 | Crohn's disease |
| 5 | F | 54 | 105 | 75 | Familial adenomatous polyposis |
| 6 | F | 35 | 105 | 105 | Crohn's disease |
| 7 | F | 28 | 85 | 90 | Crohn's disease |
| 8 | F | 44 | 75 | 120 | Crohn's disease |
| 9 | M | 21 | 90 | 30 | Visceral myopathy |
| 10 | F | 34 | 84 | 80 | Crohn's disease |
| 11 | M | 52 | 100 | 23 | Scleroderma |
| 12 | M | 29 | 85 | 60 | Volvulus |
| 13 | F | 54 | 95 | 100 | Crohn's disease |
| 14 | F | 36 | 84 | 100 | Visceral myopathy |
| 15 | F | 54 | 110 | 90 | Crohn's disease |
| 16 | F | 39 | 87 | 50 | Crohn's disease |
| 17 | F | 30 | 97 | 0 | Visceral myopathy |
| 18 | M | 34 | 88 | All | Visceral myopathy |
| 19 | F | 53 | 100 | 0 | Familial adenomatous polyposis |
| 20 | F | 42 | 100 | 0 | Visceral myopathy |

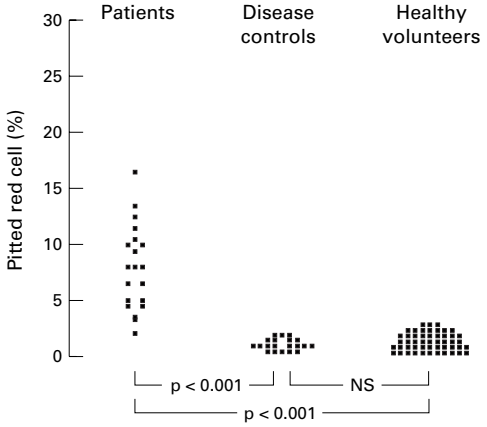


*Figure 1 Pitted red cell percentage in patients on IVN, patients with Crohn's disease, and healthy volunteers.*

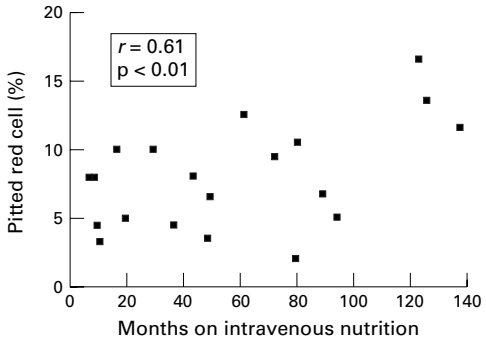


*Figure 2 Pitted red cell percentage and duration of IVN.*

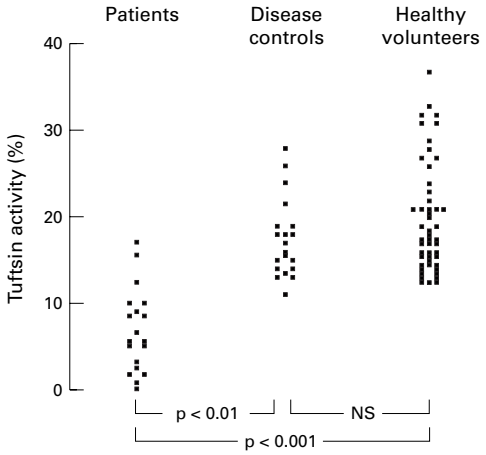


*Figure 3 Serum tuftsin activity in patients on IVN, patients with Crohn's disease, and healthy volunteers.*

receiving IVN (median age 37 years, range 18–69; seven men, 13 women), and 50 healthy volunteers (median age 40 years, range 22–64; 21 men, 29 women) were included in the study. These patients were recruited from St Mark's and St Bartholomew's Hospitals, London.

All except for two patients on long term IVN were receiving long chain triglyceride emulsion solutions (Intralipid) from a minimum of 50 g to a maximum of 350 g weekly.

