# Nutritional abnormalities in patients receiving long-term home parenteral nutrition

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A thesis submitted in accordance with the conditions governing candidates for the degree of

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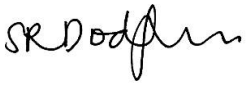
Signed:  (Sean Dodington) Date: 02 May 2018

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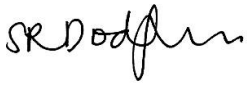
Signed:  (Sean Dodington) Date: 02 May 2018

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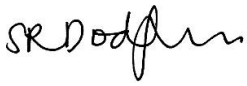
Signed:  (Sean Dodington) Date: 02 May 2018

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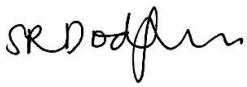
Signed:  (Sean Dodington) Date: 02 May 2018

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## SUMMARY

The last two decades have seen an increased drive to administer parenteral nutrition (PN) to patients in their home environments, thereby reducing associated hospital costs and improving patient quality of life.

The occurrence of deranged nutritional biochemistry results has baffled PN experts for years because PN additives are marketed for the general needs of patients and PN is tailored to each patient's requirements (both formulation and regimen).

This thesis documents the investigations into HPN population characteristics, the extent of nutritional abnormalities (deficiencies and excesses) in a cohort of LT PN patients in Wales. Both cross-sectional and longitudinal retrospective study designs were employed alongside small-scale laboratory efforts to investigate stability of vitamin D in PN additives using High Performance Liquid Chromatography (HPLC).

Characteristics of the HPN population in Wales were shown to be variable in terms of PN requirements for a predominantly female sample population (2:1); in whom 78.6% of patients received PN for indications relating to short bowel syndrome (SBS).

A database analysis of micronutrient test results revealed a high prevalence of deficiencies of vitamin D and selenium, as well as excesses of manganese and water-soluble vitamins; which can lead to clinically relevant effects in patients.

The sample population was shown to have impaired bone health since first receiving PN; respective sites of the femoral neck and total hip presented 58% and 60.8% of patients had osteopenia, while 28% and 19.6% had osteoporosis. Evidence in the literature links these clinical outcomes of metabolic bone disease (MBD) to patients' inadequate vitamin D status.

A final study exploring the adequacy of the trace element (TE) preparation Additrace®, found it lacking in selenium and excessive in manganese for the general requirements of the PN population. Clinician-directed supplementation of PN outside of Additrace® was associated with better micronutrient status in patients and more test results within range.

## ABBREVIATIONS

| | |
|---|---|
| AKI | acute kidney injury |
| AMA | American Medical Association |
| ANOVA | analysis of variance |
| APR | acute phase response |
| AP-spine | anterior-posterior spine |
| ASPEN | American Society for Parenteral and Enteral Nutrition |
| BANS | British Artificial Nutrition Survey |
| BAPEN | British Association for Parenteral and Enteral Nutrition |
| BMD | bone mineral density |
| BPNG | British Pharmaceutical Nutrition Group |
| BW | body weight |
| CAD | charged aerosol detection |
| C&V UHB | Cardiff and Vale University Health Board |
| CRP | C-reactive protein |
| DEXA | dual energy x-ray absorptiometry |
| EFAD | essential fatty acid deficiency |
| EN | enteral nutrition |
| ESPEN | The European Society for Clinical Nutrition and Metabolism |
| fA | (femto)-ampere |
| FRAX | Fracture Risk Assessment Tool |
| GI | gastrointestinal |
| HPLC | high performance liquid chromatography |
| HPN | home parenteral nutrition |
| IBD | inflammatory bowel disease |
| IF | intestinal failure |
| IPFR | individual patient funding request |
| IV | intravenous |
| LCMS | liquid chromatography-mass spectroscopy |
| LCT | long-chain triglyceride |
| LOD | limit of detection |
| LOQ | limit of quantitation |

| | |
|---|---|
| LT | long-term |
| MCT | medium-chain triglyceride |
| MBD | metabolic bone disease |
| MCP | multi-component preparation |
| MRI | magnetic resonance imaging |
| NHS | National Health Service |
| NICE | National Institute for Health and Care Excellence |
| NST | nutrition support team |
| pA | (pico)-ampere |
| PIS | participant information sheet |
| PN | parenteral nutrition |
| PNALD | PN-associated liver disease |
| PTH | parathyroid hormone |
| QOL | quality of life |
| R&D | Research and Development |
| RDA | recommended dietary allowance |
| REC | Research Ethics Committee |
| RNI | reference nutrient intake |
| RP-HPLC | reverse phase HPLC |
| SBS | short bowel syndrome |
| SD | standard deviation |
| S/N | signal to noise ratio |
| SE | service evaluation |
| TE | trace element |
| UHW | University Hospital of Wales |
| UK | United Kingdom |
| US | United States |
| UV | ultraviolet |
| WHSSC | Welsh Health Specialised Services Committee |

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# CHAPTER ONE

## Introduction: long-term (LT) home parenteral nutrition (HPN)

## 1.1. INTRODUCTION TO PARENTERAL NUTRITION (PN) AND HPN

### 1.1.1. PN

PN is an intravenous (IV) mode of nutritional therapy established for patients who are intolerant of, or those who cannot receive adequate nutrition via the oral or enteral route. Over the last 40 years, it has allowed patients to lead as normal a life as possible, free to continue with their day-to-day activities. It is a complex nutritional admixture composed of many different chemical entities which must be chemically, physically and microbiologically robust in order to be safely administered to the patient (White 2011). Home parenteral nutrition (HPN) refers to PN given as nutritional therapy to patients in their domiciliary home environment. This falls in line with recent NHS efforts to treat patients at home, in so doing it helps to reduce the burden placed on hospitals, reduce associated costs and improve clinical outcomes (Department of Health 2009).

PN is often referred to as "total" PN; in this sense it aims to provide the complete nutritional needs of the patient without any significant enteral intake. However PN can also be given supplementary to nutrition consumed via the gastrointestinal (GI) tract in those patients still capable of oral or enteral feeding (Rye and Nightingale 2015). HPN can be provided to patients in both these forms, as either their sole source of nutrition or supplementary to what they are capable of consuming orally or enterally (Pertkiewicz et al. 2009). In this way, the PN formulation is adapted to suit the individual needs of the patient.

The basic formula for PN includes a mixture of lipid (as an emulsion), carbohydrate (as glucose solution), amino acid solution (including essential and non-essential amino acids), vitamins, trace elements (TE), electrolytes and water, and can be produced as:

- 'Standard' bags (feeds) are pre-compounded pre-filled PN bags. These are licensed ready-to-use pharmaceutical products with a set formulation, produced for convenience to cater for more generalised nutritional needs with longer shelf lives and without the need for refrigeration. These can be given with or without subsequent additions of electrolytes and/or micronutrients (standard vs tailored regimen),

although the National Institute for Health and Care Excellence (NICE) guidelines state that all patients should receive appropriate provision of micronutrients in their PN from the outset of feeding (NICE 2006).

- 'Bespoke' bags (feeds) are compounded from individual components as per individualised patient needs, usually for patients with sensitive and/or long-term requirements (outside of remit of standard bags).

Lipid, glucose and amino acids comprise the main components (macro-nutrients) of PN. Vitamins and TE collectively are known as micronutrients, with electrolytes sometimes falling into this category as well. TE are inorganic elements included as integral parts of metabolically active organic complexes such as enzymes (e.g. iron, zinc). Vitamins split into two broad categories, either fat-soluble or water-soluble vitamins.

There is a wide range of commercially available standard bags to meet the variable needs of PN patients cared for by hospitals without or with limited compounding facilities. The general advantages of using standard prefilled PN bags are that they avoid the need to compound bags locally, and avoid the costs associated with buying in bespoke or aseptically prepared bags. Also, they do not require refrigeration, which makes them a useful option for stable home PN patients for short periods away, e.g. for holidays. As bespoke bags require sterile aseptic preparation conditions, American Society for Parenteral and Enteral Nutrition (ASPEN) have recently issued standardised competencies and safe practice recommendations regarding their order, review, preparation (including compounding) to reduce risks from differences in local procedures (Boullata et al. 2016); as yet there are no formalised recommendations for standardised competencies from European or UK PN working groups.

### 1.1.2. Indication for PN

PN is used in the treatment of patients with long-term (LT) chronic conditions. HPN patients are often maintained on PN for long periods of time and may require close monitoring and tailoring of their 'PN regimen' to suit their needs.

Throughout this report, the abbreviation 'LT PN' will be used to refer to a duration of least six months within which patients have been receiving PN.

NICE has clearly defined candidates for PN as being those who are malnourished or at risk of malnutrition as well as being either unsafe for oral nutrition or having functional problems associated with the GI tract (NICE 2006). As such, LT PN is indicated for those with chronic intestinal failure (IF), in particular IF type 3, a condition characterised by reduced intestinal absorption to the extent where HPN therapy is needed to maintain health and/or growth (Pironi et al. 2015). The most commonly implicated underlying diseases which contribute to IF are inflammatory bowel disease (IBD), complications following surgery, mesenteric vasculitis, radiation enteritis and chronic short bowel disease with severe malabsorption and dysmotility syndromes (Staun et al. 2009). The indications for LT PN for patients with chronic IF are typically short bowel syndrome (SBS), fistula, bowel dysmotility and radiation enteropathy (Nightingale 2006; Staun et al. 2009). SBS has been defined as a state of malabsorption following intestinal resection where there is less than 200cm of remaining intestinal length (Robinson and Wilmore 2001; Buchman 2006). However in practice it is when there is less than 100cm of remaining short bowel that patients risk under-nutrition and interventions requiring LT PN may be necessary (S Harwood, A Juckes, June 2015, personal communication). Practically, it can be hard to ascertain the remaining length of bowel or its remaining functionality in these patients; estimations are usually made at the end of surgical procedures. Cancer patients who experience severe malnutrition and weight loss are also candidates for PN to improve their nutritional status before surgery or therapy (Sexton et al. 2009). The chronic nature of all these conditions mean that people often require PN for long periods of time, which makes home administration very beneficial. The goal for many patients as they recover from their illness or surgery would be that they are able to consume adequate oral or enteral nutrition and not require further PN therapy; however this possibility decreases with the severity of the patients' underlying disease, the length of their remaining functional bowel and their ability to sustain themselves nutritionally;

ultimately resulting in patients necessitating the use of PN (Nightingale 2006; Van Gossum et al. 2009; Rye and Nightingale 2015).

PN is also needed for a shorter duration of time by patients in hospital who have more self-limiting conditions which put them at risk of malnutrition, as it has long been acknowledged that maintaining adequate nutrition is associated with better clinical outcomes (Studley 1936; NICE 2006). A recent national enquiry identified that 93% of patients receiving PN in hospital required PN for less than 30 days (n=1053)(Stewart et al. 2010). This figure mainly resulted from post-surgical complications for those patients requiring the need for nutritional support. It helps to show the small proportion of patients who require LT PN as an inpatient by comparison to those who receive it short-term for more acute conditions/indications.

### 1.1.3. Background for HPN

The field of HPN is a diverse setting with many differing practices and guidelines between continents (ASPEN Board of Directors and the Guidelines Clinical Task Force 2002; Staun et al. 2009). This is thought to be due to the different approaches in handling such a relatively small, complex group of patients, who have such different and individual problems in terms of their underlying disease and specific PN requirements. In the UK, HPN patients are managed out of specialised HPN centres catering for broad surrounding areas. The largest HPN centres being St Mark's Hospital in London and Hope Hospital in Salford, Manchester. In Europe, HPN practices were first initialised in specialised centres like these which developed expertise over time. However Pironi et al. (2006) expressed concern over a potential loss of expertise when their survey revealed that 50% of forty-one centres in Europe catered for less than ten patients. Another concern being the growing use of standard all-in-one PN bags with longer shelf lives over the practice of more precise prescribing on an individual basis for clinically sensitive and unstable HPN patients (bespoke PN formulations) (BPNG 2010).

Commercial homecare companies are involved in the manufacture and distribution of PN from these centres (Jones 2003). In 2010, companies such

as Calea, Baxter and BBraun provided HPN services for all new patient registrations as well as up to 94% of the patients already maintained on LT HPN (Smith et al. 2011), demonstrating the dependency of HPN patients on these services. Although providing PN in a home setting has proven expensive, it appears to cut the total management costs by around a half by comparison to hospital-based management (Howard 2006).

Although PN is an artificial source of nutrition aiming to nutritionally mimic a healthy, well-balanced oral diet, its use still carries certain risks and complications. Patients receiving LT HPN receive a fixed nutritional PN regimen with less control over their nutritional intake by comparison to healthy individuals who are not on PN. Changes to their PN regimen may only occur after clinical review or intervention. As such, abnormalities of nutritional balance are known to occur in HPN patients as their nutritional requirements change over time. Although HPN has proven a lifesaving therapy for more than four decades, its use is associated with complications that compromise patients' quality of life (QOL). Most problems relate to the presence of the venous access device to administer PN and concern line infections, sepsis, risk of pulmonary embolism or vascular occlusion; patient training is a key factor in preventing these complications. Patients are susceptible to other noteworthy complications which include metabolic complications, cholestatic liver disease, fatty liver and exacerbation of systemic inflammation (Meadows 1998; Berger 2014).

### 1.1.4. Monitoring in LT HPN

Patients on LT PN require monitoring to ensure they receive best effect from their HPN therapy. This is with particular reference to the provision of calories and fluid from parenteral nutrition to meet the individual patient requirements.

Monitoring usually occurs at the discharging base hospital (usually a HPN centre) via access to a specialised nutrition support team (Micklewright et al. 2002). It is essential for evaluation of the clinical effect of PN therapy and for management of any associated complications.

Key monitoring milestones recommended by The European Society for Clinical Nutrition and Metabolism (ESPEN) include measurement of blood biochemistry and anthropometry at all clinic visits, micronutrient measurement at intervals of at least six months and bone mineral density (BMD) assessment by dual-energy x-ray absorptiometry (DEXA) scanning at yearly intervals (Staun et al. 2009).

A stable patient would be well maintained on their PN feed and over time would show consistent blood test results within their reference range limits. Typically, the unstable patient requires closer attention and more frequent monitoring than a stable patient. For instance, closer adjustment of their PN prescription in relation to their blood test results. Monitoring for an unstable patient would occur at weekly or monthly intervals, while stable patients would be monitored at two to four monthly intervals (Staun and Pironi 2015). In practice, stable patients are monitored in the IF clinic at least every six months at the site used in this research, Cardiff and Vale University Health Board (C&V UHB).

### 1.1.5. HPN prevalence

Epidemiological data has shown that the use of HPN has grown over the last four decades, particularly in the 1990s (Van Gossum and Messing 1997; Glencorse et al. 2003; Smith et al. 2011). Causes are thought to centre around growing experience of specialised centres, increased survival of HPN patients and increased cost-effectiveness in the treatment of patients with benign disease, as well as the development of home care service provision.

It has been difficult to ascertain the point prevalence for the number of patients receiving HPN in the UK. In the UK, the British Artificial Nutrition Survey group (BANS) keep a register for the number of people on HPN (Glencorse et al. 2003). They are a committee of British Association for Parenteral and Enteral Nutrition (BAPEN) who report on data and trends of adults and children receiving LT enteral tube feeding or PN in the UK (Smith et al. 2011). The 2011 BANS report documented the point prevalence statistic

of 8.40 per million which roughly equated to 531 patients receiving HPN in the UK at that time (Smith et al. 2011). However, this figure should be interpreted carefully because the report documented gross-under reporting of HPN patients, particularly in Wales. At a recent conference, the estimated number of patients registered as receiving HPN in the UK was stated to rest somewhere around 1800-2000 (Smith 2015). More recently, the latest BANS report detailed a record for the number of patients registered as receiving HPN in UK as a period prevalence of 1360 patients in 2015, yet there are still reservations regarding its true accuracy (Smith and Naghibi 2016).

HPN patients in Wales are managed via one large beacon HPN centre in Cardiff and two smaller HPN centres in Swansea and Wrexham. After personal communications with the HPN Nutrition Support Team (NST) at C&V UHB, an accurate point prevalence was obtained for the number of HPN patients in Wales. As of July 2015, there were ninety-eight adult patients registered as receiving LT HPN in Wales (ninety-three in Cardiff, three in Swansea and two in Wrexham).

Although HPN is a small-scale service area, it is clear to see that its provision is a unique and distinctive feature of current healthcare and nutrition practice.

### 1.1.6. Homecare services

Under the Welsh Health Specialised Services Committee (WHSSC) HPN policy contract, WHSSC will fund HPN for adult and paediatric patients with LT IF who are either awaiting reconstructive surgery leading to restoration of gut continuity and function, or for those with irreversible IF (except patients who require short term feeding). The contract also allows funding for a nurse to assist with administration of PN if the carer and/or patient are unable to administer it themselves.

The process typically involves management of patients with IF within a HPN centre base hospital i.e. Hope Hospital, Salford Royal NHS Foundation Trust and University Hospital of Wales, C&V UHB. The contract permits preparation and delivery of PN from a home care company, in this instance Calea. Patients

are referred to the homecare company by the NST at the base hospital. Sometimes exceptional grounds for funding is considered when patients do not meet the explicit criteria and an Individual Patient Funding Request (IPFR) can be made to WHSSC.

### 1.1.7. Initiation for LT PN (flow diagram)

The flow diagram in Figure 1.1 depicts the standard procedure for the initiation of a patient on LT HPN.

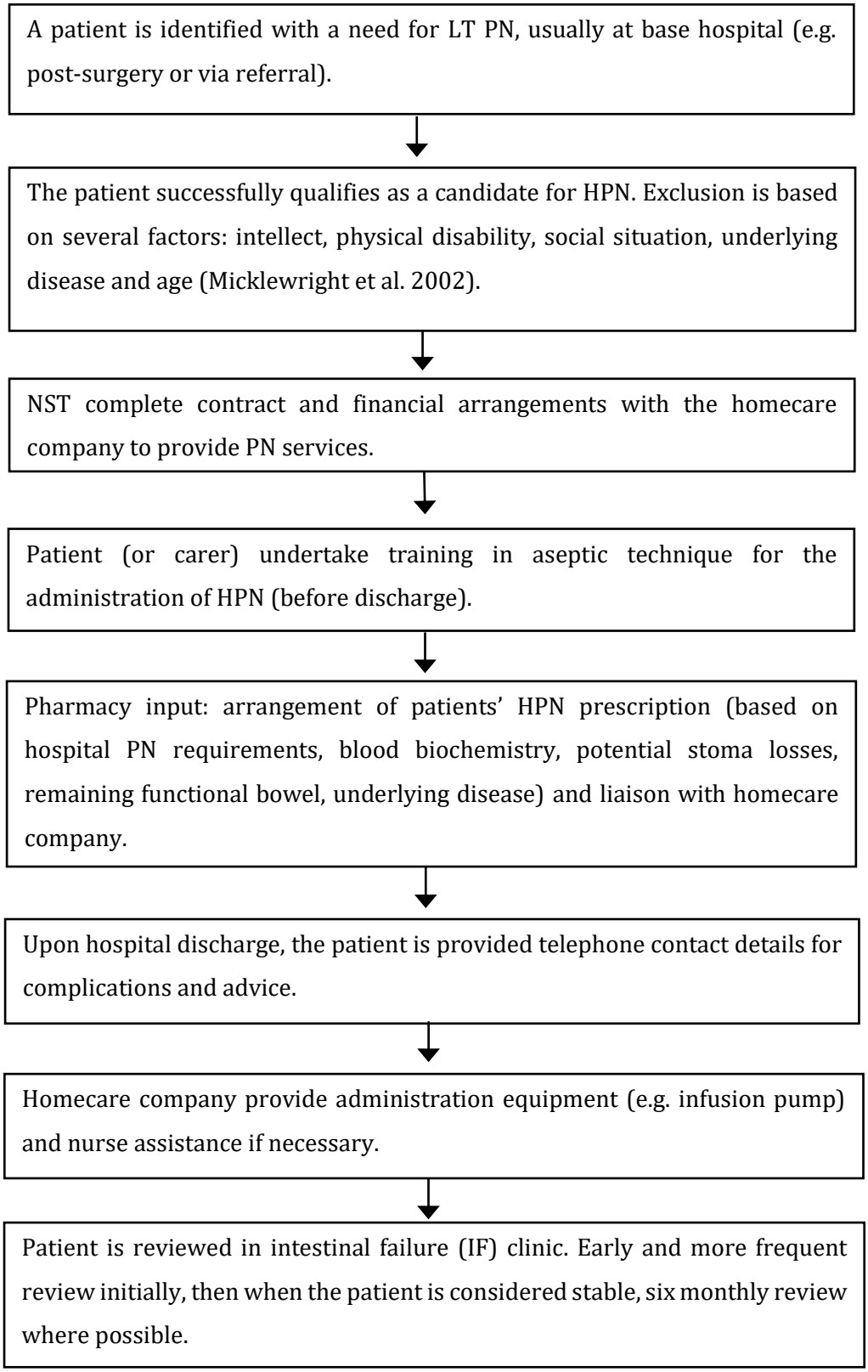


**Figure 1.1**. Flow chart to show initiation process for patients on LT HPN

## 1.2. COMPONENTS OF PN AND TYPICAL PATIENT REQUIREMENTS

For patients who are still able to consume nutrition orally, the extent should be taken into account in the first instance to gauge estimated patient requirements; alongside other factors including knowledge of their remaining gut anatomy, GI absorption, intended activity needs, underlying diseases and any fistula/stomal losses. As such, patient PN requirements vary considerably and current 'standard' bags aim to cater for a range of patient needs. For patients who are initially commenced on PN, their requirements are based on best estimates and then further refined over time according to their state of hydration and target weight as well as their personal preferences.

Table 1.1 summarises guidelines for typical adult nutritional requirements (before GI losses are taken into account). The individual requirements are further discussed throughout this chapter (Section 1.2.1.).

**Table 1.1:** Typical adult nutritional requirements for PN

| **Nutrient** | **Estimated patient requirements (before GI losses taken into account)**<br>BW=body weight | **Reference** |
|---|---|---|
| Nitrogen (excluding amino acid source) | 0.14-0.2 g nitrogen/kg BW/day | (Elia 1990) |
| Protein (amino acid) | ESPEN: unstressed adult HPN patient requires 0.8–1.0 g/kg BW per day.<br>ASPEN: 0.8-2 g/kg BW/day. | (ASPEN Board of Directors and the Guidelines Clinical Task Force 2002; Staun et al. 2009) |
| Carbohydrate (glucose) | 25–35 kcal/kg BW/day | (ASPEN Board of Directors and the Guidelines Clinical Task Force 2002; NICE 2006) |
| Lipid | Between 1 g/kg BW/day and 1 g/kg BW/week. Risk-benefit analysis between essential fatty acid deficiency and intestinal-failure associated liver disease (IFALD). | (Dupont et al. 2015a) |
| Water (fluid) | 30-35 mL/kg BW | (Tyler 1989) |
| Sodium | 1-1.5 mmol/kg BW | (Micklewright and Todorovic 2011) |
| Potassium | 1-1.5 mmol/kg BW | |
| Magnesium | 0.1-0.2 mmol/kg BW | |
| Calcium | 0.1-0.15 mmol/kg BW | |
| Phosphate | 0.5-0.7 mmol/kg BW | |
| Chloride | 1-1.5 mmol/kg BW | |

### 1.2.1. Energy sources and provision in PN

Glucose and lipid comprise the main sources of energy in PN with recommended glucose requirements set at 3-6g/kg body weight/day (Staun et al. 2009). Most patients are provided with lipid yet efforts are made to ensure its long-term provision is kept below 1g/kg body weight/day as over provision is associated with chronic cholestasis and IFALD (Cavicchi et al. 2000; Staun et al. 2009). On the other hand, caution needs to be taken with glucose provision as excess causes hyperglycaemia and likewise liver damage,

as evidenced by deranged liver function tests (LFTs) and steatosis/'fatty' liver, in which case, calculation of glucose oxidation rate gives an idea of the maximum amount of glucose an individual's body is able to utilise (Hartl et al. 2009; Rye and Nightingale 2015). Initially, patients are given glucose and lipid at a ratio of 50:50 (or 60:40), as provided by most triple chamber bags; in subsequent months this ratio is then reduced to 70:30 (or 85:15) for patients with long-term PN requirements (Staun et al. 2009). If patients show evidence of deranged liver function e.g. raised LFTs, cholestatic liver disease, then consideration is given to reducing or stopping parenteral lipid provision.

It is worth noting that the source of lipid incorporated in PN differ between manufacturers. Lipid emulsions containing long-chain triglycerides (LCT) (e.g. Intralipid®) or a mixture of LCT and medium-chain triglycerides (MCT) (e.g. Lipofundin®) have proven established use in PN. Meanwhile, more recent olive oil (e.g. SMOFLipid®, Clinoleic®) and structured lipid emulsions (e.g. Structolipid®) have also been safely used in PN, yet this area still requires significant research to consolidate their preferential use (Dupont et al. 2015a).

### 1.2.2. Carbohydrate provision in PN

Carbohydrate intake accounts for 45-55% of total dietary energy intake for most industrialised countries (Tappy 2015). Carbohydrates have a variety of forms, complex carbohydrates e.g. starch, disaccharides or simple sugars (e.g. glucose, fructose). Only glucose can be used as a carbohydrate energy substrate in PN since no enzymes exist outside the gut to break it down from more complex forms e.g. disaccharides, and fructose provision has been associated with adverse effects (Bode et al. 1973); consequently PN patients receive a greater proportion of carbohydrates as simple sugars than the general population. Glucose is used by all cells in the body and is the sole substrate used by the brain in regular, non-starved conditions. For people not requiring PN, after administration of a carbohydrate-rich meal, some glucose is temporarily stored as hepatic glycogen so that it is readily available when glucose absorption declines; however this capacity is limited to ∽100g (Tappy 2015). By comparison to administration of PN containing high amounts of carbohydrate, the temporary glycogen stores become saturated and excess

glucose is converted to fat by 'de novo' lipogenesis, in turn leading to deposition of intracellular lipids in organs and tissues, notably the liver, where over provision of glucose in PN is known to result in hepatic steatosis (Hartl et al. 2009). For this reason, carbohydrate content in PN should be optimally rationalised to prevent overprovision. Carbohydrate delivery in PN patients differs to that of a normal feeding pattern in the general population in that it is given continuously, with consequent risks of hyperglycaemia and 'de novo' lipogenesis; prevention lies in limiting excess glucose administration.

### 1.2.3. Lipid provision in PN

Healthy adults on an oral diet have recommended lipid requirements of 1-1.5g/kg/body weight/day. There exists a benefit-risk ratio analysis for the provision of lipid in PN and research suggests that lipid provision in PN should not exceed 1g/kg/body weight/day for those on LT PN (Cavicchi et al. 2000). The rationale for inclusion of lipid in PN is based upon the limited capacity for an individual on PN to oxidise glucose, alongside the provision of a calorie dense and rapidly usable energy source (9kcal/g metabolised fatty acids). Also, by supplying lipid to patients one also covers patients' essential fatty acid requirements (e.g. linoleic acid and α-linolenic acid) as well as fat soluble vitamins (Jeppesen et al. 1997). Lipids additionally have key bodily roles in phospholipid composition of cell membranes, receptor activities, cell signalling, cytokine function and gene expression (Wanten and Calder 2007). These factors lead to a consensus opinion for the advantages of lipid provision in PN over the concerns for its adverse effects, with particular reference to liver health (Dupont et al. 2015b). Lipid stability and decomposition in PN is still being researched, and as yet the clinical effects are still relatively unquantified. The potential for lipid peroxidation to occur can result in harmful labile peroxide radical species, Biesalski (2009) recommends the adequate provision of vitamin E as an antioxidant to ensure patient vitamin E requirements are met for the neutralisation of lipid peroxides.

### 1.2.4. Protein provision in PN

All adults require amino acids for various functions as structural proteins (muscle and collagen), plasma proteins (e.g. albumin, haemoglobin) and other specialised proteins (e.g. enzymes, cytokines, hormones, carrier/signalling proteins). Protein is provided in PN as solutions of mixtures of essential and non-essential amino acids; examples include Aminoven® and Intrafusin®. An amino acid intake of 0.8 g/kg/day is generally recommended for adult patients with a normal metabolism, which may be increased to 1.2–1.5 g/kg/day, or to 2.0 or 2.5 g/kg/day in exceptional cases (Stein et al. 2009). Sufficient non-nitrogen energy sources (e.g. carbohydrate and lipid) should be added in order to assure adequate utilisation of amino acids. Usually the gut mediates control of ingested protein and intermediary amino acid metabolism. When PN is administered, the protein sparing-function of the gut is bypassed; for this reason longer durations of PN administration are preferable to mimic nitrogen and protein homeostasis of regular adults (Soeters and Van de Poll 2015).

### 1.2.5. Water and electrolytes

Patient PN fluid requirements aim to ensure patients are well hydrated, taking into account any fluid and/or stoma losses as well as fluid cover for days when they do not infuse. Fluid requirements can vary greatly depending on gut anatomy and underlying disease states (Staun et al. 2009; Rye and Nightingale 2015). Initial requirements are based upon a best estimate and then further refined according to state of hydration (both patient reported and as evidenced by urea and creatinine results) as well as patient preferences in terms of length of PN administration (larger PN volumes requiring longer PN administration times) and/or nights requiring PN. Ideally, clinicians aim for patients to achieve a 1L/24 hour urine output with a random urine sodium level above 20mmol/L (Rye and Nightingale 2015).

For electrolytes, baseline requirements are calculated (see Table 1.1) and are then refined over time according to electrolyte blood test results. The guidance for estimation of baseline requirements assume normal organ function without any intestinal losses, further knowledge of anatomy helps to gauge

extra requirements. For instance, patients with greater small bowel loss will require greater amounts of sodium in their PN, or similarly, those with a jejunostomy may require magnesium supplementation. Once patient PN requirements are well-established in terms of fluid, calories and electrolytes, consistent blood tests are satisfied and the patient is deemed medically stable, the HPN prescription (and regimen) can be organised.

### 1.2.6. Micronutrients (vitamins and TE)

As they are a main feature of this PhD project, micronutrients are discussed in greater detail later in this thesis within Chapter 4 in relation to nutritional abnormalities.

In brief, micronutrients comprise the nutritional components of the human diet that are required in 'smaller' trace doses and quantities. They include vitamins and TE, each of which have essential roles in human health and physiology; contributing to the normal growth and development of living organisms (Forbes and Forbes 1997; Buchman et al. 2009). Micronutrients are supplemented to PN according to dosing guidelines and specific patient requirements (Staun et al. 2009). Micronutrient dosing is generally guided by blood serum monitoring in which the individual nutrient reference range determines whether the test result is within range. When the test result is out of range (i.e. in deficiency or excess), efforts are made to reduce or increase micronutrient provision in a patient's PN regimen.

## 1.3. COMPLICATIONS OF PN

Although the appropriate provision of parenteral nutrition has been heralded a success over the years, nevertheless there are still numerous troublesome complications associated with its use (Meadows 1998).

### 1.3.1. Central catheter care and line infections

The most frequently documented complication associated with LT PN use is infection related to the presence of the venous access device (Hartl et al. 2009; Pittiruti et al. 2009). The often high osmolarity of PN solutions require their provision via a large central vein e.g. subclavian or internal jugular vein. Tunnelled subcutaneous catheters or fully implanted ports are most frequently used for LT PN as they are associated with less infection risk over non-tunnelled catheters, which are more commonly seen for short term PN during a hospital stay. The occurrence of catheter-related infections causes significant discomfort and impairment of QOL, increases associated treatment costs as well as compromises the integrity of the catheter in situ, which may require replacement, and in more dire situations may compromise future venous access at the specific site. Treatment of bacterial infection includes the use of systemic antibiotics and successful recovery of the line is considerably variable, successfully reported in 30-80% of cases; however if fungi are shown to be present, line removal is necessary (Jeppesen et al. 1998; O'Grady et al. 2011). Patient education and training in aseptic technique as well as general barrier precautions are of the utmost importance to minimise the risk of venous access complications and/or infections (Sutton et al. 2005). Line locks with pharmacological agents such as ethanol, taurolidine, trisodium citrate (alone or in combination with anticoagulants e.g. heparin) are suggested for those experiencing repeated line infections.

### 1.3.2. Acute metabolic complications

Long-term complications of PN garner significant attention in research, yet the more acute complications still occur in some patients requiring PN. They include disorders relating to water and electrolytes which may require strict fluid restriction and control (e.g. via extra supplementation). Also, disorders related to glucose control e.g. hyper/hypo-glycaemia, in which case continual PN administration may be beneficial or reducing the rate of PN infusion, otherwise insulin administration may be necessary. Other acute metabolic complications include hypercalciuria and hypertriglyceridemia which will require optimisation of the PN feed for calcium and vitamin D, and the quantity/choice of lipid emulsion, respectively. Similarly, hypophosphataemia and hypomagnesaemia should be corrected before starting PN to avoid the onset of refeeding syndrome (see below).

#### 1.3.2.1. Refeeding syndrome

Mehanna et al. (2008, p. 1495) has defined refeeding syndrome as the 'the potentially fatal shifts in fluids and electrolytes that may occur in malnourished patients receiving nutrition'. It results from the provision of nutrition to undernourished catabolic patients, causing a quick shift to anabolism and a surge in insulin release. The insulin causes an intracellular shift in magnesium, potassium and phosphate, resulting in hypomagnesaemia, hypokalaemia and hypophosphataemia respectively. To prevent the onset of refeeding syndrome, nutrition should be given to patients at a maximum of 10kcal/kg/day and gradually increased over 4-7 days (NICE 2006). Appropriate provision of B-group vitamins (pyridoxine, riboflavin, thiamine) and electrolytes also need to be ensured to prevent associated Wernicke's encephalopathy (Mehanna et al. 2009).

### 1.3.3. Long-term metabolic complications

#### 1.3.3.1. Nutritional abnormalities

Patients sustained on LT PN experience nutritional abnormalities for specific nutrients, most commonly micronutrients; that is to say they experience a

greater degree of both extremes of out-of-range blood test results, deficiencies and toxicities/excesses (Rudman and Williams 1985; Hardy 2009; Conway et al. 2014; King 2015; Murphy and Lewis 2016). The implications of out-of-range blood test results can manifest themselves in a variety of ways depending on the individual patient and the nutrients involved (Fuhrman 2006; Shenkin 2008; Shenkin 2015).

Further detail regarding the occurrence of nutritional abnormalities (deficiencies/excesses) is given in Chapter 4. Each micronutrient is discussed in turn regarding its physiological role and what is known regarding its nutritional supplementation in PN with particular reference to any documentation of deficiencies or excesses in scientific literature.

#### 1.3.3.2. Liver steatosis and cholestatic liver disease

Liver steatosis is a common complication in LT PN (Nussbaum and Fischer 1991); it results in elevated liver aminotransferases and enlargement of the liver itself. It is usually associated with overfeeding of the glucose component of PN. Cyclic PN administration over continuous PN is thought to reduce its frequency (Kumpf 2006), as well as reducing glucose quantity in PN to match patient requirements.

Cholestatic liver disease is another complication affecting the liver with the potential to progress to cirrhosis and liver failure (Guglielmi et al. 2008). Patients present with jaundice, hyperbilirubinaemia alongside increases in γ-glutamyl transferase and alkaline phosphatase. Contributing factors are thought to relate to the decrease in the enterohepatic cycle owing to SBS, bacterial overgrowth, liver damage from lipid peroxidation products and lack of vitamin E as well as both glucose and lipid overfeeding (Sobotka 2000). In extreme cases, liver failure associated with PN administration has been associated with the need for intestinal and liver transplantation (Pironi et al. 2015).

#### 1.3.3.3. Cholelithiasis and acalculous cholecystitis

Patients who are exclusively fed parenterally are at a higher risk of developing gallbladder stasis, gallstones and gallbladder sludge (Pitt et al. 1983; Sobotka and Camilo 2009); loss of the effect of cholecystokinin, a hormone stimulated by enteral food consumption is thought to attribute to the onset of this complication (Aneta et al. 2014). Efforts to increase enteral consumption of food where possible are suggested as a treatment measure.

#### 1.3.3.4. Bone disease

Patients receiving LT PN experience metabolic bone disease e.g. osteoporosis, osteomalacia. It is associated with a loss of calcium from bone, increase in serum alkaline phosphatase and hypercalciuria as well as physical symptoms of bone pain and fractures (Klein et al. 1980; Seidner 2002; Hamilton and Seidner 2008; Pironi and Agostini 2015). It is multifactorial in nature and its relation to the provision of LT PN is not completely understood. Efforts to prevent or delay its onset focus on optimal provision of calcium, phosphate, magnesium and vitamin D in PN alongside moderate exercise (Shike et al. 1981; Sobotka 2000; Hamilton and Seidner 2008).

A more detailed introduction to bone disease in relation to LT PN is given in Chapter 7.

## 1.4. STABILITY OF PN

PN admixtures/solutions can contain as many as fifty components in a single container (Barnett et al. 2009). Each component as well as the sequence in which they are added to each other, can influence the overall stability of the resultant PN solution. Pertkiewicz et al. (2009) explains that stability testing over time ensures there are:

- No changes to the size and size distribution of lipid particles.
- No precipitation of insoluble complexes which have the potential to arise from reactions between individual components in the PN feed.
- Certifiable bioavailability of all intended PN components i.e. no degradation
- Absence of chemical reactions between components

The extemporaneous preparation of PN requires suitable practical skills, quality control and aseptic facilities to guarantee the intended composition, stability and microbiological integrity of the final PN solution (Barnett et al. 2009). Clinically relevant and well-known implicating factors contributing to PN instability include the stability of the lipid emulsion itself, calcium-phosphate precipitation, the Maillard reaction as well as reactions involving vitamins and TE. Other 'external' factors are known to influence PN, these include the type of storage material (multi-layered vs. oxygen permeable), environmental conditions (oxygen, light, temperature) and the addition of drugs (cimetidine, insulin, ranitidine). Over the last forty years a great wealth of information has been gleaned regarding the optimal stability and therapeutic use of PN and its individual components (Vanek et al. 2012; Berger 2014). Yet there is still more to consider in terms of bioavailability of individual PN components, especially in relation to nutritional abnormalities (deficiencies/excesses) in those requiring LT bespoke PN formulations.

### 1.4.1. Contamination of PN

TE contamination of PN is a known complication which occurs during compounding of the feed itself. Essentially, it refers to the inadvertent contamination (extra provision) of TE metals to PN solutions during manufacture and production (most commonly aluminium, chromium and manganese). It is believed to occur from the leaching of metal from materials used during manufacture e.g. needles, syringes, containers. Pluhator-Murton et al. (1999) reported its unquantified contribution to TE doses in PN to be potentially substantial. First acknowledged in the 1970s, TE contamination was a more-notable topic of research investigations, however in recent years its interest has waned and is demonstrated by notably less research publications (Hoffmann and Ashby 1976; Jetton et al. 1976).

## 1.5. INTRODUCTION TO THESIS

### 1.5.1. Research outline

The present PhD project investigates the provision of LT PN to a population of patients managed at Cardiff & Vale University Health Board (C&V UHB) in South Wales, a service which is commissioned by WHSSC. LT PN patients across Wales are managed from this HPN centre alongside a small minority in North Wales who are catered for by other HPN centres across the border. As such, the patients registered as receiving LT PN from C&V UHB represent the majority of patients receiving LT PN in Wales.

There is a great wealth of literature which details derangement of nutritional biochemistry in these patients. The literature documents the numerous issues to arise from these nutritional abnormalities, which include clinical and symptomatic consequences. Some well-known and more documented examples include vitamin A deficiency and night-time blindness, vitamin D deficiency and metabolic bone disease/increased risk of bone fracture, manganese toxicity and associated neurotoxicity/parkinsonian-like effects, selenium deficiency and brittle hair/nails, iron deficiency and associated iron-deficiency anaemia, to name but a few examples. Although PN is a well-established means of delivering IV nutrition to patients; the documented nutritional abnormalities in the literature show that there are issues and/or practical difficulties in providing the optimal nutritional requirements for LT PN patients (Fuhrman 2006; Buchman et al. 2009; Vanek et al. 2012; Fessler 2013).

The research in the present PhD project aims to observe the population of patients in Wales and discover whether there are similar incidences of nutritional derangement occurring in these patients by reference and comparison to what is documented in the literature. In this manner, it will be possible to observe what is happening 'in practice' to patients currently maintained on LT PN and it will be possible to explore reasons for the occurrence nutritional abnormalities in the patient population; and furthermore, assess the impact of these nutritional abnormalities on patient health.

There may be multiple possible explanations for incidences of nutritional abnormalities, for instance:

- Variation in approach to the practice of PN prescribing by different clinicians/prescribers (i.e. no formal guideline for the process of manipulation of doses of PN components)
- The frequency of patient monitoring
- The formulation or composition of PN additives (i.e. for TE and vitamins)
- Potential under-dosing resulting from unquantified PN instability or interactions between components in the PN admixture.

The initial explorative assessment of the patient population will guide further studies (i.e. subsequent chapters) throughout the PhD to research each individual avenue as a possible explanation for the occurrence of nutritional abnormalities. The results will be reviewed and evaluated in relation to the wider scope of documented nutritional abnormalities in the literature. The research findings from each chapter may suggest areas for improvement of current practice and/or help to identify ways to improve PN service provision for these patients (e.g. review of frequency of patient biochemical monitoring, revision of formulations for PN additives or a standardised approach to supplementing PN). Individual chapters will be hone in on particular areas, such as the adequacy of dosing of particular nutrients, exploration of factors which may contribute to their derangement (e.g. PN stability, dosage in PN additives) alongside further assessment (where possible) of clinical effect of the nutritional derangement on the patient (e.g. symptoms or other health outcome measurement tool). An example being vitamin D deficiency, its potential under-dosing in PN and adverse effect on patient bone health, which can be assessed by bone DEXA scanning.

Although the individual study designs and research methodologies employed throughout this PhD project may have been used before or elsewhere, they have not been researched in this level of detail, for as many nutrients, or in

such an all-inclusive population of patients maintained in Wales. As such the findings from the entire PhD project demonstrate originality and novelty. The individual study designs and methods have been used in a large and comprehensive group of patients which offer robust data over long periods of time. The over-arching research design and methodology of the entire thesis has not been employed elsewhere for a single population of patients. It offers a greater level of validity to the research as the successive findings from each chapter (i.e. each singular study) are from the same patients and give context to the greater research journey across each successive chapter (study). The inclusion of a respectable number of patients who represent the population of LT PN patients maintained in Wales (of which each patient represents a rich data source) provides value to findings and recommendations presented within this write-up. Particular sections of research (chapters six, seven and eight) have not be performed before, or documented in the literature for LT PN patients.

In brief, the PhD research journey began with an outline of the research permissions and approvals required in order to undertake the various studies within the PhD project. The research commenced with a cross-sectional description and analysis of population characteristics for the LT PN patients. This study set the scene for the PhD, succinctly displaying the population findings in terms of their patient characteristics, disease-related factors (e.g. disease state, indication for HPN) and factors related to their PN therapy. Then followed a comprehensive review of the published literature relating to the role of micronutrients in human health and physiology, alongside further related literature pertaining to nutritional deficiencies and/or excesses in PN populations. This gave an idea of reported problems or themes (of nutritional abnormalities) which may be occurring in LT PN patient populations. The findings from this literature review permitted familiarisation with the clinical field of LT PN/HPN and identified key areas of pressing clinical concern (evidenced by a greater emphasis within the literature) regarding the provision of micronutrients in LT PN. After this immersion in the literature, it was decided for Chapter 5 that an all-inclusive assessment of patients nutritional status (from their available blood test data) from the date they

were commenced on PN until the point of data collection, would best suit the data collection and analysis methodology so that one could ascertain current themes and/or trends in nutritional derangement experienced by the population of patients at C&V UHB, and whether they were in line with findings from the literature. This large study then highlighted key areas to follow-up on, namely the inadequate provision of TE (manganese toxicity and selenium deficiency) as well as a substantial proportion of patients showing inadequate vitamin D status. Attention was focussed towards the latter in Chapter 6 which aimed to investigate the stability of vitamin D in micronutrient additives to see if this could exclude its instability in PN as a potential source of under-provision for patients. Similarly, the finding of vitamin D inadequacy from Chapter 5 prompted a longitudinal assessment of LT PN patients' bone health while receiving PN (Chapter 7), since vitamin D deficiency is acknowledged to contribute to adverse bone health. Whilst the results elucidated in Chapter 5 for under/over-provision of TE were followed-up in the final study (Chapter 8) in which a service evaluation was performed to ascertain the optimal doses of TE by using a data-pairing model (prescriptions and blood test results). The research journey is brought together in the final discussion (Chapter 9) which describes, inter-links and appreciates all of the findings from the separate chapters as a whole, alongside appreciation of key literature and current recommendations. Also, in this chapter, the key findings are acknowledged, contrasted and compared against other key studies and publications from the greater literature relating to the characteristics of PN populations and the provision of micronutrients in LT PN. The thesis then closes with succinct recommendations for future work based on the research findings from the whole project.

### 1.5.2. Research question

- What are the characteristics of LT PN patients and how does the provision of LT PN affect their nutritional status and clinical health?

### 1.5.3. Aims

- To evaluate the demographic characteristics of a population of LT PN patients maintained on LT PN with C&V UHB (with reference to their patient-related, disease-related and PN-related factors).
- To ascertain the degree of the occurrence of out-of-range micronutrient blood test results in these patients.
- To establish links between micronutrient derangement (deficiency or excess) with clinical-relevant problems experienced by patients.
- To investigate causes for micronutrient derangement in patients' blood test results (e.g. doses in compound micronutrient preparations, or stability within final PN formulation).
- To evaluate the effectiveness of micronutrient dosing in LT PN patients and inform micronutrient dosing recommendations, through the correlation of PN micronutrient doses to blood test results.

### 1.5.4. Objectives

- To perform a review of the literature relating to the dosing and provision of micronutrients in LT PN/HPN patients; with particular reference to the documentation/publication of micronutrient derangement in these patients alongside current dosing recommendations and opinion.
- To use data (medical notes and test results) from LT PN patients in Wales to contribute to the research aims of this project.
- To employ both cross-sectional and retrospective longitudinal study designs to contribute to the methodological analysis of patient data.
- To use micronutrient blood test results (as depicted by the reference intervals with C&V UHB) to ascertain if there are recurring trends or themes of micronutrient derangement in LT PN patients.
- To consider (using laboratory techniques) the potential instability of micronutrients in compound multi-component PN additives as a source of under-provision of micronutrients.
- To implement a data-pairing model to correlate micronutrient doses (from each patient's PN prescription/regimen) to their recorded micronutrient blood test results, in so doing allowing the evaluation of the efficiency of micronutrient prescribing (at C&V UHB) and the available micronutrient preparations.
- To review and evaluate observed results and findings from this study against the findings of other peer-reviewed publications in this field.

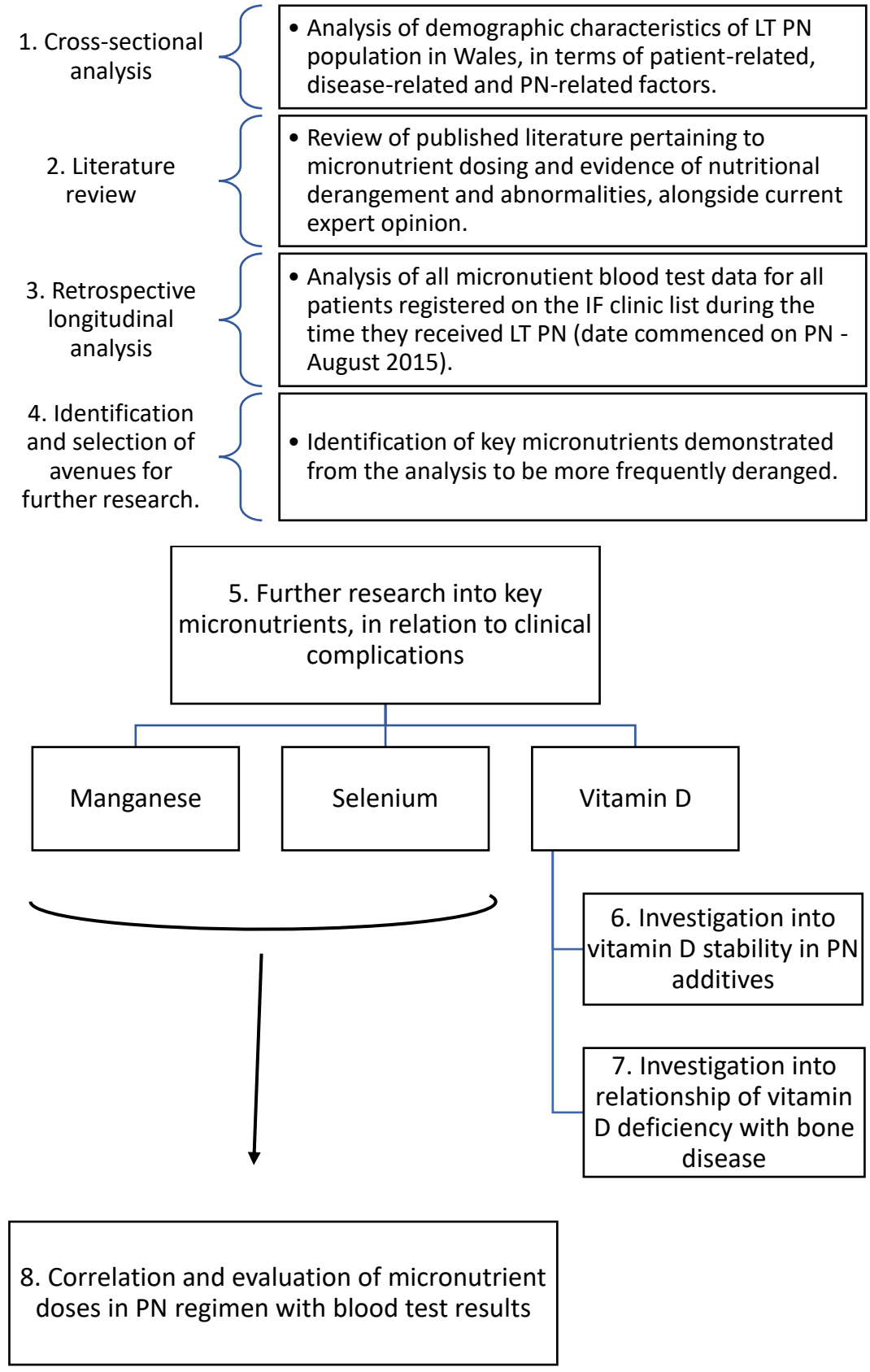


**Figure 1.2:** Flow diagram for PhD thesis.

# CHAPTER TWO:

## Ethics, research permissions and participant recruitment

## 2.1. INTRODUCTION

This chapter explains the research permissions that were gained in order to allow the individual studies and elements of research to be undertaken for submission of Ph.D. Specifically, the consented acquisition of patient data from a sample of the HPN population in Wales for research use, relevant to the project aims. The specifics of sampling and participant recruitment are detailed in this chapter.

## 2.2. RESEARCH APPROVALS AND PERMISSIONS

### 2.2.1. NHS research ethics committees **(REC)**

Proportionate ethical review was undertaken by the sub-committee of Wales Research Ethics Committee 7 (REC 7) on 08 April 2015 (see Appendix I). On behalf of the committee, the sub-committee gave a favourable ethical opinion of the research based upon the information described in the application, study protocol (see Appendix II) and supporting documentation.

### 2.2.2. National Health Service (NHS) management permission

NHS management permission, otherwise known as NHS Research and Development (R&D) approval, was granted by Cardiff and Vale University Health Board (C&V UHB) to allow the research study to be undertaken with C&V UHB as a single-site study as of 10 July 2015 (see Appendix III).

## 2.3. PARTICIPANT RECRUITMENT

### 2.3.1. Participant recruitment protocol and consent

Participants were recruited from the list of patients currently registered as routinely attending the outpatient intestinal failure (IF) clinic at C&V UHB.

Potential participants were recruited by a postal invitation to participate in the research study and a single follow-up telephone call was permitted for

non-responders two to three weeks later. These were the conditions approved by the NHS research ethics committee.

The postal invitation contained:

- An introductory letter of invitation (see Appendix IV)
- A participant information sheet (PIS) (see Appendix V)
- Two consent forms (one to keep, one to return) (see Appendix VI)
- A pre-paid return envelope.

The postal invitation was sent by Susanna Harwood, a specialist PN pharmacist independent prescriber at UHW and member of the clinical team.

The introductory letter invited the potential participants to take part in the study and also introduced the study and the researcher (Sean Dodington). The letter also directed potential participants to read the participant information sheet and make an informed decision about whether to participate.

Those who decided to participate returned the signed consent form back to the researcher (SRD) in the enclosed envelope. By agreeing to participate, participants thereby allowed the use of their hospital medical records to be used for the purposes of the study.

The population of patients who consented to participate were then assessed to see if they met the inclusion criteria (see below).

To put potential participants at ease regarding the use of their personal information, the PIS explained that no patient identifiable information was to be transferred for use in the study. Also, that the study was being undertaken in cooperation with the consultant practitioners and medical professionals responsible for their care.

### 2.3.2. Sampling

After personal communication with the HPN NST in Cardiff and as previously mentioned in Chapter 1, it transpired that there were ninety-eight patients registered as receiving HPN services in Wales as of July 2015.