Splenic function was measured by counting pitted red cells (PRC) by an investigator blinded as to the subject under investigation. Venous blood from each subject was mixed with 0.5 ml 3% buffered glutaraldehyde solution (pH 7.4). One thousand red blood cells were examined in a wet preparation (magnification ×1000) with a direct interference contrast microscope (Leitz Dialux 20, equipped with Nomarsky optics). The percentage of cells with one or more membrane abnormalities visible as "pits" under interference microscopy, was calculated. Patients with more than 4% PRC were considered hyposplenic.[16]

Tuftsin activity was measured as previously described.[17] γ Globulin (10 mg) was isolated from each subject by ammonium sulphate precipitation and dialysis in 0.1 mol/l phosphate buffer (pH 8.1) and then digested at 37°C for one hour with 0.5 mg trypsin, in a final volume of 2.5 ml 0.1 mol/l phosphate buffer (pH 8.1) to cleave tuftsin; trypsin was used as a substitute for the leukokininase that cleaves the aminolateral terminal of the $CH_2$ domain of IgG to activate tuftsin at the cellular level. Four volumes of 95% ethyl alcohol were added and the alcoholic extract was evaporated under nitrogen. The residue was dissolved in 0.25 ml Krebs-Ringer solution and the precipitate was separated by centrifugation (3000 *g*, 4°C, 30 minutes). Tuftsin was assayed by measuring its ability to stimulate phagocytosis of opsonised *Staphylococcus aureus* by neutrophilic granulocytes from healthy volunteers. Controls for tuftsin activity were: Krebs-Ringer solution; 0.3 μmol synthetic tuftsin-Krebs-Ringer solution (Sigma, St Louis, USA); and Krebs-Ringer solution containing tuftsin extracted from sera from healthy volunteers with known tuftsin activity. All assays were performed in duplicate. Slide preparations were stained by the May-Grunwald-Giemsa method. The percentage of granulocytes that contained at least one staphylococcus was calculated by counting (magnification ×1000) 1000 cells per subject by two observers who were unaware of the origin of the samples (interobserver correlation index, 0.95). Tuftsin activity for each sample was calculated by subtracting the percentage of staphylococcus positive cells in the Krebs-Ringer control.

Nutritional status was assessed as body weight expressed as percentage of ideal body weight (IBW%).[18]

### STATISTICAL ANALYSIS

Statistical analysis of results was performed by the two tailed Wilcoxon rank sum test for unpaired data and the Spearman correlation test. Data are given as median and range, unless otherwise stated.

## Results

In the IVN patient group, 17 (85%) were hyposplenic, with a PRC percentage greater than 4; 15 had received intravenous lipid infusions (fig 1). The three patients with normal PRC percentage were all receiving lipid infusions. PRC percentage in patients on IVN was significantly higher than in both disease and healthy controls; no difference was found between disease and healthy controls. PRC percentage increased with duration of IVN (fig 2); there was a significant positive correlation between PRC percentage and time on IVN.

*Table 2 Clinical characteristics and splenic function of the eight study patients who had infections*

| *Patient* | *Infection* | *Infective agent* | *Pitted cell (%)* | *Tuftsin activity (%)* |
|---|---|---|---|---|
| 1 | Septicaemia | *Streptococcus pneumoniae* | 9.4 | 5.5 |
| 2 | Pelvic abscess | Enterococcus | 4.5 | 8.5 |
| 6 | Pelvic abscess | Enterococcus | 8.0 | 6.5 |
| 7 | Septicaemia | *Streptococcus pneumoniae* | 4.5 | 8.5 |
| 10 | Subphrenic abscess | *Klebsiella pneumoniae* | 3.5 | 1.6 |
| 12 | Septicaemia | *Klebsiella pneumoniae* | 6.5 | 1.8 |
| 15 | Septicaemia | *Streptococcus pneumoniae* | 16.5 | 0.6 |
| 19 | Septicaemia | *Klebsiella pneumoniae* | 5.0 | 12.4 |

Figure 3 shows tuftsin activity in the three groups. Tuftsin activity was significantly reduced in the IVN patient group (median 6%, range 0–17%) compared both with disease controls (median 16.5%, range 11–28%), and healthy volunteers (median 17.8%, range 12.2–37%). An inverse correlation between tuftsin activity and PRC percentage was found in patients on IVN ($r_s = -0.44$, $p<0.05$).