The majority (ninety-three) of these patients were managed with C&V UHB, while the other patients were managed by minor HPN centres in Swansea and Wrexham; looking after three and two patients respectively. This cohort of patients was sampled as a representation of the HPN population in Wales. After a lengthy permissions process it became clear that data could only be realistically collected from a single HPN centre. This offered advantages in the sense that the data was representative of the majority of Wales where the standard practices were consistent for all patients in terms of monitoring, treatment and changes to PN therapy/regime; rather than differing between beacon HPN centres (if were included). There were disadvantages that the sample could not be statistically large enough or representative of the entire UK population, however the value of the long-term retrospective data from each of the participants is still sufficient to observe the main trends and patterns of nutritional status in the HPN population.

#### 2.3.2.1. Inclusion criteria

Patients were included if they satisfied the following inclusion criteria:

- Attending IF clinic and had been receiving PN for at least six months
- Able to understand and read English
- Able to give written informed consent
- Were of adult age (at least 18 years of age)

#### 2.3.2.2. Exclusion criteria

Likewise, patients were excluded according to the following exclusion criteria:

- Not been receiving PN for at least six months
- Maintained on non-nutritional/calorie-free IV electrolyte infusions (i.e.

magnesium sulphate infusions)
- Children (under the age of 18)
- Unable to understand and read English
- Unable to give consent

By ensuring that the patients who met the inclusion criteria had attended the clinic for at least six months, it guaranteed that there was a minimum of six months worth of hospital data and information for inclusion in data analysis. Also, in similar studies assessing the effect of long-term PN on patients' health, six months was used as an acceptable limit for the inclusion of patients in the study and to represent LT use of PN (Pironi 2002; Ladefoged and Jarnum 1978). Likewise, in the ESPEN guidelines on HPN in adult patients, 'long-term PN' is categorised as referring to patients who have been receiving PN in excess of at least six months (Staun et al. 2009).

Those patients who were receiving other intravenous electrolyte infusions instead of PN, most notably IV magnesium infusions were excluded on the basis that these infusions do not aim to nutritionally supplement the patients' requirements in the same way as PN. Their composition being that of a single salt in dextrose solution. The treatment aim in these patients is to replace the single electrolyte, magnesium, rather than nutritionally support patients in terms of their calorie, nutritional and fluid requirements.

By comparison to adults, children can have more complex and variable PN requirements as they develop. It would have complicated data analysis if this patient group was included in the study population. Also, the rationale for setting an age limit for participants to be at least 18 years of age was that it helped to decide an appropriate cut-off point where for research purposes the participants were assumed to have adult physiological body systems; in that they responded to the nutrients provided in their PN in a similar way. In this way, any conflicting factors associated with childhood or adolescence were avoided.

### 2.3.3. Finalised recruitment

Finalised research approval was granted by C&V UHB in July 2015 and participant recruitment promptly began by postal invitation (13 July 2015). Soon after, followed return of the participant consent forms to the researcher and the follow up phone calls to non-responders were scheduled at least two weeks afterwards.

The breakdown of participant recruitment can be seen in Figure 2.1, it shows the finalised sample recruitment after application of the inclusion criteria.

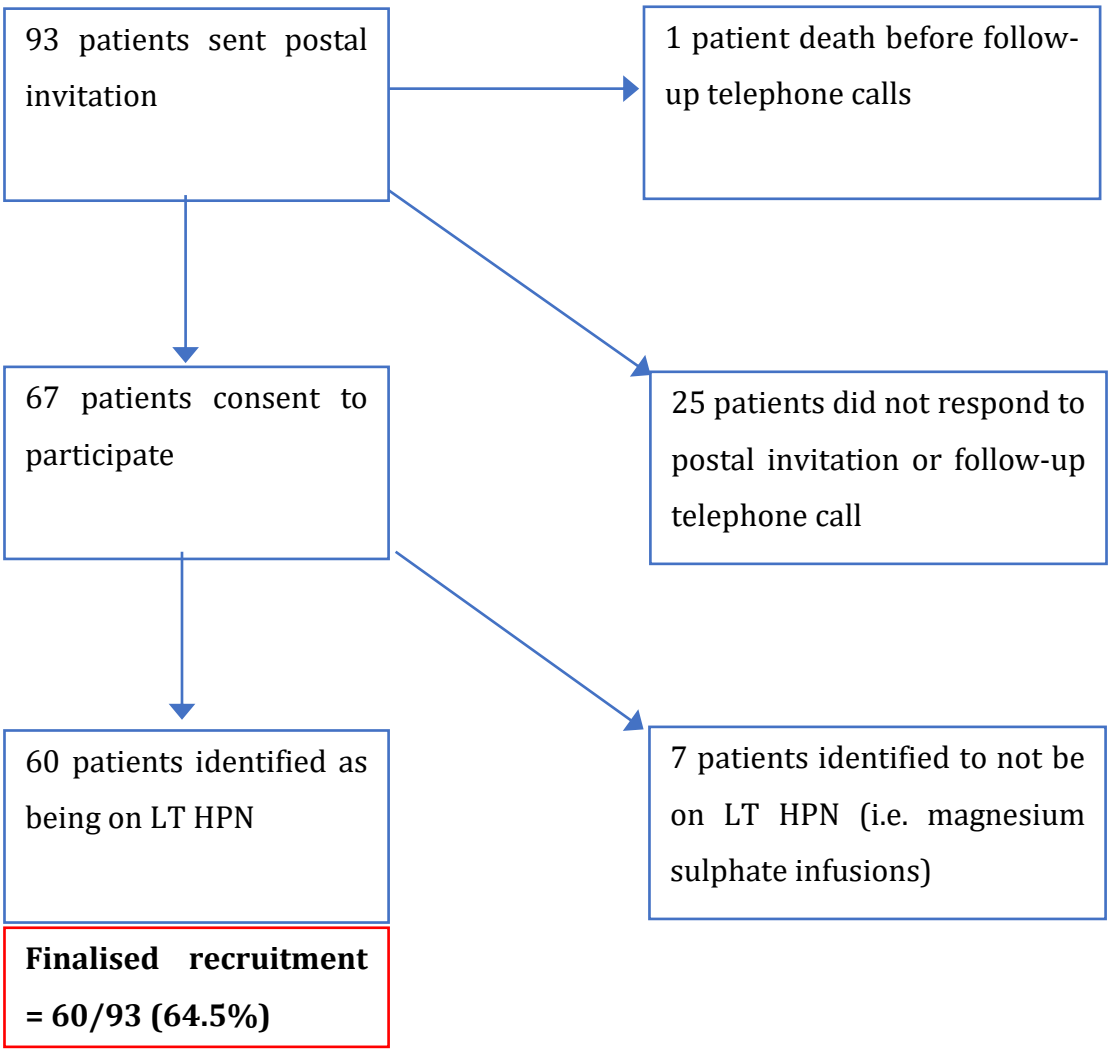


**Figure 2.1:** Flow chart to show participant recruitment

## 2.4. DATA COLLECTION

Patient data from the final participant population were eligible for use in the aims of this research project and included:

- PN Prescription records – maintained on the hospital S-drive
- Blood tests, clinic letters and other medical records – archived on the hospital 'Clinical Portal' system

The data represented a highly accurate, reliable and extensive secondary data source, abundant for potential research findings. As a secondary source of the data, it was already collected by other healthcare professionals involved in the care of these patients; not the researcher.

Specific data were collected and analysed in each separate chapter. More detail of the exact specifics of data collection is included in each chapter (i.e. methods and analysis).

### 2.4.1. Anonymisation and data security

Research permissions permitted the collection of data from the networked computer system at St Mary's Pharmaceutical Unit. It was anonymised and coded, then collated into an Excel spreadsheet, and further analysed with Microsoft Excel at Cardiff School of Pharmacy and Pharmaceutical Sciences. The anonymised data was transferred between sites on a USB device in which the files were password protected, and only accessed by members of the research team.

## 2.5. SERVICE EVALUATION APPROVAL

In the third year of the PhD project, service evaluation (SE) approval was sought from C&V UHB to evaluate the standard of micronutrient prescribing for their LT PN patients, specifically in relation to their blood micronutrient test results (see Appendix VII). This approval allowed data capture for the entire population of HPN patients registered with C&V UHB, rather than a subset sample population.

Further detail of the use of the data obtained from the SE and its related findings are given in Chapter 8.

# CHAPTER THREE:

## HPN in Wales: a cross-sectional representation of the HPN population

## 3.1. INTRODUCTION

This chapter details the prevalence of characteristics in the HPN population registered with C&V UHB.

In more detail, this chapter:

- Performed a cross-sectional analysis of the HPN population registered with C&V UHB
- Described and evaluated the characteristics of the cohort population of LT HPN patients from a single point in time with particular reference to their:
  - Patient-related factors
  - Disease-related factors
  - PN-related factors
  - Co-prescribed medicines
- Compared findings with results from other HPN population studies

### 3.1.2. Background and rationale

As mentioned previously, the point prevalence for HPN patients in the UK was most recently documented by BANS as 8.40 per million and the period prevalence as 10.02 per million over the year 2011 (Smith et al. 2011). A modest incidence by comparison to statistics reported in Europe where point prevalence has been reported to range extensively from 3.25-66 per million, yet the UK reports a similar point prevalence to the nations of New Zealand and Australia (Baxter et al. 2012). In general, countries with higher point prevalence (e.g. Denmark, Italy) presumably have greater standards in terms of patient referral pathways, access to HPN services, HPN education programmes and implementation of PN guidelines. Within the publication by Baxter et al. (2012) it was recognised that for countries with lower point prevalence (e.g. France, Spain) HPN registries were not fully available or implemented resulting in potential HPN under-reporting. It is clear to see that developments are necessary in gaining a universal standard for access to

equitable care in terms of HPN services across Europe. Interestingly, the prevalence of patients receiving HPN in South Wales has grown significantly in proportion to the last reported UK statistic and other documented demographics (Smith et al. 2011). The South Wales IF centre (managed within C&V UHB) reported a growth in service from 7.3 per million of the population in Wales (2001) to 35.9 per million as of March 2015 (personal communication, Barney Hawthorne & Amelia Juckes 2016). This is an impressive feat considering their initial target of 10-15 per million. The population supported in Wales by the Cardiff IF team (in terms of number patients per million of the population in Wales) therefore overtook the reported point prevalence statistic for the whole of the UK in 2011, according to the data published by BANS (Smith et al. 2011). However, the subsequent BANS report in 2016 noted an increase in new patient registrations for the UK to be predominantly attributable to new registrations within England (Smith and Naghibi 2016). In general, the statistics from the BANS reports detail a significant growth in service provision in UK over the last two decades and an increasing number of patients commenced on HPN, suggesting better patient access to HPN services. However, the reported statistics from BANS should be interpreted cautiously in light of significant under reporting of HPN cases.

In terms of the distribution of HPN patients across Wales, the majority reside in South Wales with most patients living close to Cardiff; alongside a smaller minority in North Wales. Reasons for this are thought to be that patients in North Wales are catered for by the geographically closer HPN centre in Salford, Manchester. Anecdotally, it has been known for patients to uproot so that they can reside within closer proximity to HPN centres (personal communication, Amelia Juckes 2016).

Although the number of patients who require LT HPN actually constitute a small proportion of all patients who receive parenteral feeding, the LT nature of their nutritional therapy marks these patients as major service users and a substantial financial burden on the NHS. This is demonstrated in the recent National Confidential Enquiry into Patient Outcome and Death (NCEPOD) in which 93% of patients in hospitals throughout the UK received PN for less than

30 days. Otherwise showing that the remaining percentage of patients constituted LT PN service users with types 2 and 3 IF (Stewart et al. 2010). The financial implications in Wales are demonstrated in the criteria for eligibility for funding and access to HPN treatment from the policy published by the Welsh Health Specialised Services Committee (WHSSC). The policy describes that funding for PN services is only permitted for patients who are awaiting reconstructive surgery (leading to restoration of gut continuity and function) or those with irreversible IF. WHSSC also funds nursing assistance for those unable to administer HPN for themselves. This funding is only available for patients in Wales via referral from the nutrition teams at either UHW or Hope hospital.

Although HPN is a vital and life-sustaining therapy in these patients, its use carries risks and complications that influence patient morbidity and prevalence statistics. A snapshot of the prevalence data from a single point in time will show the patient characteristics and requirements for the HPN population.

Few studies of population review have been performed in the field of HPN, presumably because they rely on voluntary and time-consuming data capture. Also, the logistics of data collection require considerable co-ordination between HPN centres within a unified area. However previous epidemiological studies have been performed by a harmonised and merged HPN special interest group called the Home Artificial Nutrition and Chronic Intestinal Failure (HAN & CIF) ESPEN Special Interest Group. This group performed multi-centre surveys for the prevalence of patients registered as having started HPN in the main HPN centres in Europe (Van Gossum et al. 1996; Bakker et al. 1999; Staun et al. 2004; Ugur et al. 2006). It was notable from these studies that the prevalence of HPN patients was highest in countries having the longest duration of HPN experience (Denmark, France and the UK). In the last decade there have been no new studies further investigating incidence and prevalence of HPN in Europe apart from Baxter et al. (2012) whose survey provided a global figure of HPN use in 9200 patients from 16 countries, in the year 2010. They also showed a large variation in point

prevalence of 3.25-66 patients per million of each population; but could not explain the varied result beyond differences in practices, expertise, interest, attitudes or economic provision of service. However it was recognised that HPN prevalence was under-reported in several countries.

One of the studies performed by the HAN & CIF special interest group, a survey performed in 1997, reported on the HPN prevalence, distribution of disease and health outcomes from HPN patients in Europe. They found the distribution of underlying diseases requiring HPN to be similar within Europe and the USA (Bakker et al. 1999; Howard and Ashley 2003). However, it is known that within Europe the distribution of underlying disease is more variable by comparison to USA. According to data from a survey performed in 1997 (Bakker et al. 1999), at the time the most common leading diagnosis for HPN patients in the UK was Crohn's disease, which was not in line with data from The Netherlands and Italy where cancer was the most common underlying disease state (Van Gossum et al. 1996; Smith et al. 2011). The data show differing approaches on the continent to sustain patients with PN therapy in those suffering from cancer diagnoses, in what is considered a contentious topic for clinical debate. Yet still, the UK statistic for new HPN registrations in 2010 was at 14%, up from 5% in 1997 (BAPEN report unpublished data; www.bapen.org.uk). Less than the European figure stated by Howard (2006) where as many as 40% of patients on HPN have cancer as a primary diagnosis. The variability in numbers of cancer patients treated with PN therapy suggest that there is a lack of strong evidence or direct guidance for treating these patients. The surveys performed by HAN & CIF ESPEN Special Interest Group have also explored PN-related parameters including the number of perfusions (feeds) and the types of feeds administered (aqueous/lipid) to the patients.

Previous research relating to the HPN service in Wales focused on its growth throughout the early 2000's and gave a brief overview of patient demographics (Jukes et al. 2010; Srinivasaiah et al. 2010). The present study aimed to capture information from the HPN population in Wales and document the current practices of LT PN provision; including relevant demographic, epidemiological and clinical data from the participants,

particularly in terms of the patients' diagnoses, indications and HPN requirements. The information from this study has not previously been published in such depth; and hence represents the most recent and accurate data for the sample population to draw comparisons to the findings of other similar studies.

## 3.2. METHODS

### 3.2.1. Research permissions

This study was conducted using the research permissions as described in Chapter 2.

Of note, fifty-nine out of the sixty participants that were recruited and maintained on LT HPN were eligible for this study. One patient was ineligible because at the cross-sectional point in time of data collection, the individual was no longer maintained on LT HPN (although had been previously when consented to participate).

The criterion for patients to have been maintained on HPN for at least 6 months was not necessary for this study, as data were to be collected from all participants registered as receiving HPN at the designated single point in time (cross-sectional analysis). This was in an effort to collect all relevant epidemiological data regarding the population characteristics from all participants; rather than exclude data from participants on the basis of their duration of HPN therapy in relation to their population characteristics.

## 3.3. Study design

This study was performed as a cross-sectional analysis, a form of observational research to investigate the demographic data for the Welsh HPN population in terms of patient characteristics and PN requirements.

Data was collected from a single point in time, this being the HPN records in use on the date 01 July 2015.

3.3.1. Data collection and sample population

Data was collected by the researcher from the medical records of consenting participants recruited from the outpatient clinic at C&V UHB. Specifically, relevant data were extracted from patients' medical notes (both the online

'Clinical Portal' system and written notes e.g. clinic letters, correspondence etc) and PN prescriptions.

The researcher performed data transcription checks for 10% of transcribed data and no themes/trends in errors were identified throughout data collection.

A full breakdown of the data parameters for investigation are given in Tables 3.1 and 3.2. Please note that Table 3.2 gives a more detailed list of the sub-categorisations for IF according to the ESPEN IF guidelines (Pironi et al. 2015).

### 3.3.2. Data handling, storage and analysis

Relevant data were manually transferred into a Microsoft Access database (Microsoft Office 2013 – version 15) for storage and handling, while data analysis was undertaken using Microsoft Excel (Microsoft Office 2013 – version 15).

Data were analysed using descriptive statistics, count, percentages and mean (±SD) of the total number of participants.

### 3.3.3. Data parameters

**Below Tables 3.1 and 3.2 describe the data parameters investigated in the study.**

Note, the categories for 'IF – pathophysiological classification (primary mechanism)', 'underlying disease (that causes the IF)' and 'disease state' were categorised and adapted from the same classification system used by ESPEN (Pironi et al. 2015).

The classifications which make up the category for 'Indication for HPN' were adopted from the clinical indications as described in the patients' medical notes at C&V UHB.

**Table 3.1.** The full list of data parameters for investigation in the study.

| | |
|---|---|
| **Patient-related factors** | - Gender (M/F)<br>- Age: mean (+-SD) (years)<br>- Mean duration requiring HPN (months)<br>- Patient weight before starting HPN (kg)<br>- Patient weight (July 2015) (kg)<br>- Difference between patient weight (July 2015) and at HPN initiation (kg) |
| **IF – pathophysiological classification (primary mechanism)** | - Short-bowel with jejunostomy (SBS-J)<br>- Short-bowel with jejunocolic anastomosis (SBS-JC)<br>- Short-bowel with jejunoileal anastomosis with an intact colon (SBS-JIC)<br>- Fistulas (F)<br>- Dysmotility (Mot)<br>- Mechanical obstruction (MO)<br>- Mucosal disease (MD) |
| **Underlying disease (that causes the IF) (further sub-categorisation – see table 3.2)** | - Short bowel<br>- Intestinal fistula<br>- Intestinal dysmotility<br>- Mechanical obstruction<br>- Extensive small bowel mucosal disease |

| | |
|---|---|
| **Indication for HPN (as clinically noted)** | - Short bowel syndrome (SBS)<br>- Malabsorption<br>- Obstruction<br>- Fistula<br>- Motility<br>- Failed ENT (enteral nutrition)<br>- High output (HO) stoma |
| **Disease state** | - Benign disease (BD)<br>- Active cancer (AC) |
| **PN-related factors** | - Number of days/bags administered per week<br>- Number of aqueous bags per week<br>- Number of lipid bags per week<br>- Calories per aqueous bag (kcal)<br>- Calories per lipid bag (kcal)<br>- Average calories per week (kcal)<br>- Average calories per day (kcal)<br>- Average volume of PN per day (mL)<br>- Volume of PN per week (mL)<br>- Number of micronutrient vials per week (Solivito N®, Cernevit®, Vitlipid N Adult®, Additrace®)<br>- Weekly provision of calcium from HPN (mmol) |
| **Co-prescribed medicines** | - Total number of prescribed medicines (excluding PN)<br>- Extra vitamin supplementation (outside of PN regimen)<br>- Bone health medicines<br>- Calcium<br>- Calcium and vitamin D (combined)<br>- Vitamin D<br>- Vitamin D (ergocalciferol) injection<br>- Bisphosphonates<br>- Denosumab<br>- Teriparatide |

**Table 3.2.** Full classification of underlying diseases (that cause IF) according to the type of clinical condition, as described in the ESPEN IF guidelines (Pironi et al. 2015).

| **Underlying disease (that causes the IF)** | |
|---|---|
| **Condition** | **Specific underlying disease** |
| Short bowel | *Extensive surgical resection for:*<br>• Mesenteric infarction (arterial or venous thrombosis)<br>• Crohn's disease<br>• Radiation enteritis<br>• Surgical complications<br>• Intestinal volvulus<br>• Familial polyposis<br>• Abdominal trauma<br>• Intestinal angiomatosis<br>• Necrotizing enterocolitis<br>• Complicated intussusception<br>*Congenital:*<br>• Gastroschisis<br>• Intestinal atresia<br>• Intestinal malformation<br>• Omphalocoele |
| Intestinal fistula | • Inflammatory (Crohn's disease, diverticular disease, pancreatic disease, radiation enteritis)<br>• Neoplastic (colon cancer, ovarian cancer, small bowel malignancy)<br>• Iatrogenic (operation, percutaneous drainage)<br>• Infectious disease (tuberculosis, actinomycosis)<br>• Trauma<br>• Foreign body |
| Intestinal dysmotility | • Acute (associated with critical illnesses):<br>– Post-operative<br>– Systemic inflammatory<br>– Neurological reaction<br>Chronic intestinal pseudo-obstruction (symptoms >6 months):<br>• Primary/idiopathic<br>– Neuropathic<br>– Myopathic<br>– Mesenchyopathy |

| | |
|---|---|
| | • Secondary<br>– Collagen vascular diseases<br>– Endocrine disorders<br>– Neurological disorders<br>– Medication associated<br>– Paraneoplastic<br>– Miscellaneous |
| Mechanical obstruction | • Obturation<br>• Intrinsic bowel lesions<br>• Extrinsic lesions |
| Extensive small bowel mucosal disease | • Microvillous inclusion disease<br>• Tufting enteropathy<br>• Tricho-hepato-enteric syndrome<br>• Intractable diarrhoea<br>• Severe food allergy<br>• Autoimmune enteropathy<br>• Intestinal lymphangectasia<br>• Waldman disease<br>• Common variable immunodeficiency<br>• Crohn's disease<br>• Celiac disease<br>• Radiation enteritis<br>• Chemotherapy related enteritis<br>• Congenital diseases |

## 3.4. RESULTS

Data was collected over a three-month period from July to September 2015 and the results are displayed below.

### 3.4.1. Patient-related factors

**Table 3.3.** Number and percentage of HPN patients in the sample HPN population.

| Number of patients | |
|---|---|
| Males | 20 (33.9%) |
| Females | 39 (66.1%) |
| M:F | 0.512: 1 |
| M:F (rounded) | 0.5: 1 |

**Table 3.4.** Analysis of further patient-related factors from the sample HPN population.

| | Mean (±SD) | Range |
|---|---|---|
| **Age** | 58.10 (±13.78) | 27-86 |
| Males | 59.90 (±11.89) | 35-81 |
| Females | 57.18 (±14.72) | 27-86 |
| **Duration requiring HPN (months)** | 66 (±78) | 1-344 |
| **Patient weight at HPN initiation (Kg)** | 55.39 (±16.30) | 27-107 |
| **Patient weight (July 2015) (Kg)** | 61.22 (±12.56) | 40-114 |
| **Difference between patient weight (July 2015) and at HPN initiation (Kg)** | 5.83 (±9.78) | -28-(+37) |

### 3.4.2. Disease-related factors

**Table 3.5.** Number and percentage of patients according to the ESPEN pathophysiological classification for IF.

| IF Pathophysiological Classification | Number of patients (and %) |
|---|---|
| Short-bowel with jejunostomy (SBS-J) | 33 (55.9%) |
| Short-bowel with jejunocolic anastomosis (SBS-JC) | 10 (16.9%) |
| Dysmotility (Mot) | 8 (13.6%) |
| Fistula | 4 (6.8%) |
| Mucosal disease (MD) | 3 (5.1%) |
| Short-bowel with jejunoileal anastomosis with an intact colon (SBS-JIC) | 1 (1.7 %) |
| Mechanical obstruction (MO) | 0 |

**Table 3.6.** Number and percentage of HPN patients categorised according to their underlying disease as the reason to their IF.

| Underlying disease (that causes the IF) | Number of patients (and %) |
|---|---|
| Short bowel - Crohn's disease | 14 (23.7%) |
| Short bowel - mesenteric infarction | 13 (22.0%) |
| Short bowel - surgical complications (including cancer resection) | 13 (22.0%) |
| Intestinal dysmotility – secondary* | 5 (8.5%) |
| Intestinal fistula - inflammatory (including Crohn's disease and pancreatic disease) | 3 (5.1%) |
| Short bowel - radiation enteritis | 2 (3.4%) |
| Mechanical obstruction - intrinsic lesion | 2 (3.4%) |
| Intestinal dysmotility - primary/idiopathic | 2 (3.4%) |
| Extensive small bowel disease - autoimmune enteropathy | 2 (3.4%) |
| Intestinal fistula - iatrogenic (operation) | 1 (1.7%) |
| Mechanical obstruction - extrinsic lesion | 1 (1.7%) |
| Extensive small bowel disease - congenital | 1 (1.7%) |

*Includes 4 patients with 'miscellaneous' underlying disease

**Table 3.7.** Number and percentage of patients categorised according to their indication for requiring HPN therapy, as clinically referenced by the NST at C&V UHB.

| Indication for HPN | Number of patients (and %) |
|---|---|
| Short bowel syndrome (SBS) | 43 (72.9%) |
| Motility | 6 (10.2%) |
| High output (HO) stoma | 3 (5.1%) |
| Malabsorption | 2 (3.4%) |
| Fistula | 2 (3.4%) |
| Failed ENT (enteral nutrition) | 2 (3.4%) |
| Obstruction | 1 (1.7%) |

**Table 3.8.** Number and percentage of patients according to their disease state.

| Disease state | Number of Patients (and %) |
|---|---|
| Benign disease | 56 (94.9%) |
| Active cancer | 3 (5.1%) |

### 3.4.3. PN-related factors

Results for PN-related factors of the HPN population have been expressed as a mean to show the average value for each parameter, a range to show the distribution/scale of the data and as a modal value to show the most frequently occurring values for each parameter in the population.

**Table 3.9.** Analysis of factors relating to PN administered to the sample population.

| Parameter | Mean (±SD) | Range | Mode |
|---|---|---|---|
| Total number of feeds administered per week | 5.06 (1.46) | 2-7 | 5 |
| Number of aqueous feeds per week | 4.27 (1.60) | 1-7 | 5 |
| Number of lipid feeds per week* | 1.45 (0.66) | 1-4 | 1 |
| Total calories per aqueous bag (kcal) | 1507.97 (553.03) | 200-2600 | 1800 |
| Total calories per lipid bag (kcal)* | 1886.28 (349.30) | 1350-2500 | 2000 |
| Total calories per week from HPN (non-nitrogen kcal) | 7953.36 (3255.01) | 600-14700 | 9800 |
| Average daily calories from HPN (kcal) | 1122.13 (461.60) | 85.71-2100 | 1400 |
| Lipid calories per lipid bag (kcal)* | 942.06 (170.62) | 700-1400 | 1000 |
| Average volume of PN per feed (mL) | 2466.83 (672.98) | 1297-4400 | 2000 |
| Total volume of HPN per week (mL) | 12948.90 (6595.50) | 5000-30800 | 6000 |
| Nitrogen per aqueous feed (g) | 9.77 (2.44) | 4-15.75 | 9 |
| Nitrogen per lipid feed (g)* | 10.28 (2.28) | 6.5-15.75 | 11 |
| Calcium per week from HPN (mmol) | 32.45 (22.34) | 1-105 | 35 |

Interestingly, thirty-two of the fifty-nine patients (54.2%) had a lipid bag included within their PN regimen. The result reflects the balance clinicians aim to achieve in supplying calories as both carbohydrate and lipid. The result shows that almost half of the sample population did not receive lipid PN; reasons are thought to be related to lipid intolerance, optimised glucose control (without lipid calories), absence of necessity, personal preference and its association with cholestatic liver disease (Cavicchi et al. 2000; Hartl et al. 2009; Rye and Nightingale 2015; Staun et al. 2009).

**Table 3.10.** Number and percentage of participants with each micronutrient preparation as a component of their PN regimen.

| Micronutrient preparation | Number (and %) of participants receiving micronutrient preparation in their PN regimen |
|---|---|
| Cernevit® | 53 (89.8%) |
| Solivito N® | 23 (39.0%) |
| Vitlipid N Adult® | 22 (37.3%) |
| Additrace® | 12 (20.3%) |

### 3.4.4. Co-prescribed medicines

**Table 3.11.** Analysis of medicines co-prescribed alongside PN regimen for participant population.

| Parameter | Mean (±SD) | Range | Mode |
|---|---|---|---|
| Total number of prescribed medicines | 7.71 (3.60) | 2-17 | 8 |
| Total number of bone health medicines | 1.67 (0.87) | 1-5 | 1 |

**Table 3.12.** Number and percentage of patients reported as receiving medicines relating to bone health and/or extra vitamin supplementation (outside of PN regimen).

| Medication | Number (and %) of patients receiving class of medication |
|---|---|
| Vitamin D supplementation (including combined preparations, high strength preparations and vitamin D injection)**,*** | 39 (66.1%) |
| Bisphosphonates | 18 (30.5%) |
| Calcium supplementation * | 15 (25.4%) |
| B-group vitamins | 12 (20.3%) |
| Vitamin A supplementation | 3 (5.1%) |
| Denosumab | 1 (1.7%) |
| Teriparatide | 1 (1.7%) |

* 10 patients documented as receiving combined formulations for calcium and vitamin D.
** 5 patients documented as receiving regular 3-monthly vitamin D injections.
*** 13 patients documented as receiving regular high strength vitamin D capsules (20,000 IU).

## 3.5. DISCUSSION

### 3.5.1. Patient-related factors

Prominent publications have documented the rise in incidence and prevalence of HPN cases in recent years (Van Gossum and Messing 1997; Bakker et al. 1999; Ugur et al. 2006; Wengler et al. 2006; Jukes et al. 2010). The HPN service in Wales is known to have grown considerably in this time, now equating to 35.9 per million of the population, comparably larger than the last documented statistic for the whole of the UK, and demonstrating an impressive access to HPN services in Wales. Although as previously mentioned, access to HPN services may not be as fair across the individual regions in Wales. Previous epidemiologic findings recorded in the 1990s noted a sharp increase in HPN use within the decade with causes thought to centre around the growing experience of specialised centres, increased survival of HPN patients and increased cost-effectiveness in the treatment of patients with benign disease; as well as the development of home care service provision (Van Gossum and Messing 1997).

Data relating specifically to the demographic characteristics of the sample population show that there were approximately twice as many female to male HPN patients and a similar age distribution between the sexes. It has previously been demonstrated that SBS more commonly arises in women than men (67%), reasons are thought to relate to women having shorter original bowel lengths than men (Nightingale et al. 1992). There was a large variation in the duration requiring HPN for the sample population as demonstrated by the wide range and the SD being greater than the mean value. This finding helps to support/demonstrate the varied and wide-ranging requirements in terms of PN therapy in this patient population, particularly in terms of their nutritional needs over time. It was almost an expected finding when considering that some LT PN patients may only require PN as an interim feeding measure before having restorative or corrective surgery. To give an idea of the context of these findings, nine of the patients in the sample had been commenced on HPN within the preceding year while the remaining 50 patients had been receiving HPN for periods longer than a year. The findings for sex

ratio, age distribution and duration requiring HPN are all in agreement with studies by Raman et al. (2007), Ugur et al. (2006) and Winkler et al. (2015).

France has recently published some brief yet interesting epidemiological findings from their paediatric population of HPN patients. By comparison to the findings from the adult cohort in Wales, SBS was again the dominating main indication for HPN and nearly all patients were fed tailored feeds via central venous catheters. However each centre on average managed less patients of which a higher proportion were male (56.9%) (Goulet 2016).

The results draw close comparisons to the sample in a cross-sectional study performed by (Raman et al. 2007) specifically in terms of patient-related factors. The results from our sample of 59 patients correlated very closely to their results for M:F ratio, mean age and duration requiring HPN. Although there were differences in that their sample represented only 37.5% of the estimated number of HPN patients in Canada at that time; however they did have a larger sample size of 150 patients.

As expected, patient weight was found to be higher from a cross-sectional moment in time than when the patients were initiated on HPN. Again this was similarly observed in the study by Raman et al. (2007) when they showed patient BMI increased significantly since the administration of PN. This reinforces the observations seen in practice where it is possible to observe the malnourished state of HPN patients prior to commencing HPN therapy and their improvement with LT parenteral feeding. Optimised PN therapy aims to get patients back to their ideal body weight (within realistic expectations) and natural gut adaptation (structural and functional) is also known to occur over time, helping to gradually reduce PN requirements for patients with retained ileum and colon, i.e not those with a jejunostomy (Nightingale 2006).

Very recently, HPN specialists in London conducted a survey to identify the prevalence of hospital IF and HPN services over a week-long period in the UK (Culkin et al. 2016). Of the thirteen major hospitals that participated, it was found that there were 1144 HPN patients requiring LT PN services within that

week. This was over a very broad range (5-352), exemplifying the different capacities and HPN experience between the hospitals and HPN centres. The patient-related and disease-related factors presented in this chapter were similar in distribution to the findings of this smaller scale study. The authors stressed the current pressures experienced by HPN homecare companies relating to capacity issues from the ever-growing demand for PN production services in the UK; presumably resultant from the increasing prevalence of HPN patients and greater access to clinical services.

### 3.5.2. Disease-related factors

As Nightingale (2006) explains there are three types of patients with a short bowel; jejunum-colon, jejunum-ileum and jejunostomy. Jejunum-ileum patients are uncommon and rarely require nutritional support; whereas jejunum-colon and jejunostomy patients are more frequently encountered. The results showed that those with a jejunostomy were the largest proportion of patients, depicting the group of patients in whom no gut adaptation occurs and they are completely dependent on PN for their fixed IV nutritional needs. Key findings from results relating to the disease-related factors in the sample population are summarised in Tables 3.5, 3.6, 3.7 and 3.8. The ESPEN classification for IF gives an insight into the anatomical reasons for bowel dysfunction in the patients. Collectively, it can be observed that the over-riding 'pathophysiological classification for IF' observed in the patient cohort was SBS in 44 of 56 patients; in particular for the category SBS-J (SBS with a jejunostomy). Figure 3.1 shows the graphical distribution of patients according to their pathophysiological classification for IF. This was anticipated since these patients are collectively more disadvantaged than those who have SBS-JC, who in turn are more disadvantaged than those who have SBS-JIC; a finding concordant with other studies (Gouttebel et al. 1986; Nightingale et al. 1992; Simons and Jordan Jr. 1969). It accurately displays the type of patients who require LT PN, those who have had substantial yet variable portions of their bowel resected and consequently require LT IV feeding to replace the fluid and calorie requirements that they cannot achieve themselves. However, not all SBS patients are defined by requiring lifelong PN, some are able to re-sustain themselves on oral nutrition. One of the main findings from an audit

by Gundogdu et al. (2016) showed that in the presence of an intact colon (regardless of SB remnant length), all patients should be given the chance to develop intestinal adaptation with careful nutritional management. Their audit into clinical outcomes associated with SBS found that mortality rates were greatly increased when smaller proportions of remnant bowel remained, particularly if the colon had been removed as well (100% mortality).

It was observed that mesenteric infarction, Crohn's disease and surgical complications (including cancer) were the most implicated diseases when patients were categorised in terms of their underlying disease that causes the IF according to the ESPEN IF classification; demonstrated by the larger number of patients with each disease/condition. These findings correlated closely with the disease distribution in 202 patients over a five year period in Denmark (Ugur et al. 2006). Interestingly fifteen patients (25.4%) [15/59 (14 SBS, 1F)] in the cohort were noted as having a confirmed diagnosis of Crohn's disease, comparably more than the 15% of patients diagnosed with Crohn's disease in the European survey by Van Gossum et al. (1996).

The next greatest number of patients fell into the category for 'intestinal dysmotility – secondary', where the motile function of the intestines do not function as expected, secondary to another condition. Patients are often diagnosed after exclusion of other disease states, namely obstruction and mucosal disease, and cause attributed to either other conditions or undefined idiopathic aetiology (Paine et al. 2013). A growing number of patients are diagnosed with intestinal dysmotility and require HPN, the percentage of patients from this study is appreciably similar to other studies (10-14%) (Lal et al. 2006; Mullady and O'Keefe 2006).

Again, SBS was the prevailing manifestation when the participant cohort were categorised according to their 'indication for requiring HPN therapy', in terms of the single most attributable reason for which they required HPN as clinically documented in medical notes (see Figure 3.2). The remaining patients were evenly spread out across the other indications, with the exception of a small minority categorised as needing HPN therapy for problems relating to gut

motility. Conditions observed here included diverticular disease, Erhlers-Danlos syndrome and chronic pancreatitis, alongside idiopathic causes for GI dysmotility. These results support those observed by Raman et al. (2007) and Van Gossum et al. (1996) in which the largest amount of patients had SBS as the indication for HPN, 60% and 31% respectively. In the UK and USA respectively, the BANS group and SUSTAIN registry both report SBS as the main indication for HPN (Smith et al. 2011; Winkler et al. 2016).

Over the last two decades, there has appeared a disparity in the diagnoses and indications for HPN between the UK and the rest of the Europe. In a European survey performed in 1993 from 488 patients, the leading underlying disease for HPN was noted to be cancer (42%), of which 67% of patients were from Italy and only 9% resided in the UK. This difference in HPN therapy for malignant diseases can still be observed with our results and has been corroborated by others in the field (Van Gossum and Messing 1997; Van Gossum et al. 1996; Pironi et al. 2007). For the findings of the present study, nearly all patients were considered to be in a 'benign disease' state as opposed to being in a state of 'active cancer' (in terms of the current status of their disease and conditions). This is thought to be a reflection of the funding allocation for patients on HPN since the WHSSC do not fund patients with active cancer relating to their HPN therapy. Understandably this is with the view that these patients do not suitably meet the criteria for LT HPN therapy, the guideline only stipulates funding allocation for chronic conditions or as an interim measure whilst awaiting reconstructive surgery (WHSSC Complex Conditions Management Group 2014).

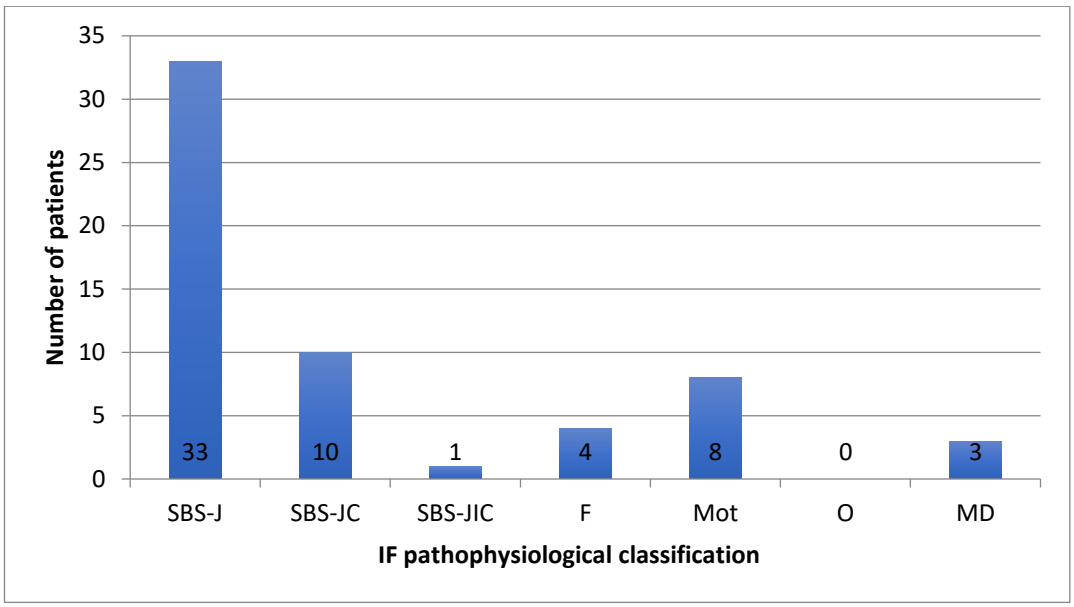


**Figure 3.1:** Patients categorised according to their pathophysiological classification for IF.

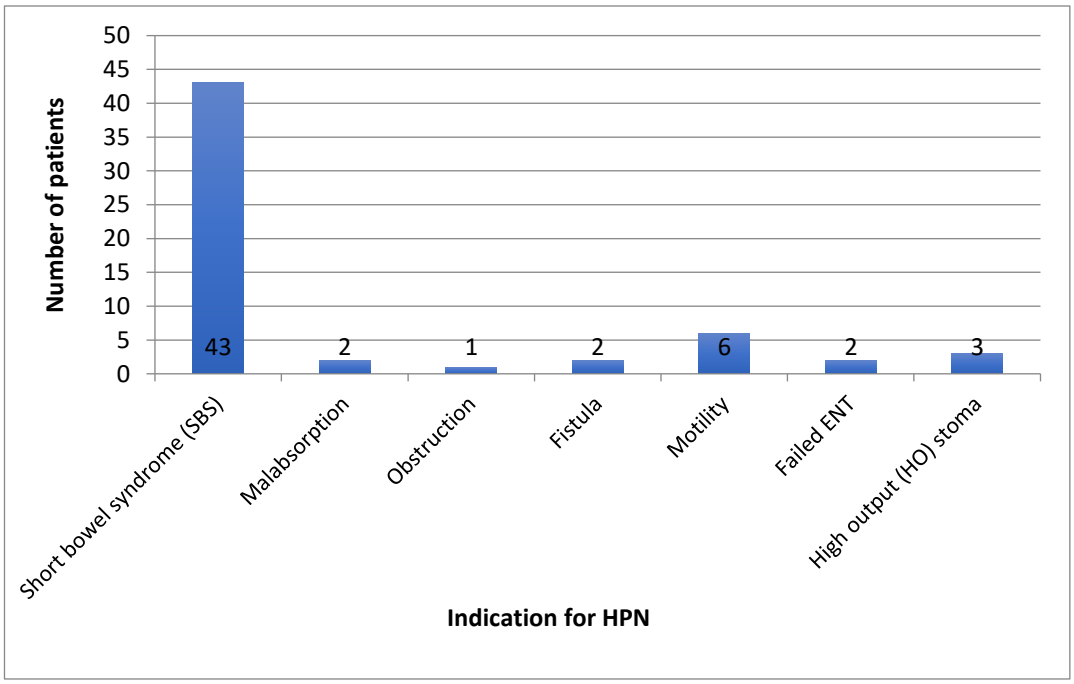


**Figure 3.2:** Patients categorised according to their indication to receive PN.

### 3.5.3. PN-related factors

The characteristics of the population shown in terms of their HPN therapy are as set out in Tables 3.9 and 3.10. Essentially, they depict the variable requirements of the population in terms of their fluid and calorie requirements. The Scientific Advisory Committee on Nutrition (SACN)

approximate that the daily estimated average requirement (EAR) for adults aged 55-64 is 2581 kcal for men and 2079 kcal for women (Scientific Advisory Committee on Nutrition 2011). One observes that the average calories in the aqueous and lipid feeds closely resemble the approximate requirements for the general population when allowances/considerations are given for modest oral (or enteral) feeding outside of the PN regimen.

On average, patients received more aqueous PN than lipid PN as part of their PN regimen over the course of the week. Our findings closely resemble those of another survey performed by the HAN & CIF ESPEN group whose patient cohort also only included LT PN patients. They found the mean duration of HPN was 7 years and the mean weekly number of nutritional bags was 5.6 including a mean of 1.6 lipid bags per week (Van Gossum et al. 2016). The reasoning thought to be that HPN teams are more reserved in the administration of lipid emulsions in clinical practice over the concern of the development of intestinal failure associated liver disease (IFALD) associated with their use (Cavicchi et al. 2000). IFALD is also more commonly termed as PN associated liver disease however there exists dispute whether factors relating to PN or the IF itself are implicated as the root causative factors for the onset of liver disease. Also, ESPEN HPN guidelines recommend that lipid should constitute only 15-30% of the PN regimen for LT patients (Staun et al. 2009). Dated studies have shown that 3-4.5% of total calories as fat appear to prevent essential fatty acid deficiency (EFAD) and that cholestasis is common when 500mL of Intralipid® is given more than three times a week (Barr et al. 1981; Cavicchi et al. 2000). As one can observe, there is a fine balance between providing sufficient lipid and creating adverse effects from optimal or overprovision. On one end of the spectrum minimal fat provision is recommended to be no less than 1g/kg of body weight per day (in healthy adults) to avoid essential fatty acid deficiency; no standards have been set for LT HPN patients but it is expressly considered to be no more than this amount over fears for inducing hepatic toxicity (Dupont et al. 2015a). In reality, current patients are estimated to receive less than this recommendation for lipid requirement (0.3-0.9 g/kg body weight per day) (Chambrier et al. 2004; Pironi et al. 2003; Reimund et al. 2000; Reimund et al. 2005; Vahedi et al.

2005). Unfortunately, studies are sparse that relate to determining appropriate lipid requirements in LT HPN, particularly with reference to the different lipid emulsions and their relative efficacy, safety and toxicity profiles. Ultimately, decisions regarding the inclusion of lipid in patients' PN regimens result from a risk-benefit analysis between patients acquiring essential fatty acid deficiency or developing IFALD. As mentioned previously, the area of lipid inclusion in PN provision requires significant research, in terms of both the types of lipid included in PN (e.g. LCT, LCT:MCT, olive, soy or structured lipids) and lipid dosing requirements for patients (Calder et al. 2010; Dupont et al. 2015b; Raman et al. 2017).

In the participant population, on average the total calories in the feeds containing lipid were higher than in those without (the aqueous feeds). Although this is a likely occurrence as in general fat emulsions carry more calories per volume than glucose (dependent on formulation and individual PN components); reasons are thought to relate to ensuring patients receive substantial provision of lipid in the feed because they are less frequently administered (on average 1.45 per week). A rather delicate compromise between giving sufficient lipid calories to reduce the likelihood of developing EFAD and reducing liver complications from overprovision. Another factor which contributes to this finding is the overall physical stability of the PN feed. Often and in particular for large volume feeds, greater quantities of lipid are required in proportion to the aqueous components to ensure the physical stability of the resultant emulsion (fat in water).

The amount of calcium in the patients' weekly PN regimen was included to give an insight into the provision of calcium solely from the PN regimen of these patients, so that the results could give an indication to whether they are receiving adequate dosages. As the results show there is a large variation in the amount of calcium patients receive from their HPN regimen. However, with hindsight, it is difficult to extrapolate conclusions from this data parameter when the amount prescribed in the patients' regimen are dictated by the levels in their blood i.e. whether or not they are within the normal reference interval and require more or less calcium supplementation in PN.

Also, this result should be interpreted cautiously in relation to calcium provision and its effect on bone health because as with other nutrient components, it is not known how much calcium these patients consume or receive from their oral and/or enteral diet outside of their PN regimen.

Table 3.10 shows that the vast majority of patients received the preparation Cernevit® to provide their fat and water-soluble vitamin requirements within their PN regimen. As the usual preparations of choice for addition to lipid feeds, Vitlipid N Adult® and Solivito N® were used in just over a third of the sample populations' PN regimens; this was reflected by the total number of patients in the sample who had lipid feeds within their PN regimen (n=32). Interestingly, only 12% (20.3%) of patients within the sample cohort received TE from the preparation Additrace® which is marketed for LT use to provide a daily dose of the nine essential TE. The direction to include these preparations in patients' PN regimens follows assessment of their nutritional status from micronutrient blood tests i.e. patients showing deficiencies of micronutrients would benefit from their supplementation and those with excesses or toxicities would indicate removal of the compound preparation from their PN regimen. This finding suggests that a large proportion of patients are not being routinely supplemented with the preparation, are unsuitable for TE supplementation or have already had the preparation removed from their PN regimen.

### 3.5.4. Technical aspects

On average all patients infused PN over 12-14 hours nocturnally and were all fitted with a form of central venous catheter to allow administration of the PN feed; most frequently a single-lumen broviac tunnelled catheter.

### 3.5.5. Co-prescribed medicines

The data show the wide range for the extent of polypharmacy in the sample population, particularly in terms of the number of co-prescribed medicines the patients administer alongside their HPN therapy. Reasons for this variation are thought to relate to the variable medication needs of patients within the

average age group of the sample cohort, the burden of co-existing medical conditions and GI-related pharmacologic treatment needs (e.g. high dose loperamide and codeine to help slow intestinal transit) (Nightingale 2006).

When medication data were subcategorised according to the different classes of bone health medicines, it was clear to see that declining bone health is a complication in the patient group. Results showed that 66.1% of patients required vitamin D supplementation outside of their PN regimen to help maintain bone health through its natural role in maintaining calcium and phosphorus serum concentrations to promote mineralization of bone (Holick 1996). Also, 30.5% of patients required bisphosphonate treatment in an effort to improve BMD; these patients have a defined diagnosis of osteoporosis to necessitate treatment with bisphosphonates. However the possible implication of PN therapy warrants further investigation considering the multifactorial nature of the onset of osteoporosis (Cohen-Solal et al. 2003). Of note, all patients receiving calcium supplements were also maintained on vitamin D supplements, whether in combined supplement form (e.g. Adcal $D_3$® ), or administered separately.

### 3.5.6. Other discussion points

This present study on the HPN population in Wales has been a unique opportunity to capture the demographics and characteristics of the population as a whole. However, there exist certain limitations to the study, the sample cohort only represented approximately 60% of the total population of HPN patients in Wales and was a small sample in itself considering an estimated UK prevalence of 1600-1700 patients. Although the results from the study give an accurate representation of HPN in Wales, they may not be generalizable to the whole HPN population in the UK. There are other technical considerations between different HPN centres in terms of their routine practices, approach to managing patients and their nutritional requirements. On the other hand, the advantages of this study over other studies is that all the patients were uniformly recruited from a single centre by a single research team, and as such there were no inconsistencies in data entry or their interpretation; all tests were performed according to local guidelines and protocol (without varying

practices between different centres). The accurate nature of the patient medical records as a data source strongly supports the validity of the findings. Its thoroughness and completeness as data source means that all relevant data from all participants was collected to give the most accurate representation of the population. The end result of the study being a comprehensive presentation of characteristics for the HPN population in Wales with a detailed/in-depth analysis.

## 3.6. CONCLUSIONS

In summary, this small-scale study has confirmed current findings for disease characteristics and shed light on PN requirements in HPN populations. The specialised nature of the LT HPN field and its niche attributes have been presented with associated research findings to add to the body of HPN work and inform those of interest, patients and service providers alike. This chapter has highlighted some avenues for follow-up in subsequent chapters, including the use of compound TE additives and their suitability for the LT PN population (e.g. if micronutrient preparations are marketed for the daily needs of all LT PN patients, then why are more patients not receiving them). Another notable finding and potential research avenue realised during data collection was the number of patients receiving treatment for bone disease, an area of pressing clinical concern that warrants further research into PN-related parameters which can affect patient bone health. Further studies will undoubtedly show that HPN practices will continue to evolve as the HPN knowledge-base grows and as clinicians aim to provide optimal HPN therapy for LT patients, especially those with IF. By documenting and evaluating HPN patients and their associated PN characteristics it helps to better LT PN management and clinical monitoring of the effectiveness of PN provision.

# CHAPTER FOUR:

## Micronutrient abnormalities in LT PN: a review of the literature

## 4.1. INTRODUCTION

This chapter serves as an introduction for information relating to micronutrients, their monitoring and dosing in HPN patients while also providing a concise review of the relevant literature pertaining to each micronutrient in relation to the occurrence of nutritional abnormalities in LT PN patients.

### 4.1.1. Micronutrients - background

Micronutrients are essential components of nutrition and therefore LT PN. As a group they comprise both TE and vitamins. They are vitally important for body cellular functions at biological, chemical and molecular levels. Their roles include mediation of biochemical reactions (as co-factors for enzymes), acting to stabilise or conform protein structures as well as receptor-site interactions (Prashanth et al. 2015). These processes require micronutrients and occur in the natural body systems including immune, antioxidant, inflammatory and metabolic functions. Humans have ongoing constant micronutrient requirements to keep body levels 'within range' so they are provided in patients' PN regimens to ensure they do not become deficient. Over time, micronutrient abnormalities have been shown to occur in LT PN patients causing nutritional deficiencies and toxicities (Rudman and Williams 1985; Forbes and Forbes 1997; King 2015; Shenkin 2015c); possibly due to concurrent medical conditions and/or over or under provision of micronutrients in the PN regimen.

### 4.1.2. Micronutrient status in HPN

As mentioned previously, there is a variable degree of dependency on HPN; some patients rely completely on PN to sustain themselves whereas others can have variable or uncertain nutritional intake from GI absorption of what limited diet they are able to consume orally. For this same reason there also exists variable micronutrient requirements in PN between the individual LT PN patients.