No relation was found in the group of patients on IVN between PRC percentage or tuftsin activity and type of disease, IBW%, residual length of small intestine, or administration (quantity and frequency) of lipid emulsion. Eight patients on IVN had serious infections within the 12 months preceding the study, not related to the central line as cultures of catheter tips were negative (table 2). In this group, percentage of PRC (median 5.7%, range 3.5–16.5%), tuftsin activity (median 6.0%, range 0.6–12.4%), and duration of IVN (median 48 months, range 6–123) did not differ from those of 12 patients without evidence of infections in the 12 months preceding the study (percentage of PRC: median 9%, range 2.0–13.5%; tuftsin activity: median 7.2%, range 0.0–17.0%; duration of IVN: median 52 months, range 8–126). No episodes of serious infections were recorded in the control group in the 12 months preceding the study.

## Discussion

Lipid emulsions are currently recommended as a component of parenteral nutrition regimens, both in hospitalised patients and home fed patients. Not only do these emulsions provide a source of essential fatty acids, but because of their relative caloric density and isotonicity they have been promulgated as an effective energy source.[19 20] However, it has been suggested that Intralipid may compromise human host defence mechanisms and put patients at risk of invasive bacterial disease.[13–15] Confirming these results, IVN has been associated with an increased incidence of infection,[21 22] and higher mortality has been reported in patients who received preoperative IVN in whom half of the non-protein energy was provided as lipid emulsion.[23]

Animal studies suggest that the intravenous administration of long chain triglyceride emulsions may impair RES function and clearance of bacteria, depending on both source and quantity of lipid administered.[24 25] Liver histology in patients receiving long term soybean oil emulsion has revealed lipophagosomes in the most hypertrophic Kupffer cells and this accumulation has been hypothesised to inhibit the clearance capacity of cellular components of the RES.[26 27] On the other hand, IVN in rats, even without lipids, may be immunosuppressive through the release of prostaglandin $E_2$ from splenic macrophages following a septic challenge.[28] In addition, bowel rest and IVN have recently been shown to produce alterations in host resistance to injury with an enhanced counter-regulatory hormone and splanchnic cytokine response to lipopolysaccharide in humans.[29]

Splenic function and tuftsin activity have never been evaluated before in patients receiving IVN; furthermore, RES function has not been studied in patients receiving IVN, with or without long chain triglycerides, for periods longer than a few days.[14 15] In patients with intestinal failure, receiving long term home IVN, we found significant impairment of splenic function as studied by PRC counting and measurement of tuftsin activity. Of the 17 hyposplenic patients, two had never received long chain triglyceride emulsion infusion. These two patients had the highest PRC percentages (13.5% and 16.5%) and had been on home IVN for a long period (126 and 123 months) suggesting, in accordance with the experimental data of Nussbaum *et al*,[28] that factors other than long chain triglyceride infusion may be relevant in promoting splenic hypofunction after IVN.

Eight patients were on home IVN because of short bowel syndrome following several operations for Crohn's disease, and an impaired splenic function has been described in this condition.[30 31] However, no relation was found in our study between the disease and the degree of splenic function. Neither splenic hypofunction or tuftsin deficiency was found in the 20 patients with unoperated Crohn's disease that we studied as disease controls.

Overwhelming sepsis caused by encapsulated bacteria is a well known complication of splenectomy.[32–36] The protective role of the spleen may be achieved because of the release of tuftsin. Therefore, in the absence of the spleen or in splenic hypofunction, tuftsin deficiency is a crucial factor in determining susceptibility to major infection, and the protective effect of synthetic tuftsin in post-splenectomy sepsis has been confirmed experimentally.[37]

In our study, eight patients on IVN had serious infections within the 12 months preceding the study, which were not related to the central line. This observation may be extremely important, because it shows that the increase in PRC percentage, together with the related

decrease in tuftsin activity, could have real clinical relevance. All the microorganisms involved in these infections were encapsulated bacteria, which are the most frequently involved in infective processes in patients with splenic hypofunction. Furthermore, to support the presence of an increased risk of infection in patients on long term IVN, a recent survey of the prognosis of long term home IVN has shown a very high mortality and, among the causes of death, 22% were due to sepsis.[38]

Further investigation is required to identify the factors which lead to splenic hypofunction in these patients. It may be that components of parenteral nutrition other than long chain triglycerides are involved in this process, although, so far, we have not been able to hypothesise what these could be. However, this study suggests that it may be worthwhile monitoring splenic function in patients on long term IVN, and to consider the need for vaccination against encapsulated bacteria in these patients.

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