Patients receiving LT PN fulfil their micronutrient needs (both TE and vitamins) from commercial micronutrient preparations supplemented directly into the PN feed. The micronutrient preparations available in the UK have generally been developed to provide more than basal amounts of all micronutrients; the rationale being that catabolic patients would require increased micronutrient provision, or others may have increased losses (e.g. high-output stoma) or some patients may already present with deficiency states (Shenkin 2015b). The daily doses present in the daily unit vials are generally more than the oral dietary reference intakes and should be more than sufficient to meet patients' needs, particularly since IV administration bypasses GI absorption and its associated essential losses. (Panel of Dietary Reference Values 1991). The recommended requirements and doses of micronutrients in PN have recently been disputed by Vanek et al. (2012); their recommendations will be discussed in relation to the study results later in the chapter.

### 4.1.3. Commercial micronutrient preparations

Tables 4.1 and 4.2 show the different doses of micronutrients in the currently available micronutrient preparations. Worthy of note, these preparations are trademarked under different names in Europe e.g. Solivito/Soluvit, Vitlipid N Adult/Vitalipid N.

Elsewhere in Europe, the preparation Additrace® has been replaced with a newer product called Addeven® containing less zinc, copper and manganese, alongside more selenium. Similarly, a longstanding but now discontinued preparation called Decan® has been replaced in some countries by Nutryelt®, which contains less copper, manganese and fluorine, alongside increased provisions of selenium and iodine. Unfortunately these newer preparations face opposition against routine inclusion in PN amongst homecare PN suppliers in the UK.

**Table 4.1**: Trace element product compositions and international recommendations.

| **Trace element (µmol)** | **RNI (µmol)** | **RDA (µmol)** | **ESPEN recommended daily doses (µmol)** (Pironi et al. 2016) | **ASPEN recommended requirements (µmol)** (Vanek et al. 2012) | **Additrace® µmol** | **Addeven® µmol** | **Decan® µmol** | **Nutryelt® µmol** | **Tracutil® µmol** |
|---|---|---|---|---|---|---|---|---|---|
| Chromium | 0.5 | 0.6 | 0.2-0.3 | 0.2-0.3 | 0.2 | 0.2 | 0.289 | 0.19 | 0.2 |
| Cobalt | NR | NR | NR | NR | -- | -- | 0.025 | -- | -- |
| Copper | 19 | 14 | 4.7-.9.6 | 4.7-7.8 | 20 | 6.3 | 7.55 | 4.7 | 12 |
| Fluoride | 200 | 158 | NR | NR | 50 | 50 | 79 | 50 | 30 |
| Iodine | 1 | 1.2 | 0.5-1.2 | NR | 1 | 1 | 0.012 | 1 | 1 |
| Iron | 9.5 | 8 | 17.9 | NR | 20 | 20 | 17.9 | 18 | 35 |
| Manganese | 26 | 42 | 1.1-1.8 | 1 | 5 | 1 | 3.64 | 1 | 10 |
| Molybdenum | 0.5-4.0 | 0.5 | NR | NR | 0.2 | 0.2 | 0.261 | 0.21 | 0.1 |
| Selenium | 0.75-0.95 | 0.7 | 0.2-0.8 | 0.75-1.25 | 0.4 | 1 | 0.887 | 0.9 | 0.3 |
| Zinc | 145 | 170 | 38-61 | 46-77 | 100 | 77 | 153 | 153 | 50 |

Note: RDA, Recommended dietary allowance (USA); RNI, Reference nutrient intake (UK); NR, no recommendation.

**Table 4.2:** Vitamin product composition and international recommendations

| **Vitamin (units)** | **RNI** | **DRI** | **ASPEN recommended requirements** (Vanek et al. 2012) | **Vitlipid N Adult®** | **Cernevit®** | **Solivito N®** |
|---|---|---|---|---|---|---|
| Vitamin A (µg) | 700 | 1000 | 990 | 990 | 1050 | -- |
| Vitamin E (mg) | 5 | 10 | 10 | 9.1 | 10.2 | -- |
| Vitamin D (µg) | -- | 5 | 5 | 5 | 5.5 | -- |
| Vitamin K (µg) | 70 | 80 | 150 | 150 | -- | -- |
| Biotin (µg) | 100 | 150 | 60 | -- | 69 | 60 |
| Folic acid (µg) | 200 | 200 | 600 | -- | 414 | 400 |
| Niacin (mg) | 16 | 19 | 40 | -- | 46 | 40 |
| Vitamin B1 (thiamine) (mg) | 0.9 | 15 | 6 | -- | 3.51 | 3.1 |
| Vitamin B2 (riboflavin) (mg) | 1.3 | 1.7 | 3.6 | -- | 4.14 | 3.6 |
| Vitamin B6 (pyridoxine) (mg) | 1.4 | 2 | 6 | -- | 4.53 | 4 |
| Vitamin B12 (cyanocobalamin) (µg) | 1.5 | 2 | 5 | -- | 6 | 5 |
| Vitamin C (mg) | 40 | 60 | 200 | -- | 125 | 100 |

Note: DRI, Dietary Reference Intake; RNI, Reference Nutrient Intake.

### 4.1.4. Nutritional abnormalities - background

As previously mentioned, it has long been well-established that LT HPN patients are at risk of developing nutritional abnormalities (King 2015; Rudman and Williams 1985; Shenkin 2015b; Staun et al. 2009; Van Gossum et al. 2009). This is particularly notable for the micronutrient components of PN i.e. vitamins and TE, because it can be difficult to gauge the individual requirements for the diverse and complicated HPN population (Shenkin 2015b). In this chapter where the term 'nutritional abnormality' has been used in this context, it refers to the result of a recorded blood test for a particular micronutrient going out of its specified reference range in an individual HPN patient, either into deficiency or excess. The effects of having a nutritional abnormality can be diverse; in the context of deficiency they are known to relate to ineffective function relating to each individual nutrient and their associated physiological roles. Toxicity states can act similarly, disrupting physiological function but also causing cellular damage and potential deposition in tissues. It has long been considered that micronutrient abnormalities in PN may be associated with specific symptoms, for example selenium deficiency and hair loss, vitamin A deficiency and night-time blindness, manganese toxicity and neurological movement disorders or zinc deficiency and skin rash; the evidence for each varies considerably (Daniells and Hardy 2010; Hardy 2009; Maskarinec and Fowler 2016; Sidana et al. 2015; Vanek et al. 2012).

In the late 1990s it was recognised that there was a lack of investigation into how nutritional deficiencies occur, their extent and clinical significance (Van Gossum and Neve 1998). Much of the research that has been performed over subsequent years has been difficult to place in clinical context because it mostly relates to individual nutrients and is limited to case reports of nutrient deficiencies or toxicities. Of the research performed, nutritional abnormalities have been most notably demonstrated for the vitamin, TE and electrolyte components of PN; constituting the micronutrient components of PN (Shenkin 2015b; Sobotka 2011; Staun et al. 2009). They are required in far smaller quantities with usual daily requirements of less than 100mg yet are still vital nutritional components (Prashanth et al. 2015). The reason for the occurrence

of nutritional abnormalities is unclear and difficult to comprehend since HPN is tailored to the individual needs of each patient. Although it is thought that their smaller dosage requirements and less frequent monitoring, alongside the potential for patient requirements to fluctuate may play a role in the occurrence of nutritional abnormalities.

Most reports of deficiencies in HPN patients relate to the omission of a particular nutrient from the PN admixture, which in turn results in that particular deficiency. In previous years shortages of injectable multivitamin preparations has been reported to be responsible for nutritional abnormalities arising from the rationing of supplies of vitamins in IV preparations in patients from the US (Centre for Disease Control and Prevention 1989; Centre for Disease Control and Prevention 1997; Hanson et al. 2012). It is generally accepted that the longer a patient receives a set (defined) PN formula, the higher the risk that the formula will not match the nutritional needs of the patient (Fuhrman 2002; National Advisory Group on Standards and Practice Guidelines for Parenteral Nutrition 1998).

The stated micronutrient requirements for HPN differ markedly between USA and Europe (US Dietary Reference Intakes set by the Food and Nutrition Board versus the Panel of Reference Nutrient Intakes set by the Department of Health). Vanek et al (2012) published a comprehensive report on TE and vitamin requirements in PN. A notable point discussed in this report was the difference between the recommended oral and intravenous intakes of micronutrients. They explained the efficiency of intestinal absorption and homeostatic control/regulation of micronutrient levels from an oral diet; by comparison to the provision of IV nutrition to HPN patients which bypasses homeostatic control. The resultant variable IV nutrient requirements for HPN patients is additionally confounded by inter-patient variation, conveying the difficulty in accurately gauging micronutrient requirements in LT PN patients. It is worth noting that guidance for nutritional reference intakes are intended for a fit and healthy population rather than those with complex medical needs as demonstrated by patients on LT PN, their use as an acceptable and accurate comparative standard could be cause for debate.

TE supplementation by prescribers is usually directed by serum TE concentrations and it has been recognised that interpretation of the results from a patient's TE biochemistry can be complicated. It is thought that there is poor correlation of serum TE concentrations with tissue stores of TE (Btaiche et al. 2011). This can make it difficult to calculate the exact amount of TE that a patient requires, especially if they have other medical problems affecting TE clearance from the body e.g. cholestatic liver disease can lead to the accumulation of manganese (Hardy 2009). On the other hand, TE levels have been known to fall in situations of infection or metabolic stress which may complicate analysis by not truly indicating a deficiency state (Meadows 1998). The clinical accuracy and reliability of results from tests for blood biochemistry are clearly confounding factors in the correct interpretation of micronutrient status in LT PN patients.

Some possible explanations which may help to explain how nutritional abnormalities come to occur are as follows:

- Instability or compatibility issues arising during compounding or storage of PN may be responsible for reduced nutrient doses being delivered to patients (Ferguson et al. 2014).
- Contamination of materials used during the manufacture of the PN admixture could increase the content of certain micronutrient components (Bohrer et al. 2001). For instance, individual solutions or aseptic materials (e.g. metal needles or tubing) can result in excess provision of aluminium, chromium and manganese via contamination (Btaiche et al. 2011; Hardy 2009; Leung 1995).
- Errors or mistakes associated with the PN composition and regimen could be responsible for nutritional abnormalities. For example, the final PN feed not containing the correct composition of nutrients or the inadvertent omission of necessary changes to PN formulation during clinical review.
- Long-term administration of a set PN regimen (of the same composition) may result in a patient's nutrient levels gradually going out of range. For instance, even doses that are only slightly high or low could result in nutrient levels going out of range when given over a long period of time (Fuhrman 2002).

- Patient monitoring (tests/clinic visits) not occurring as frequently as clinically necessary. Individual patient's nutritional requirements may change over time and the PN formulation may gradually become less appropriate for the patient. The PN is not changed in time with the patient's nutritional needs (Shenkin 2008).
- Patient-specific factors may influence the distribution and utilisation of the PN components within the body (Fessler 2013; Shenkin 2008; Staun et al. 2009).

Some well-known factors that put HPN patients at risk of nutritional deficiency include:

- **Deliberate removal of compound micronutrient preparations from a patient's PN regimen.** Some micronutrients are only available in specific combination products and certain clinical situations may necessitate the removal of the product from the regimen altogether. For instance impaired liver excretory function can lead to accumulation of manganese and of copper, which is of concern in patients on HPN given that standard micronutrient preparations may contain too much of several metals for long-term intravenous administration (including manganese and copper). Removal of the compound preparation from the patient's PN regimen may in turn result in patients becoming unavoidably depleted of other essential micronutrients (Fuhrman et al. 2000; Spiegel and Willenbucher 1999; Staun et al. 2009).
- **The variation in PN regimen.** Some patients may receive PN therapy only a few days of the week to supplement their oral intake, by comparison to others who require it every day. There must be some residual gut function for these patients to absorb macronutrients and micronutrients on the days when they do not receive PN. However it depends on the composition of food and balance of micronutrients in the patient's oral diet whether the patient can meet their requirements in terms of TE and vitamins (Shenkin 2015b). Likewise, some patients may not receive lipid emulsion-containing PN as frequently as aqueous PN, and consequently there may be limited provision of fat-soluble vitamins.

- **Instability of micronutrients within the PN admixture.** The instability of micronutrients within PN admixtures has been well-documented and can result in less than the intended prescribed nutrient dose being administered to patients. Some examples include the oxidation of ascorbic acid (vitamin C) when oxygen permeable bags are used or the ability of copper to complex with some amino acids, resulting in reduced bioavailability (Allwood and Kearney 1998; Dupertuis et al. 2005; Thibault 2014). Another being the photo-degradation of retinol (vitamin A) by ultraviolet light (Allwood and Plane 1984). For these reasons surrounding micronutrients and the risk of instability within the PN formulation, it is common practice to make additions of micronutrients to PN immediately before infusion (Baines et al. 2001). However, this is not always the case for HPN patients who often have their formulations compounded remotely and delivered at weekly or two weekly intervals to their home residence.

### 4.1.5. Guidelines and monitoring of micronutrient status

Guidelines recently published by ESPEN recommend that LT PN patients are regularly monitored. This includes anthropometry (body size measurements), blood biochemical measurements at each clinic visit, annual bone dual energy X-ray absorptiometry (DEXA) scanning and micronutrient testing (for TE and vitamins) at least every 6 months (NICE 2006; Staun et al. 2009). Over time, the results from these tests have revealed nutrient abnormalities in some patients. A nutritional abnormality is considered to be when the result of a particular test has gone outside of its normal reference range, resulting in deficiency or excess for the specific nutrient. Healthcare professionals involved in the care of LT PN patients need to be aware of how to monitor, manage and resolve nutrient abnormalities.

ESPEN guidelines recommend that micronutrient assessment should be performed for HPN patients at initiation and then at six monthly intervals (Staun et al. 2009). Clinical management of the patient's micronutrient status can then be performed. For instance, further supplementing the PN formulation to correct any deficiencies or reducing doses in PN to correct any nutrient excesses.

Forbes and Forbes (1997) have previously shown that micronutrient status may not be optimal even with attempts to supplement the micronutrient needs of the patient. Nevertheless it has now generally been considered that so long as there is regular review of a patient's regimen to ensure adequate micronutrient provision, the event of a patient developing clinically relevant levels of deficiency should be rare (Shenkin 2015a). This can appear confusing when blood test results indicate values outside the reference range, which in turn continues to raise questions around the stability of micronutrients in PN and the adequacy of their provision in patient formulations. Shenkin (2015c) has also explained how the safety margin between the adequacy of provision and toxicity is large, and that it is difficult to over-provide micronutrients to patients in their feeds. However this is not the case for copper, manganese and vitamin D where there appears to be a fine balance between adequate and over/under-provision (see further detail under sections '4.2.1.1.' '4.2.1.3.' and '4.2.2.4' respectively).

#### 4.1.5.1. Accuracy of micronutrient assessment

There are difficulties in gauging exact TE requirements for LT PN patients based on current assessments for nutritional status. For instance, there exists poor correlation of serum TE concentrations with tissue TE stores (Btaiche et al. 2011), as well as underlying conditions that can affect TE balance e.g. copper accumulation in hepatic cholestasis or selenium/zinc losses via GI stomal fluids.

A hot topic within the literature was the lack of confidence in the correlation between micronutrient dosing, serum/plasma levels and tissue/body stores, particularly in the accuracy of micronutrient biochemical tests to define specific nutritional status (Btaiche et al. 2011). In practice, this proves troublesome for practitioners who are trying to interpret the values and their clinical context for each individual scenario. It also emphasises the need for newer and simpler biomarkers for use in the assessment and interpretation of micronutrient status in the clinical setting (Daniells and Hardy 2010).

There are some difficulties involved in the assessment of micronutrient status for HPN patients. For instance, it can prove challenging for clinicians to identify micronutrient abnormalities because the signs and symptoms of a deficiency or toxicity are neither specific nor sensitive for each micronutrient (Fuhrman 2002; Fuhrman 2006). Also, the time taken for a deficiency to develop can vary widely, ranging from several weeks in the case of iron to months or years for copper or selenium; with only extreme deficiencies leading to the development of clinical symptoms (Gallitelli 1995). It therefore makes the clinical determination of which nutrient needs to be reduced, omitted or increased problematic to decipher.

Another issue to consider is the choice of laboratory test for the suspected deranged micronutrient. The predicament being which sample to test (e.g. blood, serum, tissue, urine, hair) and the individual reliability and accurateness of each sample (Gallitelli 1995; Fuhrman 2006). This large variation in samples and tests makes it difficult to standardise or interpret deficiency or toxicity states. A good example being the novel biomarkers for selenium which have now increased the complexity of assessing selenium status and requirements (Nève 2000).

Another well-documented factor which affects interpretation of micronutrient test results is the acute phase response (APR). It is a plasma protein response which is a part of a complex series of physiological, haematological and biochemical events that make up the inflammatory response which occurs after tissue injury, illness or infection (Nichol et al. 1998). The size and duration of the APR are related to the nature and severity of the injury, as well as the presence of sepsis (Davies and Hagen 1997). The plasma concentrations of micronutrients such as selenium, copper, iron and zinc alter during active inflammation, depicted by raised C-reactive protein (CRP) and give an inaccurate presentation in biochemistry test results (Fraser et al. 1989). HPN prescribers are advised to be aware of the effect of the APR on assessment of micronutrient status in HPN patients, especially when implementing dose changes in their PN (Shenkin 2008; Staun et al. 2009).

4.1.5.2. Contamination of PN admixtures.

A relatively recent discovery is that PN admixtures can become contaminated from the individual solutions used in the manufacturing process. An unintentional occurrence by which TE are present as ubiquitous contaminants in various solutions; the degree of PN contamination made worse via its manipulation with various equipment during PN manufacture. A study by Pluhator-Murton et al. (1999) demonstrated that there was the potential for trace element toxicity from contaminated PN solutions. They identified that measured concentrations of TE in combined TE additives were higher than stated values and the relative amount of contaminated TE delivered to the patient could be substantial (Pluhator-Murton et al. 1999). This stresses the need for thorough sampling, handling and measurement techniques in the preparation of PN admixtures to ensure avoidance of contamination (Buchman et al. 2009). The micronutrients most notably implicated as contaminants of PN solutions include aluminium, chromium, iodine and manganese; such contamination could contribute or be responsible for TE toxicity states (Bohrer et al. 2001; Hak et al. 1998; Hardy 2009; Kruger et al. 2013; Moukarzel 2009). Where possible, monitoring and assessment of nutritional status should factor in the complication of PN contamination.

## 4.2. REVIEW OF RELEVANT LITERATURE

A review of the relevant literature relating to micronutrient abnormalities (deficiency and toxicity of both vitamins and TE) was performed between the years 2015 and early 2017. Subscription to online literature publication alerts allowed notification for the latest relevant publications during this period.

Rather than use an explicit and detailed search strategy, an unrestricted non-specific literature search was performed using Google Scholar. This enabled full control over all the relevant literature to be included, without unintentional omission of pertinent literature via a restrictive and complex search strategy. For example, many different scientific and colloquial terms exist for subject themes related to the literature review; micronutrients can be known under different names and/or abbreviations (e.g. vitamin B12, hydroxycobalamin, cobalamin), as different biological forms (e.g. vitamin D, vitamin $D_3$, cholecalciferol, colecalciferol, 1,25-dihydroxycholecalciferol, 25-OH vitamin D, activated 7-dehydrocholesterol), or selectively termed together (e.g. B-group vitamins or water-soluble vitamins). Additionally, terminology for expressing nutritional abnormalities can differ (e.g. toxicity/excess). A restrictive search strategy may have resulted in important literature being overlooked. Selective identification of relevant literature was also found via a 'snowball' style technique in which relevant publications were identified (and chosen) from the bibliography of another publication. While not the conventional choice of evidence-based literature review, the following literature appraisal provides an adequate introduction for the topic of nutritional abnormalities in LT PN patients. Although it aimed to include all relevant publications and scientific text, a degree of selective bias cannot however be excluded.

### 4.2.1. Noteworthy micronutrient abnormalities - TE

There are nine essential TE (chromium, **copper**, fluoride, iodine, **iron**, **manganese**, molybdenum, **selenium**, **zinc**), five of these (in bold font) are of greater clinical interest because there are concerns surrounding their safe supplementation in PN and they will each be discussed in greater detail. For the same reason they are the only TE routinely monitored by C&V UHB.

#### 4.2.1.1. Copper

Copper is essential for cell metabolism, having high concentrations in the liver and brain. It acts as a co-factor for many vital enzymes involved in energy metabolism, immune functioning, iron metabolism and wound healing (respective examples of enzymes being cytochrome C oxidase, copper-zinc superoxide dismutase, caeruloplasmin and lysyl oxidase) (Collins and Klevay 2011).

Deficiency is a well-known occurrence, particularly in LT PN when the copper provision is less than the necessary adult requirements (Fessler 2013). The most common risk factors for deficiency include malabsorption following surgery, excessive zinc provision, burns injuries and increased GI losses (Shike et al. 1981; Berger et al. 1992; Prodan et al. 2009; Shike 2009). It has been shown that shortages of micronutrient preparations have been responsible for copper deficiency in the past (Pramyothin et al. 2013). Deficiency presents itself with neurological abnormalities as well as haematological features such as anaemia and neutropenia (Kumar et al. 2004; Juhasz-Pocsine et al. 2007; Prodan et al. 2009). Authors have discussed the late presentation of copper deficiency since serum copper is initially replenished from hepatic stores, an example being up to nine years post-operative (Juhasz-Pocsine et al. 2007).

Copper toxicity is thought to result in oxidative damage to cells (Gaetke et al. 2014). Gaetke et al. (2014) showed symptoms relating to copper toxicity from contaminated water to be abdominal pain, vomiting and diarrhoea. It has been known to occur in LT PN through excessive provision in the feeds. Normally homeostatic mechanisms prevent copper accumulation in the body, however

impaired biliary excretion and cholestatic liver disease are known to contribute to copper toxicity (Blaszyk et al. 2005; Howard et al. 2007). Blaszyk et al. (2005) showed that elevated hepatic copper levels (>35μg/g) were reported in 89% of adults with abnormal liver enzymes levels who were on LT PN. It is important to note that in this study the authors believed it was PN-induced chronic cholestatic liver disease which lead to the increased copper levels and that it was unlikely to be a direct overload of copper from the PN regimen; thus displaying the multi-factorial nature of nutritional abnormalities.

Assessment of copper status and the related interpretation of patient requirements are complicated by the fact that the current available biomarkers are unreliable; due to insensitivity and the potential for false readings of toxicity from confounding factors. A well-known example being the effect of the acute phase response (APR), which stimulates hepatic synthesis of caeruloplasmin during related inflammation (resulting from conditions themselves e.g. Crohn's disease) which in turn gives an increased serum copper concentration, regardless of the true copper status in patients (Collins and Klevay 2011). Also for this reason, results that are within range during inflammation cannot reliably exclude deficiency. Another reason for poor reliability of measurement of serum copper is that it is known to correlate inadequately with tissue accumulation i.e. patients with copper toxicity may still have results within the reference range (Blaszyk et al. 2005). Therefore it is apparent that the full clinical picture should be taken into account, particularly if symptoms of deficiency or toxicity are present.

As with other TE, copper is a known contaminant of PN but without intended supplementation it is not thought to result in sufficient dosing for LT patients due to the numerous case reports of deficiency (Karpel and Peden 1972; Dembinski et al. 2012; Pramyothin et al. 2013; Frankel 2016).

Over the last few decades, there has been dispute over the recommended dose of copper required by patients on LT PN, resulting in updated dosing guidance from ASPEN in 2002 (Mirtallo et al. 2006). The guidance recommends a copper

dose of 0.3-0.5 mg/day (4.74-7.90 μmol); approximately two-thirds lower than previous recommendations (ASPEN Board of Directors and the Guidelines Clinical Task Force 2002). Regrettably, in a review undertaken by ASPEN the current multi-component TE preparations in Europe and the USA have been shown to provide up to twice the necessary copper requirements in LT PN, potentially causing toxicity (Vanek et al. 2012). Overall, it is clearly important to note the tendency for both the late presentation of deficiency, its reversible nature with corrective supplementation and also the potential for toxicity in patients with cholestatic liver disease.

#### 4.2.1.2. Iron

Iron is a vital TE in humans, it is contained within the biomolecule heme which is found in both haemoglobin and myoglobin. Both are complex proteins which bind iron and oxygen in blood and muscle, respectively (Lieu et al. 2001). Iron is also functionally associated with bodily enzymes such as cytochromes, catalases and peroxidases (Prashanth et al. 2015). It is usually only absorbed from food when necessary and binds to the transport iron-protein called ferritin. Iron absorption and metabolism is unique in that absorption is only mediated when body stores are deplete, iron excretion not being regulated (Vasudevan et al. 2013). Clinical iron deficiency results in severe disorders, one of the most notable being iron-deficiency anaemia (Lieu et al. 2001). Diffuse hair loss has been postulated to be associated with iron-deficiency anaemia in LT PN however the authors noted the limited evidence and data to support the connection (Daniells and Hardy 2010).

Forbes and Forbes (1997) noted that approximately 30% of HPN patients in their study developed iron-deficiency anaemia. Suggestions to the reasons for its occurrence being that micronutrient preparations and patient formulations contain low doses of iron and are limited in the amount that can be supplemented in each feed by its physical solubility (Koletzko et al. 2005; MacKay et al. 2009). In patients for whom this does occur, iron dextran infusions can be given to correct clinical deficiency. Vanek et al. (2012) explained that iron deficiency can occur simply due to short bowel, especially since iron is absorbed in the duodenum where some patients may have had

extensive surgical resections. They also stressed the difficulty in gauging a single recommendation for iron requirements in PN because of the variable requirements in menstruating women or patients who require frequent blood draws which have the potential to induce a negative iron balance (Burns et al. 1996). Also, it is not routine practice in the US to supplement lipid-emulsion containing PN with iron based on the theory that trivalent cations ($Fe^{3+}$) can destabilise the lipid emulsion (Fessler 2008), alongside the potential for IV administered iron to cause adverse anaphylactic reactions. By comparison to the US, iron deficiency in PN patients in the UK is marginally less of a concern since iron is routinely provided in multicomponent TE preparations (20 μmol) which are supplemented in PN; however it is rarely achievable to supplement much more than this dose in PN admixtures due to solubility limitations. In these situations, patients would then require further parenteral iron supplementation outside their PN regimen e.g. iron dextran.

#### 4.2.1.3. Manganese

As an essential micronutrient manganese plays a key role as a component of various enzymes needed in the synthesis of glycosaminoglycans and glycoproteins, required as components of connective tissue. Manganese is also needed for tissue maintenance, wound healing and energy metabolism; also as a co-factor for mitochondrial enzymes, especially superoxide dismutase.

The number of patients presenting with manganese toxicity has been a troubling concern for many years because of its clinical presentation of neurotoxicity and associated parkinsonian-like symptoms resulting from deposition in the brain (Bertinet et al. 2000; Fell et al. 1996; Reynolds 1994; Reynolds 1998). It is thought to relate to the presence of manganese as a ubiquitous contaminant in commercial IV admixtures and from general over provision in PN (Reynolds 1994; Bertinet et al. 2000; Hardy 2009; Conway et al. 2014). Complications relating to manganese toxicity are more renowned; they include cholestatic liver disease and iron deficiency. Around 90% of manganese is excreted in bile and as such patients with cholestatic liver disease accumulate manganese (from reduced excretion); a clinical situation made worse with excessive over provision of manganese (Hambidge et al.

1989). Also, iron competes with manganese for absorption and in the event of iron deficiency, there is more manganese available for absorption, leading to toxicity (Kim and Park 2014). The potential clinical consequences of manganese toxicity (hypermanganesaemia) is the development of subclinical tissue accumulation (symptoms not always readily observable) which can lead to possible irreversible neurotoxicity and parkinsonian-like effects if not dealt with promptly (Bertinet et al. 2000; Dickerson 2001; Hardy et al. 2008; Santos et al. 2014).

While there have been many reports relating to manganese toxicity; manganese deficiency is considered a very rare occurrence. Only one patient has been documented as deficient in manganese, presenting with weight loss, osteoporosis and abnormal blood clotting (Norose et al. 1992). Hardy et al. (2008) and Santos et al. (2014) have further stated the scarcity of manganese deficiency and that there is little evidence of its occurrence in human populations.

Manganese is a known contaminant of PN, occurring during compounding of the PN admixture (Hardy 2009). The study by Pluhator-Murton et al. (1999) showed that contamination is increasingly likely to occur with commonplace PN solutions of calcium gluconate, magnesium sulphate, sodium chloride and potassium chloride but were unable to give reasons for its occurrence. A dated view by some experts suggested that owing to the extreme unlikelihood of deficiency, manganese requirements were likely to be met by manganese contamination alone in the PN admixture (Dickerson 2001). However this standpoint has been recently contested. ASPEN currently recommend an adult dose for manganese supplementation in PN as 55 μg (1 μmol)/day (Vanek et al. 2012). This is considerably less than the current dose in the widely-used preparation Additrace, 265 μg (5 μmol)/day. Rationale for the decreased recommendation from ASPEN comes from a study in which the reduced dose maintained blood manganese results within the reference interval, without detectable changes in magnetic resonance imaging (MRI) signal intensity (a measurement of toxicity) (Takagi et al. 2002). The evidence base for this decision was backed by a recent systemic review by Baker et al (2016); yet in

light of their concise review of the evidence, they stated there was limited evidence behind the rationale for not supplementing manganese in LT PN patients. In recent years, many experts have recommended the intravenous provision of manganese in current additives to be reduced in line with recommendations from 265μg (5μmol) to 55μg (1μmol) per day (Shenkin 2001; Hardy 2009; Vanek et al. 2012).

To clinically manage manganese toxicity, often the multi-TE preparation must be removed entirely from the PN regimen and the other TE administered separately to the PN feed (where a suitable product exists) or infused separately. This practice can incur consequential effects in that patients miss out on other TE where there is no alternative preparation for supplementation e.g. iodine or chromium. Management of safe and optimal manganese provision in LT PN is labour intensive and clearly indicates a need for more suitable multi-trace element components.

#### 4.2.1.4. Selenium

Selenium is a physiological component of selenoproteins in the body (Lu and Holmgren 2009). These proteins have roles in antioxidant defence, decreasing inflammation, regulation of thyroid hormone metabolism and regeneration of reduced vitamin C. Glutathione peroxidases are a well-researched category of selenoproteins and an important group of antioxidant enzymes.

Selenium deficiency has been noted as a clinical problem, responsive to selenium supplementation (Van Rij et al. 1979; Baker et al. 1983; Levander 1984; Abrams et al. 1992; Shenkin 2009; Etani et al. 2014; Chen et al. 2016). Clinical implications of selenium deficiency result from impaired activity of selenoproteins with resultant impairment of antioxidant system and immune system. During LT PN, deficiency also clinically presents as cardiomyopathy and muscle weakness (Burke and Opeskin 2002; de Berranger et al. 2006). Further clinical features of selenium deficiency and toxicity are described in Table 4.3; note that states of toxicity are significantly less reported in LT PN patients than states of deficiency.

**Table 4.3:** Causes and clinical features of abnormal selenium status.

| Causes for deficiency | Clinical features of deficiency | Causes of toxicity | Clinical features of toxicity |
|---|---|---|---|
| – Acute illness<br>– Burns<br>– Decreased dietary intake<br>– GI losses<br>– Insufficient PN supplementation<br>– Renal replacement therapy<br>– Medications (e.g. steroids, sodium valproate, clozapine)<br>– Smoking | – Anaemia<br>– Cardiomyopathy<br>– Growth retardation<br>– Hair loss<br>– Macrocytosis<br>– Myopathy<br>– Muscle weakness<br>– Risk of infection<br>– White nail beds | – Excessive selenium provision and intake<br>– Excessive exposure in salt, soil, food or water<br>– Over-provision in PN<br>– Occupational exposure (rare) e.g. airborne via metal industry, chemical processes or painting trades | – Altered mental status<br>– Fatigue<br>– Garlic breath<br>– Hair loss<br>– Nausea<br>– Vomiting<br>– Abdominal pain<br>– Diarrhoea<br>– Peripheral neuropathy<br>– Tender and/or discolored fingernails |

After many reports of selenium deficiency there have been concerns over the adequacy of its dose in the extensively used preparations Additrace® and Decan® (Abrams et al. 1992; Gramm et al. 1995; Burke and Opeskin 2002; Chariot and Bignani 2003). For instance Additrace® provides only 0.4µmol/day and it has been suggested that this is not enough to correct depleted status or maintain selenium status in patients with greater needs (Malone et al. 1989). Shenkin (2015b) has suggested that intravenous requirements in PN should be within the range 0.75-1.25µmol/day. For those requiring increased doses in their PN feeds, larger doses of sodium selenite can be included to supplement the dose provided by TE preparations.

In practice, measurement of serum selenium is most widely used method for assessment of selenium status. Measuring serum selenium during acute illness (acute phase response) has the limitation that the result may be up to 30% lower than the true value; resulting in unreliable interpretation of selenium status in critical illness (Stefanowicz et al. 2014). This response during illness

is caused by redistribution of selenium from the bloodstream into tissues to support antioxidant defence, protein synthesis and cell proliferation (Steinbrenner and Sies 2009). Selenium content in red blood cells has also been used for selenium assessment, however as standard assessment technique it is not routinely used by UK HPN centres. Yet it has been proposed as a more reliable nutritional marker, particularly during critical illness (Stefanowicz et al. 2013).

Numerous reports convey selenium deficiency associated with insufficiently supplemented PN (Fessler 2013). Interestingly the interval from commencement of selenium-free PN to presentation of clinical symptoms ranged from three months to two years; however biochemical features of deficiency are thought to occur earlier. Symptoms relating to selenium deficiency include those indicative of congestive cardiomyopathy related to Keshan disease where there is inadequate dietary provision of selenium (Burke and Opeskin 2002); see Table 4.3 for further symptoms.

Selenium toxicity associated with PN administration has not been reported (Fuhrman 2006). It is thought that the amounts provided in PN admixtures are less than the requirements of many patients and certainly less than the tolerable upper limit for dosing; alongside the fact that any excesses are thought to be excreted in urine (Livingstone 2016). However contamination of PN components with selenium has been demonstrated but at amounts that are too low to be of concern (Pluhator-Murton et al. 1999). There is the potential for selenium toxicity to occur when increased supplemental doses are given LT to patients with renal problems, potential symptoms are described in Table 4.3.

In 2012, ASPEN proposed new recommendations for selenium requirements in PN patients (60-100 μg/d, 0.77-1.28 μmol/day), because it was consistently shown that previous doses recommended by ESPEN and the Task Force for the Revision of Safe Practices for PN did not maintain serum selenium concentrations in many patients (Mirtallo et al. 2004; Braga et al. 2009; Vanek et al. 2012). Baines and Shenkin (2002) showed that provision of selenium

from standard micronutrient preparations in post-operative patients was inadequate to restore antioxidant status; implying that further supplementation is required in critical illness. This is also the case for patients with increased GI losses (e.g. stomal losses), burns or acute kidney injury (AKI). It has been postulated that current multi-trace element products do not provide enough selenium to meet the needs of LT PN patients; some not meeting standard recommendations. These shortcomings can be overcome with further selenium supplementation (of sodium selenite injection) in addition to the standard multi-component TE products added to the PN admixture.

#### 4.2.1.5. Zinc

Zinc is the most abundant TE in the body playing a vital role in many systems, most notably in human growth and the functioning of the immune system. It is also required by transcription factors in gene expression and as an essential component of many enzymes involved in energy metabolism, protein synthesis and free radical clearance.

Zinc requirements in PN are stated to be around 2.5-5mg/day with larger requirements in those with significant GI losses (e.g. fistula, stoma or diarrhoea) and burns (Mirtallo et al. 2004; Jeejeebhoy 2009). Deficiency occurs when requirements are not maintained, when there is insufficient absorption and via increased bodily losses. The clinical consequences are unsurprising considering zinc's extensive role in the body. They include poor or stunted growth, skin rash, impaired wound healing and susceptibility to infection (Golden et al. 1978; Underwood 1977; Yanagisawa 2004).

Assessment of zinc status in PN relies on measurement of serum zinc and NSTs need to be aware of the limitations. Serum zinc measurement lacks sensitivity in deficiency, exhibits wide biological variation in patients and inaccurate results may be obtained from potential contamination during the sampling process (in the collection tubes themselves) (Livingstone 2015). Clinical assessment must accompany interpretation of serum zinc results given its insensitivity in early stage deficiency. Also, the APR has an effect on

interpretation of serum measurement of zinc because in acute illness zinc redistributes into cells giving false results of deficiency (Braunschweig et al. 1997). For this reason, reliable interpretation can only be assumed when inflammatory markers are low (Duncan et al. 2012).

Zinc deficiency has been shown to be responsive to supplementation in PN after clinical symptoms of a rash quickly resolved in a patient previously maintained on zinc-free PN (Kay et al. 1976). Likewise, rapid clinical responses were observed with zinc replacement therapy in LT PN patients experiencing hair loss (Daniells and Hardy 2010). These factors indicate the importance of optimal zinc supplementation. Furthermore, experts have stated that up to 12-17mg of additional zinc may be required per litre of GI fluid losses (Vanek et al. 2012), particularly in SBS as most absorption occurs in the upper small bowel. This would necessitate further supplementation of the feed or additional separate IV infusions. Unfortunately, current multi-component TE preparations are uncompromising for zinc dosing in individual LT PN patients and it is hoped that future preparations will cater for their needs.

#### 4.2.1.6. Other notable TE abnormalities

##### *4.2.1.6.1. Aluminium*

As a TE, aluminium is not believed to be an essential nutrient and subsequently it is not included as an active component of TE preparations. Yet concerns exist relating to its toxic effects as a contaminant of PN admixtures (Bohrer et al. 2001; Kruger et al. 2013; Lima-Rogel et al. 2014). Its known toxic effects for LT PN patients include CNS toxicity and accumulation in bone from being taken up during the bone mineralisation process, resulting in osteomalacia. These effects were proven in a study by Bishop et al. (1997) in which preterm neonates receiving aluminium contaminated PN had significantly lower developmental scores than neonates receiving aluminium-free PN.

The concentration of aluminium in PN admixtures has been shown to be consistently above the FDA recommended concentration limits and is largely the result of three additives, calcium gluconate, inorganic phosphates and

cysteine hydrochloride (Aiticho et al. 2011; Hernandez-Sanchez et al. 2013). Kruger et al. (2013) demonstrated ($P<0.0001$) that there was a higher aluminium content in the bones of LT adult PN patients versus control patients.

These widespread concerns of aluminium contamination in IV parenteral formulations prompted a new regulation from the FDA, "Final Rule for Al", mandating a limit on the aluminium content in liquid parenteral products to be no more than the safe upper limit of 25 mcg/L (Department of Health and Human Services 2003). Labelling requirements were also introduced in the U.S. to reflect this rule and inform people of the risk of CNS toxicity and bone toxicity associated with aluminium accumulation (Department of Health and Human and Services 2000). Ultimately, a systematic review of aluminium in PN performed by Hernandez-Sanchez et al. (2013) decided that the absence of a universal approach to lower aluminium concentration between manufacturers, along with imprecise information on aluminium content and high lot-to-lot variation result in poor regulation with aluminium concentration limits.

#### *4.2.1.6.2. Chromium*

Stearns (2000) described the debate over the essentiality of chromium as a TE since no enzyme or co-factor had been characterised. There are reports of chromium toxicity related to deliberate chromium supplementation in PN while trying to avoid deficiency states (Malone et al. 1989; Moukarzel et al. 1992). Contamination of PN with chromium is a known issue; it is especially associated with amino acid solutions (Hak et al. 1998). There is no evidence to suggest harm associated with excess chromium provision in adults; a ten year follow-up study showed no adverse events associated with elevated serum metals post metal-on-metal total hip replacement (Grubl et al. 2007). Yet others have expressed a need to lower the recommended amount included in PN admixtures because it is thought that patients on LT PN receive ample chromium from contamination of products used during PN manufacture; not from direct supplementation in the formulation (Moukarzel et al. 1992; Moukarzel 2009).

It is generally accepted that total body chromium concentration controls the absorption of chromium in the gut, where it has poor bioavailability and absorption. Its main role is in the regulation of insulin action and deficiency states induce a syndrome of glucose intolerance similar to that of diabetes, corrected with chromium supplementation (Anderson 1998). Chromium insufficiency has therefore been hypothesised as a contributing factor in the development of type II diabetes (Mertz 1993; Jeejeebhoy 1999).

#### *4.2.1.6.3. Fluoride (fluorine)*

Since fluoride deficiency has not been described in the literature there appears no basis for monitoring patient fluoride status (Nielsen 2009), however it is still regarded as an essential nutrient giving physiologic resistance to the enamel of teeth (Nielsen 2009). Its toxicity has been shown to result in dental fluorosis characterised by porous enamel (Whitford 2006). Studies have shown that PN provides relative amounts of fluoride by comparison to daily oral recommended doses of 1-4mg, since the IV route bypasses intestinal absorption by ∽50% (Forbes and Forbes 1997; Bouletreau et al. 2006; Fessler 2013). Nielsen (2009) explained that when determining fluoride intake, its provision from drinking water should also be assessed as it is thought to bring fluoride levels into normal range. A theory supported by Bouletreau et al. (2006) who expressed concern for the potential for fluoride toxicity from amounts given in PN alongside amounts in orally consumed water and tea. Although not a pressing concern for micronutrient dosing in HPN patients, more research needs to be performed to ascertain the optimal dose of fluoride needed by PN patients, considering the additional provision from drinking water.

#### *4.2.1.6.4. Iodine*

Iodine deficiency is still a well-documented occurrence in the general population, having adverse effects on growth, development and thyroid hormone production; deficiency is also notable in LT PN patients receiving unsupplemented PN (Zimmerman 2009a; Zimmerman 2009b; Zimmerman

2010). A dated study by Moukarzel et al. (1992) found thyroid function remained normal even without the provision of iodine in PN and they concluded that routine addition of iodine to PN was not necessary; stating that sufficient iodine provision was achievable from both potential contamination of PN and the use of povidone-iodine as an antimicrobial agent for safe care of the central venous catheter site. However, more recently (Guidetti et al. 2014) showed that HPN patients generally had a low intake of iodine as displayed from urine iodine concentrations. They found evidence of subclinical hypothyroidism in approximately a quarter of patients and concluded revision of the lower ESPEN reference range limit for iodine may be necessary especially in light of the decreased use of iodine containing antiseptics. Recently, a recommendation was made at a micronutrition research event, experts came to the decision that the addition of 70-150mcg/day of iodine to adult formulas is necessary, following the decreased use of cutaneous povidone-iodine (Buchman et al. 2009).

More recently in 2014, a notable case of iodine deficiency was reported in a PN-dependent adolescent (Mortensen et al. 2014). The authors stated the current strategy of limiting lipid dosing to PN patients to prevent PN-associated liver disease may play a role in the prevalence of iodine deficiency, especially since the iodine content of Intralipid (Fresenius Kabi®) has been estimated at 15.1mg/L, which may appear minimal but should not be considered insignificant (Belfort et al. 2012; Mortensen et al. 2014).

Current evidence suggests iodine supplementation may be beneficial in some patients and perhaps review of its requirements in PN is necessary. Mortensen et al. (2014) also expressed the need for an individual iodine preparation to cater for more variable requirements. Unfortunately, iodine levels are not routinely monitored at C&V UHB for their population of HPN patients.

#### *4.2.1.6.5. Molybdenum*

The essentiality of molybdenum supplementation in PN is arguable (Leung 1995). A single report of deficiency in LT PN exists which was related to

intolerance to amino acid solutions, corrected by an infusion of ammonium molybdate (Abumrad et al. 1981). High doses of molybdenum (>0.5mg/day) are thought to cause significant urinary copper losses, however far lower doses exist in current TE preparations (Deosthale and Gopalan 1974).

### 4.2.2. Noteworthy micronutrient abnormalities – vitamins

Comparable to TE, the dosing and monitoring of some vitamins are of greater clinical concern in HPN patients. Below, five key vitamins are discussed in greater depth as they are noted as being of current topical interest within the HPN community, especially regarding their optimal dosing and associated clinical implications. As such, these vitamins are routinely monitored by HPN centres and are directly monitored in biochemical blood tests (in relation to PN supplementation). Other vitamin abnormalities are discussed in less depth.

#### 4.2.2.1. Vitamin A (retinol)

Vitamin A belongs to a group of compounds called retinoids that are essential for vision, growth, functioning of cellular processes such as development, reproduction and the immune system (Olson 1987). It is found naturally in dairy, fish, liver and eggs. However in those receiving PN, dietary insufficiency, fat malabsorption and zinc deficiency can all predispose patients to vitamin A deficiency, resulting in poor outcomes in any of the vitamin A–related functions (Vanek et al. 2012). Symptoms of deficiency present themselves as night-time blindness, xerophthalmia and changes in T-cell immune function (Stephensen 2001). Serum retinol is monitored to assess vitamin A status, similarly measurement of retinol-binding protein (RBP) is also used but to a lesser extent than serum retinol. As with other micronutrients, during periods of infection or stress, measurement of retinol is unreliable; in these situations, measurement of RBP would be more desirable (Rosales and Ross 1998).

Regarding nutritional deficiencies of retinol in PN, most data relates to PN administration to neonates as they require higher relative doses due to low stores and increased needs for growth and development (Greer 2001; Haas et al. 2002); further researched because of known stability problems and implications of under-dosing retinol in PN delivery systems (Shenai et al. 1981; Allwood and Plane 1984; Thomas et al. 1991; Allwood and Martin 2000; Ord et al. 2016). Hack et al. (1990) found retinol deficiencies in 52% of their post-operative neonates requiring PN for longer than two weeks. Besides dated publications reporting retinol deficiency in 26-43% of LT PN patients,

no recent studies have investigated retinol deficiencies or excesses in adult PN patients (Howard et al. 1980; Dempsey et al. 1987; Labadarios et al. 1988). However, it is worthy of note that in the aforementioned papers, the vitamin preparations were added prior to storage rather than just before infusion, allowing more time for potential retinol instability to occur e.g. via photo-degradation or adsorption.

Vitamin A toxicity has been shown to be less common but sometimes observed in those with renal failure or liver dysfunction and those receiving over provision in IV nutrition (Gleghorn et al. 1986; Shenkin 2008; Vanek et al. 2012). Bone abnormalities have been described in relation to toxicity, though are thought to result from vitamin A antagonism of vitamin D at receptor level (Rohde et al. 1999), which in turn results in net bone resorption (Johansson and Melhus 2001).

#### 4.2.2.2. Vitamin B9 (folate/folic acid)

Folate is a naturally occurring essential micronutrient with bodily functions in the synthesis and repair of DNA and RNA. Other roles include production of red blood cells, enhancing brain activity; as well as assisting cell division and growth (The British Dietetic Association 2016). Folic acid is a closely related yet synthetic compound used for vitamin B9 supplementation in both modern food sources (e.g. flour, cereals) and PN additives (Jacques et al. 1999).

Therapeutically it has an established role in the treatment of folate deficiency anaemia that is characterised by fatigue, irritability and weight loss (NICE 2015). It is also recommended to be given to women of childbearing age to prevent the occurrence of neural tube defects in the foetus (Wilson et al. 2003).

Folic acid has been a traditional component of PN admixtures for many years with established stability in a range of formulations. Current multi-component additives contain ~400µg to meet daily requirements; outside this, manual additions can be made to the PN admixture to treat deficiency states. Dated reports of deficiencies exist with folate dose of 100-200µg (Nichoalds et al. 1977; Anon 1983; Barker et al. 1984). However nutritional abnormalities

relating to folate have not been published in recent years, presumably because the revised dose in PN additives is sufficient and excesses are readily excreted.

#### 4.2.2.3. Vitamin B12 (cobalamin)

As a water-soluble vitamin, vitamin B12 is essential for normal blood formation and normal neurologic function (Food and Nutrition Board. Institute of Medicine 1998). Naturally vitamin B12 is only available from animal sources e.g. meat, fish, eggs and dairy, while other fortified food sources and cereals exist for those with limited intake e.g. vegans. Vitamin B12 is a co-factor for two enzymes involved in methyl transfer which contribute towards DNA synthesis. Both vitamin B12 and folate are involved in methyl transfer and as such vitamin B12 deficiency mirrors folate deficiency in terms of haematological effect (Vanek et al. 2012). It presents itself as anaemia, neutropenia and thrombocytopenia; deficient individuals show symptoms of being pale, tired and short of breath, as well as neurologic symptoms of peripheral neuropathy e.g. loss of sensation, numbness and tingling.

In terms of monitoring, serum/plasma vitamin B12 is the most common test performed to assess vitamin B12 status (Selhub et al. 2008). Unfortunately results indicating deficiency often develop late after blood levels are already depleted and similarly, false normal results can result in situations of recent vitamin B12 intake (Green 2011).

Multicomponent preparations provide ~5μg of vitamin B12 per daily unit vial, as based on oral RDA requirements, yet it is worth noting that only 50% is absorbed from oral dosing (Chenarin 1979). Consequently in LT PN patients, IV dosing of vitamin B12 is known to result in elevated serum results, suggesting that the vitamin B12 doses in multicomponent preparations may be excessive. However, experts believe that no dosage adjustment is necessary as no evidence for vitamin B12 toxicity syndrome exists and high serum levels can reflect recent parenteral infusion from PN rather than levels in tissue stores (Vanek et al. 2012).

Cobalamin deficiencies while on supplemented PN are unlikely due to wide use of supplemented PN but have still been documented in dated studies (Van Spreeuwel et al. 1988; Compher et al. 2001; Compher et al. 2002). Lambert et al. (1997) investigated vitamin B12 status of twenty patients on LT HPN and found four patients (20%) to be deficient, although no patients showed metabolic signs of deficiency and patients were supplemented with vitamin B12 injections rather than supplemented PN. Even though there is the view that excesses of vitamin B12 are not harmful, there are reports suggesting an unnecessary over-provision of vitamin B12 in LT PN patients (Elkhatib et al. 2010). This belief was also recently surmised in a study by Żyła et al. (2015) in which the median value for vitamin B12 was consistently higher than the upper limit of the reference interval in neonates. The authors stated that the multi-component preparations Cernevit® and Solivito® contain too much vitamin B12 and attributed the excessive results to these doses.

#### 4.2.2.4. Vitamin D (chole/ergo-calciferol)

Vitamin D is discussed in greater detail over other micronutrients as it forms a key component of PN that is researched within the scope of this PhD project.

##### *4.2.2.4.1. General information and physiological role*

Vitamin D is a seco-steroid compound and its role in relation to bone health is particularly well established, particularly, its deficiency along with other minerals (calcium, phosphorus) being known to give rise to rickets in children and osteomalacia in adults. It is required during the bone mineralisation process to create osteoid tissues e.g. bone and teeth (Francis et al. 2013). Its physiological role is to regulate calcium and phosphorus levels (as well as regulating levels of iron, magnesium and zinc) in the blood by promoting their absorption from food in the intestines. It also promotes reabsorption of calcium in the kidneys which in turn enables normal mineralisation of bone. It has garnered more attention in recent years as its low status in human populations has been linked to various diseases and conditions e.g. heart disease, high blood pressure, some cancers and diabetes to name but a few (Autier et al. 2014). However the evidence surrounding these relationships is

often not well-established; uncertainty exists as to whether it is the low vitamin D status or the disease itself that is to blame. Lately the role of vitamin D has been considered outside of skeletal function and Holick (2007) has even explained its emerging potential role in the prevention of cancer, multiple sclerosis, type 1 diabetes and Crohn's disease.

Despite its human essentiality, it is only obtained from few natural dietary sources (e.g. oily fish, egg yolk and mushrooms); hence it is supplemented in various foods such as cereals, margarine, infant formula and dairy alternatives (Pearce and Cheetham 2010). In the UK, the main source of vitamin D is via skin exposure to ultra-violet B (UV-B) light within the months of April to September, showing our dependency on dietary sources over the winter months when the UK has insufficient exposure to UV-B wavelengths required for vitamin D synthesis (Pearce and Cheetham 2010).

#### *4.2.2.4.2. Activation, metabolism and monitoring of vitamin D status*

Vitamin D exists in several forms (vitamers) as prohormones which are activated in the body when required. Ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$) being the two major compounds required by the body. They can be ingested from the diet and supplements, as well as being synthesised cutaneously from exposure to ultraviolet light. They are very chemically similar and prove difficult to resolve from each other in chromatographic investigations (The National Institute of Standards and Technology 2015). When taken orally, vitamin D is absorbed in lipid micelles and incorporated into chylomicrons, dietary fat is therefore needed to absorb vitamin D. For this reason supplements are advised to be taken with the largest meal of the day. Vitamin D that is ingested and cutaneously produced, undergoes a series of conversion steps within the body to its physiologically active form. Vitamin D, beginning as either ergocalciferol (D2) or cholecalciferol (D3), is first hydroxylated by the liver to calcidiol (25-hydroxyvitamin D2/D3). Calcidiol is then further hydroxylated by the kidneys to its active form calcitriol (1, 25-hydroxyvitamin D2/D3). It is this form of vitamin D which circulates as a hormone, regulating the levels of calcium and phosphate in the bloodstream, thereby exerting their effect on bone

mineralisation and remodelling. In serum, only a fraction of calcidiol is converted the active calcitriol metabolite when required. Calcitriol has a short half which complicates accurate assessment of vitamin D status (Wootton 2005). For this reason, measurement of total calcidiol is considered best to assess total body stores of vitamin D; although essentially a pro-hormone, it gives an approximation of the amount of vitamin D obtained from food, oral supplements and that produced in the skin; as well as an indirect approximation of the amount of activated vitamin D in the body (Heaney 2011).

#### *4.2.2.4.3. Vitamin D in relation to bone health*

Optimal provision of vitamin D in HPN patients has been a longstanding and troubling issue, particularly with reference to the complication of metabolic bone disease (MBD). Many studies note its deficiency in HPN populations and its exact cause is still uncertain (Compher et al. 2007; Corey et al. 2009; Thomson and Duerksen 2011), although its onset is thought to be a combination of both patient-specific factors and PN-related factors (Foldes et al. 1990; Klein and Coburn 1991; Verhage et al. 1995). On the other hand, the study by Verhage et al. (1995) suggests that overprovision of vitamin D alongside suppression of normal parathyroid responses could play a role in the development of MBD. However, it is more likely that the inadequate vitamin D status has a greater input to the development of MBD in HPN populations as conveyed in a paper by ASPEN (Vanek et al. 2012). Interestingly, vitamin D deficiency has also been noted as a problem in younger PN populations, suggesting that its onset may be a more pronounced problem for all patients alongside potential under-provision in PN (Diamanti et al. 2014; Wozniak et al. 2015).

#### *4.2.2.4.4. Vitamin D deficiency*

Interestingly, it has been supposed that the general population themselves do not achieve adequate vitamin D status, especially during the winter months. Estimations of the prevalence of vitamin D deficiency in Europe range from 2-30% and one wonders how the vitamin D status of the general population

would compare to the population of LT HPN patients, within the UK (Spiro and Buttriss 2014).

Symptoms and signs associated with vitamin D deficiency are almost non-existent in mild deficiency; however in severe cases symptoms have been reported as muscle aches and cramps, joint pain, tiredness, increased risk of infection and bone pain (Soliman et al. 2014; Galesanu and Mocanu 2015), clinically evidenced by hypocalcaemia, hypophosphataemia, muscle weakness and demineralisation of bone/osteoporosis. The authors of a review article, Spiro and Buttriss (2014), mentioned the difficulty in recommending adequate levels of vitamin D intake because of the varied definitions of adequate or optimal vitamin D status; stressing the need for more standardised definitions to allow a better evidence based approach to measuring and assessing vitamin D status. They offered their own recommendations around the areas of dietary provision, food fortification, vitamin D supplementation and sensible sun exposure; they also explained the need to take into account national, cultural and dietary habits relating to vitamin D.

#### *4.2.2.4.5. Vitamin D toxicity*

Despite widespread concern of deficiency, vitamin D toxicity is also of clinical concern. Excessive intakes that result in toxicity can cause increased intestinal absorption of calcium and mobilisation of calcium from bone, leading to hypercalcaemia (Vieth 2006; Jones 2008). This in turn results in increased calcium deposition in soft tissue, bone demineralisation as well as both renal and cardiovascular toxicity. Efforts should be made to reduce vitamin D provision if there is evidence of hypercalcaemia (Tebben et al. 2016).

#### *4.2.2.4.6. Vitamin D in PN additives*

There has been dispute over the adequacy of the recommended daily dose of vitamin D for HPN patients (Vanek et al. 2012). The current longstanding market leading preparations Cernevit® and Vitlipid N Adult® only contain ~5µg (~200 IU) of vitamin D (cholecalciferol and ergocalciferol, respectively);

it is a contentious issue whether this dose is sufficient to meet the needs of LT PN patients considering the numerous reports of deficiency and recent changes in recommendations for vitamin D dosing (DeLuca 2009; Thomson and Duerksen 2011; Vanek et al. 2012).

#### *4.2.2.4.7. Review of vitamin D dosing recommendations*

In the UK, a safe upper limit (SUL) indicates an intake that can be consumed daily over one's lifetime without significant risk to health. There was insufficient evidence to establish an SUL for vitamin D, as such, using limited data a guidance level (GL) was set of 25μg (1000IU) per day for adults, signifying an intake not expected to cause adverse effects (Expert Group on Vitamins and Minerals 2003). However in the US, the equivalent parameter, the tolerable upper intake level (UL), was set at 100μg (4000IU) daily for adults; likewise the European Food Safety Authority (EFSA) recommends the same UL (Institute of Medicine 2011; European Food Safety Authority 2012). The European guidelines are considered appropriate and help to demonstrate the high upper limit for vitamin D dosing as well as the disparity between dosing recommendations. Considering the more commonplace occurrence of deficiency, patients are unlikely to attain these daily upper limits unless they have already demonstrated a justified need for treatment of deficiency.

Public Health England and the Food Standards Agency directed the latest National Diet and Nutrition Survey which reported higher than expected levels of vitamin D deficiency among the general population (all age and sex groups) (Bates et al. 2011). These findings have precipitated the recent review of dietary recommendations, both the US Institute of Medicine (IOM) and the UK Scientific Advisory Committee on Nutrition (SACN) have increased their RDA and DRI recommendations for vitamin D dosing for people of all ages, to 600IU (15μg) daily and 400IU (10 μg) respectively; yet PN additives do not mirror these revised dosing recommendations for vitamin D in the general population, even though HPN patients are conceivably at a greater risk for vitamin D deficiency and related bone health problems (Institute of Medicine 2011; Scientific Advisory Committee on Nutrition 2016). Especially since previous RDA values assumed no exogenous sources of vitamin D from

sunlight exposure. Datta and Stone (2016) explained that around 80-90% of vitamin D is known to be produced cutaneously from exposure to sunlight with only 10-20% derived from dietary sources; this fact demonstrates the ease of onset of vitamin D deficiency. As such, the recommendations did not consider intradermal vitamin D production from sun exposure to skin due to the variability and complexity in the number of factors which can affect it. In the wider scope, they have finally helped to indicate appropriate baseline dosing for the general population which can be generalised for the needs of LT PN patients. The revised recommendations show that the current PN vitamin additives are not in keeping with recommendations for the general population, even though HPN patients are conceivably at a greater risk of vitamin D deficiency and related bone health problems.

Recently the form of vitamin D given for treatment or supplementation has been shown to be an important dosing consideration. Previously, both ergocalciferol ($D_2$) and cholecalciferol ($D_3$) were thought to be equivalent and interchangeable, yet recently, cholecalciferol has been proven to be more potent and to exhibit greater bioefficacy in raising serum 25-OH vitamin D levels (Houghton and Vieth 2006; Boullata 2010). One wonders whether the differences in biological form of vitamin D ($D_2$/$D_3$) could contribute towards reports of deficiency in LT PN patients.

#### 4.2.2.5. Vitamin E (tocopherol)

Vitamin E exists in eight isomeric forms which all exhibit variable biological activity and associated biological effects; α-tocopherol is the naturally occurring form with the highest vitamin E activity and is also the isomer included in PN additives (National Center for Biotechnology Information 2016). Vitamin E is a component of all cell membranes and acts as a potent peroxyl radical scavenger; to date its primary known role is to protect cell membranes from lipid peroxidation and oxidative damage (Rizvi et al. 2014). Vitamin E has a strong affinity for free radicals and is able to interrupt the chain reaction (via formation of a resonance stabilised tocopherol radical, before reconversion back to vitamin E by ascorbic acid) (Biesalski 2009).

Research has been undertaken to establish the antioxidant role of vitamin E in PN. Both Pironi et al. (1998) and Reimund et al. (2000) noted an increase in malondialdehyde (MDA), a lipid peroxidation marker in the presence of reduced plasma tocopherol concentration.

In the absence of genetic causes for vitamin E deficiency, it usually results from under-provision and fat malabsorption, with early deficiency being asymptomatic. Later symptoms of deficiency are neurologic in nature and include ataxia and general weakness (Biesalski 2009). There are few critical studies published regarding vitamin E deficiency in PN; yet Porter et al. (2005) presented a case of clinical vitamin E deficiency in a patient with visual symptoms and signs of macular degeneration. The symptoms were completely resolved within 3 weeks following vitamin E supplementation (no explanation of dose given).

However, it has been stressed that care should be taken not to provide vitamin E in excess as Miller et al. (2005) showed that high-dose tocopherol supplements (greater than or equal to 360mg/day) may be associated with increased all-cause mortality, yet these doses are far greater than the doses in PN vitamin preparations (∽5-10mg/dose). This finding is contrary to those of (Biesalski 2009), who commented that data regarding toxicity from parenteral vitamin E does not exist and that studies of large oral supplemental use had shown no consistent adverse effects (references not included). Also it worth noting that the study by Miller et al. (2005) stressed that the high-dose vitamin E studies were small and often performed in those with chronic diseases. It is more likely that reduced supplemental intake below the norm is a more probable occurrence over toxicity.

Both Europe and the US recommendations for vitamin E dosing in LT PN are set at 10IU (9.1mg) per day (Nutrition Advisory Group 1979; Greene et al. 1988; ASPEN Board of Directors and the Guidelines Clinical Task Force 2002). It is debatable whether this dose is sufficient for LT PN patient needs, Forbes and Forbes (1997) measured vitamin E status in thirty-two LT PN patients and found seventeen had deficient vitamin E levels. Another study detected high

levels of pentane (an indicator of lipid peroxidation) in the breath of HPN patients and found association with low vitamin E status (Lemoyne et al. 1988).

More recently, some revolutionary findings were published by Ng et al. (2016) showing that vitamin E plays an important hepatoprotective role in preventing PNALD, presumably against the liver injury-inducing nature of phytosterols (from lipid PN components). However these were findings extrapolated from pig studies and the authors stated the need for further pre-clinical studies to definitively show proof of the liver toxic effects of phytosterols and the hepatoprotective effects of vitamin E.

Overall experts have stressed the need to monitor vitamin E levels closely to ensure adequate status by its appropriate provision in PN, and supplemental dosing where necessary; thereby ensuring patients have sufficient antioxidant capacity and free radical protection (Biesalski 2009).

#### 4.2.2.6. Other notable vitamin abnormalities

##### *4.2.2.6.1. Vitamin B1 (thiamine)*

The biologically active thiamine (as pyrophosphate) acts as a key coenzyme in the generation of ATP. As previously mentioned, product shortages have been implicated in nutrient deficiencies. In a recent case report, after a rationing of a patient's vitamin supply during a time of nationwide shortage thiamine, a PN-dependent patient developed thiamine deficiency; presenting with septic shock, metabolic crisis and hyperlactataemia. The patient's condition rapidly resolved following thiamine supplementation (Da Silva et al. 2015). Prior to these events, the patient was reduced from daily doses of thiamine to a thrice weekly regimen which precipitated the deficiency. The reduced dose was notably less than the recommended weekly intake for adults, 18mg/week instead of 42mg/week (Vanek et al. 2012). The occurrences from this case study are in line with the expected symptoms for thiamine deficiency and show the significance of its role as a cofactor in the body (Kreisberg 1980; Centre for Disease Control and Prevention 1997). This case showed that

thiamine deficiency can be considered a differential diagnosis for PN patients presenting with symptoms of acidosis, neuropathy or encephalopathy (Da Silva et al. 2015). However, it should be additionally noted to be just a singular case report and unusual to only have occurred in a single patient during the nationwide shortage; it is more likely that there was significant under-reporting and/or monitoring of patients. It is more likely that other nutritional abnormalities occur in regard to vitamin B1 supplementation, but a lack of investigation into its supplementation and monitoring in PN makes the interpretation of its adequacy of dosing in PN rather difficult.

#### *4.2.2.6.2. Vitamin B2 (riboflavin)*

The literature review yielded a single report for vitamin B2 deficiency, a French case report in a Crohn's patient on LT PN for approximately 3 months (Duhamel et al. 1979). Aside from little concern for vitamin B2 dosing in PN, Laborie et al. (1998) has explained its potential paradoxical role with vitamin C in the photoinduction of harmful peroxide radicals in PN.

Aside from oral or enteral vitamin B2 provision and any problems associated with GI absorption, the instability of vitamin B2 within PN admixtures could potentially result in less being delivered to patients. Chen et al. (1983) explained that although most B-group vitamins are stable in the presence of light in PN, vitamin B2 was shown to be sensitive to both indirect and direct sunlight (47% and 100% destruction respectively within eight hours); the sensitivity of vitamin B2 to light was further corroborated by Allwood and Kearney (1998).

#### *4.2.2.6.3. Vitamin B3 (niacin/nicotinic acid)*

An older study by Howard et al. (1983) showed that vitamin B3 supplementation twice weekly in PN did not result in micronutrient abnormalities. However (Labadarios et al. 1988) reported vitamin B3 deficiency in 6% of blood tests in those receiving standard vitamin additives in LT PN. Likewise, a study assessing water-soluble vitamin status in cancer patients noted niacin deficiency to be the most prevalent, present in 40% of

patients (Inculet et al. 1987). Of late, there appears less concern for nutritional abnormalities associated with vitamin B3 as it is not routinely monitored by HPN centres and no recent reports of deficiency or toxicity have been documented.

#### *4.2.2.6.4. Vitamin B6 (pyridoxine)*

PN supplemented with standard vitamin additives was demonstrated to improve vitamin B6 status (Stromberg et al. 1981). Apparent "safe" vitamin B6 supplementation was further consolidated by Howard et al. (1983) with a twice weekly vitamin dosing schedule in PN. A dated finding from a study by Dempsey et al. (1987) found high rates of deficiency and excess (18% and 36% respectively) but from only a total of twenty-eight vitamin tests. On the other hand, little to no reports of toxicity exist, except for an abstract showing that chronic renal insufficiency can precipitate pyridoxine toxicity (Craig et al. 2017).

Again physical instability of pyridoxine within PN admixtures could result in potential under-dosing in PN, Chen et al. (1983) found that 86% of vitamin B6 was destroyed by direct sunlight in standard PN admixtures within eight hours.

#### *4.2.2.6.5. Vitamin B7 (biotin)*

Studies have confirmed biotin deficiency in patients with short bowel receiving PN without biotin supplementation; patients presented with lethargy, dermatitis and hair loss which grew back once adequate supplementation was commenced (Innis and Allardyce 1983; Khalidi et al. 1984; Velazquez et al. 1990). No recent reports of deficiency exist and is probably the result of routine supplementation of biotin in PN from multi-component compound additives e.g. Solivito®.

#### *4.2.2.6.6. Vitamin C (ascorbic acid)*

Like vitamin E, vitamin C is a strong antioxidant and also a co-factor for many enzymes, including collagen formation, neurotransmitter synthesis and cholesterol metabolism (Berger 2009). Circulating levels drop in surgical shock, trauma and sepsis; these patients have larger requirements due to oxidative stress and wound healing.

LT HPN patients are usually in stable clinical condition with lower vitamin C requirements than the critically ill. Yet it has been shown that in prolonged PN, plasma concentrations can fall below normal range (Labadarios et al. 1988). In relation to nutritional abnormalities in PN, instances of deficiency prevail over toxicity giving symptoms of scurvy (tiredness, muscle and join pain) (Levavasseur et al. 2015).

Numerous chemical and physical stability issues are known to affect vitamin C in PN, including oxygen, temperature, light, pH (<4) and the presence of other micronutrients (copper, iron); all of which could result in reduced amounts administered to patients (Berger 2009). Berger (2009) explained that 200mg daily is considered "quite reasonable" for HPN patients, yet it is twice the dose of current preparations available in the UK. In the US, the FDA revised their vitamin C dose recommendations from 80-100mg to 200mg daily (FDA 2005) and the preparations for PN still do not reflect these recommendations.

There have been no reports of vitamin C toxicity in PN patients; the upper limit (UL) is currently set at 2g daily and is unlikely to be achieved from its provision in LT PN (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000). However caution is expressed in patients receiving high doses of vitamin C with co-existing renal failure as there is the potential to cause renal stones and nephrolithiasis (Pena de la Vega et al. 2004; Handelman 2007).

It is generally considered that one dosing recommendation cannot fit all patients for vitamin C, dosing should parallel the degree of oxidative stress which is variable amongst patients (Berger 2009). Some recent studies have tried to quantify the oxidative effect of vitamin C in PN. Kuwabara et al. (2016) tested a 500mg daily intervention but found no change in inflammatory markers for oxidative stress between the intervention and control group, other than restoring vitamin C status back to normal range for those with deficiency. Yet in another vitamin C intervention study following GI surgery, it was found that vitamin C may decrease post-surgical oxidative stress; 8-

isoprostane, an oxidative stress marker was significantly lower in the group treated with the higher vitamin C dose (Yamazaki et al. 2011).

#### *4.2.2.6.7. Vitamin K (phyllo/mena-quinones)*

Vitamin K is an essential human co-factor for peptide conversion in specialised proteins e.g. Gla-proteins. When body stores are deficient, it is the well-known culprit for bleeding syndromes when the body is unable to synthesis active coagulation factors II, VII, IV and V; as such, deficiency symptoms include active bleeding and bruising (Shearer 2009). Treatment of deficiency relies on corrective supplementation with preparations of phylloquinone. Clinically, vitamin K is also an established treatment to antagonise the effects of warfarin therapy when necessary (e.g. bleeding, bruising, high INR).

Anecdotally, vitamin K deficiency in LT PN appears to be a known clinical problem yet is not well substantiated in research publications. Phylloquinone is a natural source of vitamin K in the lipid emulsion component of PN feeds; it is available in varying amounts depending on the lipid source e.g. soybean, safflower etc (Lennon et al. 1993; Shearer 2009; Singh and Duerksen 2003). Duerksen and Papineau (2000; 2004) explored the prevalence of coagulation abnormalities in PN patients receiving lipid emulsions and found that coagulation defects were five times higher in those receiving the lipid emulsion containing less phylloquinone; however no significant bleeding incidents were reported (Duerksen and Papineau 2000; Duerksen and Papineau 2004). Logically it would appear that LT PN who do not receive lipid PN or micronutrient preparations containing vitamin K (e.g. Cernevit®) are at a definitive risk of vitamin K deficiency. A more substantial publication by (Chambrier et al. 1998) investigated the long-term relationship between vitamin K intake from lipid emulsions on plasma phylloquinone concentrations showed that an average weekly intravenous supply of 255µg (36µg/day) was sufficient to maintain phylloquinone concentrations within the reference range in HPN patients. Findings that were in keeping with other studies that vitamin K content solely from lipid PN is sufficient to meet daily requirements i.e. without micronutrient preparations including vitamin K (Goulet et al. 1990; Lennon et al. 1993; Drittij-Reijnders et al. 1994). For those not receiving sources of vitamin K in their PN, Shenkin (2015b) advises a

separate IV vitamin K injection once a week. Chambrier et al. (2004) further established that a LCT emulsion is able to maintain plasma vitamin $K_1$ status moreover a MCT/LCT combination. Interestingly EFA deficiency does not preclude deficiencies of fat-soluble vitamins e.g. vitamin K, in SBS. Edes et al. (1991) stated that requirements for lipid and fat-soluble vitamins should be determined independently.

There are situations where it is not appropriate for LT PN patients to receive vitamin K and in 1979 the Nutritional Advisory Group in the US issued a recommendation that patients should not receive vitamin K supplementation if they receive anticoagulant therapy (e.g. warfarin for thrombo-prophylaxis) (Nutrition Advisory Group 1979). As such, there still exist vitamin preparations without vitamin K included (e.g. Cernevit®) and ASPEN recommend that preparations with and without vitamin K continue to be available for such situations (Vanek et al. 2012).

Vitamin K deficiency also happens to be common in those with cholestatic liver disease as bile salts are required for its uptake. This situation is further complicated by the cautious attitude of prescribers to give lipid PN to patients with liver disease over concerns of its contributory effect to PNALD.

Generally adults require ~100μg daily to maintain hemostasis (Shearer 2009). In 2000, the US FDA revised their guidelines and mandated that adult IV vitamin preparations should provide 150μg phylloquinone per day (previously 100μg). With these revised guidelines it could be possible for some patients to have vitamin K daily doses in excess of 300μg from both the lipid emulsion and the vitamin preparation (Singh and Duerksen 2003). Overall, the revised dosing guideline is generally considered beneficial for most patients; however it could be harmful for others, such as patients receiving anticoagulants (Helphingstine and Bistrian 2003). Singh and Duerksen (2003) stressed that NSTs should be conscious of vitamin K provision from all sources in LT PN patients. Unfortunately serum vitamin K tests (as phylloquinone) are not performed at C&V UHB, assessment of vitamin K status relies on

interpretation of any presenting symptoms (e.g. bruising) and/or associated coagulation tests (e.g. prothrombin time, international normalised ratio).

## 4.3. SUMMARY

In summary, pertinent findings from published data regarding nutritional abnormalities for almost all micronutrients have been demonstrated. The non-specific search strategy yielded a great deal of published studies and case reports relating to nutritional abnormalities. However, the publications varied greatly in study design, reflecting a hierarchical difference in evidence base for the data findings from each publication. The review still elucidated relevant up-to-date information to summarise the current body of knowledge representing nutritional abnormalities in LT PN. The great variation in study design, study setting (e.g. US vs UK) and study dates limit the generalisability of known findings from the literature review; with particular reference to the different clinical dosing and monitoring practices in different geographical settings and points in time.

Specifically, the following notable points were realised from the literature review:

- There were few recent studies incorporating a range of micronutrients from a substantial HPN population cohort over longer time periods.
- Although clinical correlations have been made for LT PN patients e.g. increased prevalence of vitamin D deficiency and poor bone health/osteomalacia, the long-term clinical implications relating to micronutrient abnormalities in PN have still not been fully characterised, particularly for dose or length of exposure dependent studies.
- There is scope for optimisation and improvement of micronutrient dosing in LT HPN patients.

The review has shown that research is in favour of the aforementioned points and these will form the basis of the current PhD study schedule; to evaluate the extent of nutritional abnormalities in LT HPN patients, explore their relation to the prevalence of clinical issues experienced by patients and finally to provide research based recommendations to optimise provision of micronutrients in patients' HPN.

# CHAPTER FIVE:

## Assessment of micronutrient abnormalities in LT HPN patients (vitamins and trace elements)

## 5.1. INTRODUCTION

This chapter concerns the retrospective assessment of nutritional abnormalities (both deficiency and excess) in a cohort of HPN patients in Wales. The chapter ends with a critical analysis of the foremost findings from the study in relation to current expert opinion and previous studies.

### 5.1.1. Chapter aims

This research chapter aimed to:

- To investigate the extent of nutritional deficiency and accumulation experienced by the population of LT HPN patients at C&V UHB
- To identify the nutrients which are most commonly implicated as being deranged in this population of patients
- To identify any patterns or trends in nutritional abnormalities experienced by patients with similar diagnoses or underlying conditions
- To identify potential factors which could be implicated in or contribute to micronutrient derangement

### 5.1.2. Rationale

A review of the literature has demonstrated the numerous types of nutritional abnormalities that can occur during LT PN; the extent to which they occur is made more complex by the many components that comprise PN. Particularly since there is still more to learn regarding the stability of individual components, their effects upon each other (especially at different concentrations and/or temperatures) and the overall physical stability of the PN feed. The review of the literature has given rationale for the present study performed in this chapter.

There is more to find out about how nutritional abnormalities come to occur, the extent to which they occur and for what reasons. By researching these considerations in greater depth it is hoped that further recommendations will

be made and best practices implemented to help reduce the potential for their occurrence.

Although ESPEN and ASPEN publish guidelines on patient PN requirements, nutritional assessment and monitoring, each HPN centre have their own experiences and practices. Murphy and Lewis (2016b) supported this theory when they said that the recommended published guidelines that dictate patient requirements and intervals for biochemical monitoring are moreover said to be based on experience rather than evidence-based literature. Perhaps in undertaking new research examining trends in nutritional abnormalities experienced by C&V UHB, it will help to bring to light new information that will benefit the body of HPN knowledge as a whole.

Biochemical blood test records represent a wealth of concise and accurate data sources. Their use in this study may help to bring to light new findings which may help to reduce the burden of these nutritional complications on secondary care; perhaps even reducing associated mortality rates, costs and clinic waiting times. It was already demonstrated at Hope hospital in Manchester that two-thirds of all readmissions for HPN patients were for complications of HPN rather than the underlying condition or surgery (Jones 2003).

By reviewing the biochemical data from the HPN patients maintained at C&V UHB in the manner of this preliminary study, it will allow future studies such as audit and service evaluation to be undertaken by developing standards of practice; ultimately helping to provide methods of overcoming micronutrient dosing problems that contribute to occurrence of nutritional abnormalities

This study represents a large-scale cohort study of several micronutrients. One of the first of its kind that attempts to quantify the extent of out of range (either deficient or excessive) nutritional states experienced by LT PN patients.

### 5.1.3. Reference range definition

The reference range (or more accurately defined reference interval) is applied to continuous data and is used to describe the limits (or reference interval) for blood test results (Lab Tests Online 2009). By explaining its definition, one is able to understand the context of the results in relation to the reference interval and their interpretation. Essentially, when a biochemical investigation is performed, the result is assessed against a reference point, usually what is expected in a healthy individual and the range of values seen in healthy individuals is termed the 'normal range' (as depicted by the normal distribution observed with continuous data). In some situations, it might be more appropriate for the comparable reference point to be the values expected in a symptomatic individual. The analyte reference intervals (normal range) are traditionally defined on the basis of measurement of the analyte in a sufficiently large sample of individuals from an appropriate healthy population (age, sex, ethnicity). For data having a Gaussian (normal) distribution, the results are normalized so that the sum over all values gives a probability of one. This then gives the definition for the normal range as the range of values lying between the limits specified by two standard deviations below the mean and two standard deviations above, encompassing approximately 95% of the values found in a sample (Marshall 2008). This gives the implication that the great majority of healthy people will have a value for the analyte within this 'normal' range (95%); leaving the 2.5% of values either side of this range to represent states of deficiency and toxicity.

It can appear misleading to apply the normal range to a sample population of patients who are considered by some to be "abnormal" or "not normal", interpretatively meant as in that they have chronic health problems and require LT IV feeding. However the ultimate aim is to achieve the same nutritional status as a 'healthy' individual (without GI issues or requiring PN) and hence the reference interval from a 'healthy' reference population is used as a reference guide.

## 5.2. METHODS

### 5.2.1. Research permissions

This study was conducted using the research permissions as described in Chapter 2. All sixty participants recruited and maintained on LT HPN were eligible for this section of research.

### 5.2.2. Study design

This study was performed as a retrospective longitudinal database analysis to investigate the incidence of micronutrient abnormalities experienced in a cohort of participants maintained on LT HPN at C&V UHB. Specifically, whether the results were deficient, in range or in excess (toxic) as depicted by the local C&V UHB reference limits.

#### 5.2.2.1. Data collection and sample population

Data were collected from the medical records of consenting participants recruited from the outpatient clinic at C&V UHB. Specifically, this was achieved via manual data transcription of the blood test results from the online 'Clinical Portal' system which stores records of the patients' micronutrient blood tests results.

Data were collected from the date which patients were initiated on HPN up to and including August 2015. Data collection was limited to the medical records available online for the participants. For instance, some longstanding patients preceded the online storage of medical blood test results pre-2007/2008.

As already stated, micronutrient assessment in this population of patients is recommended at least six monthly. By collecting these data, there were at least two sets of blood test results per participant for each year they were maintained on HPN. Sensitive or more closely monitored patients may have required more frequent testing, particularly if repeat tests were required to confirm absence of deficiency or toxicity after changes to PN formulation were implemented e.g. removal of compound micronutrient preparations. A full

breakdown of the data parameters investigated are given in Tables 5.1. and 5.2.

Where older blood test results for serum 25-hydoxyvitamin D (pre-2012) were collected but stated in ng/mL on the hospital computer system, these were converted to nmol/L using the calculation stated in 'Section 5.2.2.3.1.'.

#### 5.2.2.2. Data handling, storage and analysis

The relevant data were manually transferred into a Microsoft Access database for storage and handling, while data analysis was undertaken using Microsoft Excel. Participants were anonymised and coded to maintain their confidentiality throughout.

The results from the patients' blood tests were categorised as deficient, in range or in excess according to the hospital reference interval limits in use on the local C&V UHB intranet 'Clinical Portal' system. Table 5.1 describes the local reference ranges implemented by C&V UHB for the chosen data parameters. Please note the separate classification and interpretation of results for vitamin D (see Section 5.2.2.3.1. and Table 5.4).

#### 5.2.2.3. Data parameters

In terms of individual data parameters (Table 5.1), data collection was limited to the blood tests that are routinely performed at C&V UHB and the micronutrients that can be directly assayed from blood samples i.e. correlating directly with the provision of micronutrients from the PN feed.

Electrolytes (e.g. sodium, potassium, calcium, chloride, phosphorous and magnesium) are measured at each clinic visit for HPN patients, however they do not constitute micronutrients and are more accurately referred to as principle elements or macro elements (Prashanth et al. 2015). Their daily requirements in adults are above 100mg/day and deficiency usually results in fatal consequences. Equilibrium of serum electrolytes does have the potential to fluctuate especially between body cell stores and blood volume, but true

deficiency is unlikely and doesn't warrant investigation, hence their exclusion from this study.

On the other hand, ferritin was included in data collection to give an indication of iron provision from PN; although technically a biological molecule and not directly assayed in biochemical blood tests. Its biological structure incorporates an iron core that may contain as many as 4000-4500 iron atoms and its concentration in plasma is positively correlated with the size of total body iron stores (in absence of inflammation). Therefore, it can be assumed that blood plasma levels of ferritin directly correlate with dietary iron provision (WHO 2011). However, it is worthy of note that a low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses.

**Table 5.1:** Local micronutrient reference intervals implemented by C&V UHB.

| Micronutrient | Reference Interval (and units) |
|---|---|
| Copper (Cu) | 11.0 - 22.0 µmol/L |
| Manganese (Mn) | 70 - 210 nmol/L |
| Selenium (Se) | 0.80 - 1.40 µmol/L |
| Zinc (Zn) | 8.0 - 17.0 µmol/L |
| Ferritin | 15 - 300 µg/L |
| Folate | 3.1 - 20.0 µg/L |
| Vitamin A | 1.10 - 2.60 µmol/L |
| Vitamin B12 | 130 - 900 ng/L |
| Vitamin D (25-OH-vitamin D) | As per BMJ classification, section 5.2.2.3.1. |
| Vitamin E | 11.00 - 47.00 µmol/L |

The reference intervals for vitamin D are discussed in 'Section 5.2.2.3.1.' because classification of vitamin D status required further categorisation than the intervals stated by C&V UHB, to allow an evidence-based interpretation for vitamin D deficiency.

**Table 5.2:** Categories for further sub-classification of micronutrient blood test results according to: A. IF pathophysiological classification, B. Underlying disease (that causes IF), and C. Indication for HPN (as clinically noted).

| **A. IF – pathophysiological classification (primary mechanism)** |
|---|
| - Short-bowel with jejunostomy **(SBS-J)** |
| - Short-bowel with jejunocolic anastomosis **(SBS-JC)** |
| - Short-bowel with jejunoileal anastomosis with an intact colon **(SBS-JIC)** |
| - Fistula **(F)** |
| - Dysmotility **(Mot)** |
| - Mechanical obstruction **(MO)** |
| - Mucosal disease **(MD)** |
| **B. Underlying disease (that causes the IF) (main 5 categories)** |
| - Short bowel |
| - Intestinal fistula |
| - Intestinal dysmotility |
| - Mechanical obstruction |
| - Extensive small bowel mucosal disease |
| **C. Indication for HPN (as clinically noted)** |
| - Short bowel syndrome (SBS) |
| - Malabsorption |
| - Obstruction |
| - Fistula |
| - Motility |
| - Failed ENT (enteral nutrition) |
| - High output (HO) stoma |

Micronutrient blood test results were sub-categorised according to the same classification used in Chapter 3 for pathophysiological IF classification, underlying disease and indication for HPN, the same classification systems implemented by ESPEN (Pironi et al. 2015).

#### *5.2.2.3.1. Classification for vitamin D status*

The reference interval/classification described by C&V UHB for results of vitamin D (serum 25-hydroxyvitamin D) was not deemed to be sufficiently explicit enough for interpretation of deficiency. It merely stated "levels less than 50 nmol/L are indicative of deficiency". As Marshall (2008) has explained when reference ranges give no direct cut off for data values in this manner, the term 'reference/normal range' is misleading and it is more appropriate to define target values, depending on the overall classification of risk of vitamin D deficiency. Consequently, a more precise and descriptive classification was sought for results of the blood test for serum 25-hydroxyvitamin D.

As corroborated in the review by Mithal et al. (2009), the definition and classification of vitamin D deficiency and insufficiency vary considerably between studies. A resultant effect is observed in which it becomes difficult to interpret the results of vitamin D blood test results, particularly between different studies, countries and/or institutions, and the different units being used. Table 5.3 gives an overview of key recommendations for vitamin D classification from distinguished organisations; note the variation in vitamin D classification.

The blood test result values for serum 25-hydroxyvitamin D are sometimes stated in both ng/mL and nmol/L. Results for tests at C&V UHB are stated in nmol/L and for consistency these units have been used throughout this chapter. To convert between the units the following calculation is used: nmol/L = 2.5 x ng/mL (Holick et al. 2011; U.S. Centre for Disease Control and Prevention 2012; Vitamin D Council 2016a).

**Table 5.3:** Studies showing the variable classifications for vitamin D deficiency and insufficiency by different institutions.

| Reference | Institution/ Organisation | Vitamin D classification limits | Author remarks and recommendations |
|---|---|---|---|
| (Dawson-Hughes et al. 2010) | International Osteoporosis Foundation (IOF) | Deficiency: <25nmol/L<br>Insufficiency: either <75 or <50 nmol/L<br>Optimal target: ≥75nmol/L | Suggest 20-25µg (800-1000IU) per day in older adults. Efficacy of doses not yet evaluated in RCT, premature to recommend such doses. |
| | The Endocrine Society (a Clinical Practice Guideline) | Deficiency: < 50nmol/L<br>Insufficiency: 52.5-72.5nmol/L | To raise above 75nmol/L, may require 25 µg (1000IU) per day. |
| (Drezner 2015) | UpToDate® (evidence-based clinical decision resource) | Deficiency: <50nmol/L<br>Insufficiency: 50-75nmol/L<br>'normal' (optimal): > 75nmol/L | Unanimous agreement that ≤30nmol/L defines deficiency in US. |
| (WHO Scientific Group on the Prevention and Management of Osteoporosis 2003) | World Health Organisation (WHO) | Insufficiency: <50nmol/L | Daily intake of 10-20µg (400-800IU) is a straightforward, safe and inexpensive means of prevention (of deficiency). |
| (Vitamin D Council 2016a; Vitamin D Council 2016b) | Vitamin D Council (US non-profit organisation) | Deficient: 0-100nmol/L<br>Sufficient: 100-200nmol/L<br>High Normal: 200-250nmol/L<br>Undesirable: > 250nmol/L<br>Toxic: > 375nmol/L | Suggests that patients should aim for an ideal level of 50ng/mL (125nmol/L). |
| (Pearce and Cheetham 2010) | British Medical Journal (BMJ) | Deficiency: <25nmol/L<br>Insufficiency: 25-50nmol/L<br>Adequate: 50-75nmol/L<br>Optimal: > 75nmol/L | 10mcg (400IU) daily dose only provides sufficient vitamin D as a prevention of osteomalacia. Inadequate to attain optimal status without skin synthesis as well. High dose calciferol treatment necessary <25nmol/L. |

The classification system for vitamin D status based on serum 25-OH vitamin D as recommended by the BMJ was the chosen and preferred classification system for use in the study (see Table 5.4) (Pearce and Cheetham 2010). Rationale was based on the following reasons:

- The BMJ considered multiple sources of evidence from the literature which were of higher grade of evidence.
- The BMJ classification system had similar cut-off points to the other well-recognised classification recommendations e.g. International Osteoporosis Foundation.
- It depicts the same cut off limits between insufficient and adequate levels as C&V UHB.
- It reflects the most stringent criteria for classification of vitamin D status and as such the results reflect the best-case scenario for vitamin D deficiency and insufficiency.

**Table 5.4:** BMJ classification of vitamin D status (Pearce and Cheetham 2010).

| Vitamin D status | Serum 25-hydroxyvitamin D concentration (nmol/L) |
|---|---|
| Deficient | < 25 |
| Insufficient | 25 - 50 |
| Adequate | 50 - 75 |
| Optimal | > 75 |

One may notice that reference values for toxicity have not been given, this has been previously explained in section '4.2.2.4. Vitamin D'.

5.2.2.4. Methods of analysis

The data was analysed using descriptive statistics as follows:

- To show the total number (and percentage) of recorded micronutrient blood tests that were deficient, in range and in excess for the entire cohort of patient participants. In so doing, identify trends or themes in micronutrient abnormalities occurring in the patient population.
- To perform comparative analyses by classifying the blood test results according to the patient's pathophysiological classification for IF, underlying disease (causing the IF) and indication for HPN. Results were shown as total number blood tests that were deficient, in range or in excess per patient group within each category to see if there were any particular nutritional abnormalities associated with individual diagnoses or indications.

Data transcription checks were performed for 10% of transcribed data to ensure no errors were made during the process of data transcription and collection. In result, no patterns or trends in error were identified throughout data collection.

## 5.3. RESULTS

### 5.3.1. Total results for participant cohort.

5.3.1.1. Trace elements and ferritin

**Table 5.5:** Number (and percentage) of TE blood test results that were deficient, in range or in excess.

| | **Copper (Cu)** | **Ferritin** as an indication of iron (Fe) stores | **Manganese (Mn)** | **Selenium (Se)** | **Zinc (Zn)** |
|---|---|---|---|---|---|
| Deficient | 69 (12.4) | 34 (7.4) | 0 (0) | 187 (32.8) | 32 (5.8) |
| In range | 446 (79.9) | 332 (72.5) | 262 (49.4) | 350 (61.4) | 456 (82.6) |
| In excess | 43 (7.7) | 92 (20.1) | 268 (50.6) | 33 (5.8) | 64 (11.6) |
| Total | 558 | 458 | 530 | 570 | 552 |

5.3.1.2. Vitamins

**Table 5.6:** Number (and percentage) of vitamin blood test results that were deficient, in range or in excess.

| **Blood test Classification** | **Vitamin A** | **Vitamin B9 (folate)** | **Vitamin B12** | **Vitamin E** |
|---|---|---|---|---|
| Deficient | 77 (21.8) | 4 (0.7) | 0 (0) | 28 (7.9) |
| In range | 214 (60.4) | 429 (79.3) | 343 (64.5) | 315 (89.3) |
| In excess | 63 (17.8) | 108 (20) | 189 (35.5) | 10 (2.8) |
| Total | 354 | 541 | 532 | 353 |

*5.3.1.2.1. Vitamin D*

**Table 5.7:** Number (and percentage) of serum 25-hidroxyvitamin D blood test results that were classed as deficient, insufficient, adequate or optimal.

| | Deficient | Insufficient | Adequate | Optimal | Total |
|---|---|---|---|---|---|
| **Number of tests (and %) for vitamin D (serum 25-hydroxyvitamin D)** | 28 (5.9) | 114 (24.2) | 152 (32.3) | 177 (37.6) | 471 |

5.3.1.3. Mean (±SD) and range for all micronutrient blood test data

**Table 5.8:** Mean (±SD) and range of micronutrient blood test results that were deficient, in range or in excess.

| | Mean | ±SD | Min | Max | Reference range |
|---|---|---|---|---|---|
| **Copper** (µmol) | 15.36 | 4.68 | 0.78 | 29.7 | 11.0-22.0 |
| **Manganese** (nmol) | 227.82 | 108.07 | 77 | 780 | 70-210 |
| **Selenium** (µmol) | 0.92 | 0.31 | 0.1 | 1.89 | 0.80-1.40 |
| **Zinc** (µmol) | 12.45 | 4.13 | 3.3 | 26.7 | 8.0-17.0 |
| **Ferritin** (µg/L) | 217.18 | 245.57 | 4 | 1517 | 15-300 |
| **Folate** (µg/L) | 11.75 | 6.41 | 1.4 | 25.6 | 3.1-20.0 |
| **Vitamin A** (µmol) | 1.79 | 0.86 | 0.1 | 5.26 | 1.10-2.60 |
| **Vitamin B12** (ng/L) | 864.02 | 446.32 | 174 | 2000 | 130-900 |
| **Vitamin D** (nmol/L) | 69.14 | 34.97 | 10 | 211 | BMJ class. |
| **Vitamin E** (µmol) | 23.84 | 11.16 | 5.40 | 81.85 | 11.00-47.00 |

### 5.3.2. Comparative group analyses

5.3.2.1. IF pathophysiological classification

**Table 5.9:** Number (and %) of micronutrient blood test results subcategorised according to the patient's IF pathophysiological classification.

| | | IF pathophysiological classification [Num. of tests (and %)] | | | | | |
|---|---|---|---|---|---|---|---|
| **Micronut.** | **Test classif.** | **SBS-J** | **SBS-JC** | **SBS-JIC** | **F** | **Mot** | **MD** |
| Copper | Deficient | 7 (3.2) | 35 (17.8) | 13 (29.5) | 0 (0) | 11 (17.2) | 3 (20.0) |
| | In range | 182 (84.3) | 155 (78.6) | 29 (65.9) | 21 (95.5) | 47 (73.4) | 12 (80.0) |
| | In excess | 27 (12.5) | 7 (3.6) | 2 (4.6) | 1 (4.5) | 6 (9.4) | 0 (0) |
| Manganese | Deficient | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | In range | 101 (48.6) | 83 (44.6) | 32 (74.4) | 9 (42.9) | 36 (60.0) | 1 (8.3) |
| | In excess | 107 (51.4) | 103 (55.4) | 11 (25.6) | 12 (57.1) | 24 (40.0) | 11 (91.7) |
| Selenium | Deficient | 43 (19.1) | 77 (39.1) | 22 (47.8) | 2 (8.7) | 34 (54.0) | 9 (56.3) |
| | In range | 159 (70.7) | 117 (59.4) | 23 (50.0) | 17 (73.9) | 27 (42.9) | 7 (43.7) |
| | In excess | 23 (10.2) | 3 (1.5) | 1 (2.2) | 4 (17.4) | 2 (3.1) | 0 (0) |
| Zinc | Deficient | 4 (1.9) | 9 (4.7) | 1 (2.2) | 1 (4.5) | 9 (14.5) | 8 (53.3) |
| | In range | 168 (78.1) | 175 (90.6) | 43 (95.6) | 19 (86.4) | 44 (71.0) | 7 (46.7) |
| | In excess | 43 (20) | 9 (4.7) | 1 (2.2) | 2 (9.1) | 9 (14.5) | 0 (0) |
| Ferritin | Deficient | 14 (5.7) | 4 (3.8) | 3 (13.6) | 0 (0) | 8 (20.5) | 5 (23.8) |
| | In range | 165 (67.4) | 87 (82.1) | 14 (63.6) | 23 (92.0) | 27 (69.2) | 16 (76.2) |
| | In excess | 66 (26.9) | 15 (14.1) | 5 (22.8) | 2 (8.0) | 4 (10.3) | 0 (0) |
| Folate | Deficient | 1 (0.4) | 0 (0) | 0 (0) | 0 (0) | 1 (1.8) | 2 (10.0) |
| | In range | 202 (87.1) | 124 (74.3) | 19 (48.7) | 27 (100.0) | 41 (73.2) | 16 (80.0) |
| | In excess | 29 (12.5) | 43 (25.7) | 20 (51.3) | 0 (0) | 14 (25.0) | 2 (10.0) |
| Vitamin A | Deficient | 17 (12.7) | 32 (25.4) | 1 (3.4) | 0 (0) | 21 (58.3) | 6 (33.3) |
| | In range | 82 (61.2) | 93 (73.8) | 7 (24.1) | 9 (81.8) | 12 (33.3) | 11 (61.1) |
| | In excess | 35 (26.1) | 1 (0.8) | 21 (72.4) | 2 (18.2) | 3 (8.4) | 1 (5.6) |
| Vitamin B12 | Deficient | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | In range | 143 (63.0) | 131 (78.4) | 3 (7.7) | 20 (74.1) | 29 (53.7) | 17 (94.4) |
| | In excess | 84 (37.0) | 36 (21.6) | 36 (92.3) | 7 (25.9) | 25 (46.3) | 1 (5.6) |
| Vitamin D | Deficient | 10 (4.9) | 9 (5.7) | 3 (10.7) | 0 (0) | 5 (9.8) | 1 (7.7) |
| | Insufficient | 43 (21.2) | 45 (28.7) | 3 (10.7) | 8 (42.1) | 12 (23.5) | 3 (23.1) |
| | Adequate | 60 (29.6) | 55 (35.0) | 2 (7.1) | 6 (31.6) | 23 (45.1) | 6 (46.1) |
| | Optimal | 90 (44.3) | 48 (30.6) | 20 (71.5) | 5 (26.3) | 11 (21.6) | 3 (23.1) |
| Vitamin E | Deficient | 1 (0.7) | 12 (9.6) | 0 (0) | 0 (0) | 5 (13.9) | 10 (55.6) |
| | In range | 128 (95.5) | 113 (90.4) | 26 (89.7) | 10 (90.9) | 30 (83.3) | 8 (44.4) |
| | In excess | 5 (3.7) | 0 (0) | 3 (10.3) | 1 (9.1) | 1 (2.8) | 0 (0) |

5.3.2.2. Underlying disease (that causes IF)

**Table 5.10:** Micronutrient blood test results subcategorised according to the patient's underlying disease that causes IF.

| | | Underlying disease (that causes IF) [Num. of tests][%] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Micronut.** | **Test Classif.** | **Short bowel** | | **Intestinal fistula** | | **Intestinal dysmotility** | | **Mechanical obstruction** | | **Extensive small bowel mucosal disease** | |
| | | **Num** | **%** | **Num** | **%** | **Num** | **%** | **Num** | **%** | **Num** | **%** |
| Copper | Deficient | 55 | 12.6 | 0 | 0 | 11 | 18.3 | 0 | 0 | 3 | 20.0 |
| | In range | 348 | 79.8 | 21 | 95.5 | 46 | 76.7 | 19 | 76.0 | 12 | 80.0 |
| | In excess | 33 | 7.6 | 1 | 4.5 | 3 | 5.0 | 6 | 24.0 | 0 | 0 |
| Manganese | Deficient | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | In range | 211 | 50.8 | 9 | 42.9 | 33 | 58.9 | 8 | 30.8 | 1 | 8.3 |
| | In excess | 204 | 49.2 | 12 | 57.1 | 23 | 41.1 | 18 | 69.2 | 11 | 91.7 |
| Selenium | Deficient | 140 | 31.3 | 2 | 8.7 | 33 | 55.9 | 3 | 12.0 | 9 | 56.3 |
| | In range | 281 | 62.9 | 17 | 73.9 | 25 | 42.4 | 20 | 80.0 | 7 | 43.7 |
| | In excess | 26 | 5.8 | 4 | 17.4 | 1 | 1.7 | 2 | 8.0 | 0 | 0 |
| Zinc | Deficient | 14 | 3.2 | 1 | 4.6 | 9 | 15.5 | 0 | 0 | 8 | 53.3 |
| | In range | 367 | 85.0 | 18 | 81.8 | 42 | 72.4 | 22 | 88.0 | 7 | 46.7 |
| | In excess | 51 | 11.8 | 3 | 13.6 | 7 | 12.1 | 3 | 12.0 | 0 | 0 |
| Ferritin | Deficient | 19 | 5.4 | 1 | 3.8 | 7 | 20.0 | 2 | 8.3 | 5 | 23.8 |
| | In range | 254 | 72.2 | 24 | 92.4 | 24 | 68.6 | 14 | 58.4 | 16 | 76.2 |
| | In excess | 79 | 22.4 | 1 | 3.8 | 4 | 11.4 | 8 | 33.3 | 0 | 0 |
| Folate | Deficient | 1 | 0.2 | 0 | 0 | 1 | 2.0 | 0 | 0 | 2 | 10.0 |
| | In range | 327 | 77.9 | 28 | 100.0 | 36 | 70.6 | 22 | 100.0 | 16 | 80.0 |
| | In excess | 92 | 21.9 | 0 | 0 | 14 | 27.4 | 0 | 0 | 2 | 10.0 |
| Vitamin A | Deficient | 45 | 16.4 | 1 | 8.3 | 20 | 60.6 | 5 | 29.4 | 6 | 33.3 |
| | In range | 173 | 63.2 | 9 | 75.0 | 11 | 33.3 | 10 | 58.8 | 11 | 61.1 |
| | In excess | 56 | 20.4 | 2 | 16.7 | 2 | 6.1 | 2 | 11.8 | 1 | 5.6 |
| Vitamin B12 | Deficient | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | In range | 264 | 63.5 | 21 | 75.0 | 27 | 55.1 | 14 | 66.7 | 17 | 94.4 |
| | In excess | 152 | 36.5 | 7 | 25.0 | 22 | 44.9 | 7 | 33.3 | 1 | 5.6 |
| Vitamin D | Deficient | 22 | 6.0 | 0 | 0 | 5 | 10.4 | 0 | 0 | 1 | 7.7 |
| | Insufficient | 86 | 23.4 | 8 | 40.0 | 12 | 25.0 | 5 | 22.7 | 3 | 23.1 |
| | Adequate | 108 | 29.3 | 7 | 35.0 | 20 | 41.7 | 11 | 50.00 | 6 | 46.1 |
| | Optimal | 152 | 41.3 | 5 | 25.0 | 11 | 22.9 | 6 | 27.3 | 3 | 23.1 |
| Vitamin E | Deficient | 13 | 4.8 | 0 | 0 | 5 | 15.1 | 0 | 0 | 10 | 55.6 |
| | In range | 252 | 92.3 | 11 | 91.7 | 28 | 84.9 | 16 | 94.1 | 8 | 44.4 |
| | In excess | 8 | 2.9 | 1 | 8.3 | 0 | 0 | 1 | 5.9 | 0 | 0 |

5.3.2.3. Indication for HPN (as clinically noted)

**Table 5.11:** Micronutrient blood test results subcategorised according to the patient's indication for HPN.

| | | Indication for HPN (as clinically noted) [Num. of tests] [%] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Micronut.** | **Test classif.** | **SBS** | | **Mal-abs.** | | **Obst.** | | **Fistula** | | **Motility** | | **Failed ENT** | | **HO stoma** | |
| | | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** |
| Copper | Deficient | 55 | 12.9 | 1 | 8.3 | 0 | 0 | 0 | 0 | 11 | 21.1 | 0 | 0 | 2 | 18.2 |
| | In range | 370 | 87.1 | 11 | 91.7 | 1 | 25.0 | 5 | 100.0 | 38 | 73.1 | 13 | 100.0 | 8 | 72.7 |
| | In excess | 0 | 0 | 0 | 0 | 3 | 75.0 | 0 | 0 | 3 | 5.8 | 0 | 0 | 1 | 9.1 |
| Manganese | Deficient | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | In range | 218 | 49.4 | 0 | 0 | 3 | 75.0 | 2 | 40.0 | 30 | 62.5 | 6 | 46.1 | 3 | 30.0 |
| | In excess | 223 | 50.6 | 9 | 100.0 | 1 | 25.0 | 3 | 60.0 | 18 | 37.5 | 7 | 53.9 | 7 | 70.0 |
| Selenium | Deficient | 140 | 29.6 | 8 | 61.5 | 1 | 25.0 | 2 | 40.0 | 32 | 62.7 | 2 | 15.4 | 1 | 9.1 |
| | In range | 304 | 64.3 | 5 | 38.5 | 2 | 50.0 | 2 | 40.0 | 18 | 35.3 | 11 | 84.6 | 9 | 81.8 |
| | In excess | 29 | 6.1 | 0 | 0 | 1 | 25.0 | 1 | 20.0 | 1 | 2.0 | 0 | 0 | 1 | 9.1 |
| Zinc | Deficient | 14 | 3.1 | 8 | 66.7 | 0 | 0 | 0 | 0 | 9 | 18.0 | 0 | 0 | 1 | 9.1 |
| | In range | 389 | 85.1 | 4 | 33.3 | 2 | 50.0 | 5 | 100.0 | 36 | 72.0 | 11 | 84.6 | 9 | 81.8 |
| | In excess | 54 | 11.8 | 0 | 0 | 2 | 50.0 | 0 | 0 | 5 | 10.0 | 2 | 15.4 | 1 | 9.1 |
| Ferritin | Deficient | 21 | 5.5 | 5 | 29.4 | 1 | 25.0 | 0 | 0 | 3 | 10.3 | 4 | 40.0 | 0 | 0 |
| | In range | 277 | 72.3 | 12 | 70.6 | 3 | 75.0 | 2 | 50.0 | 22 | 75.9 | 6 | 60.0 | 10 | 90.9 |
| | In excess | 85 | 22.2 | 0 | 0 | 0 | 0 | 2 | 50.0 | 4 | 13.8 | 0 | 0 | 1 | 9.1 |
| Folate | Deficient | 1 | 0.2 | 2 | 11.8 | 0 | 0 | 0 | 0.0 | 1 | 2.3 | 0 | 0 | 0 | 0 |
| | In range | 357 | 79.5 | 13 | 76.4 | 5 | 100.0 | 4 | 100.0 | 31 | 70.4 | 9 | 75.0 | 19 | 86.4 |
| | In excess | 91 | 20.3 | 2 | 11.8 | 0 | 0 | 0 | 0 | 12 | 27.3 | 3 | 25.0 | 3 | 13.6 |
| Vitamin A | Deficient | 48 | 16.9 | 6 | 40.0 | 1 | 33.3 | 0 | 0 | 20 | 66.7 | 1 | 12.5 | 1 | 11.1 |
| | In range | 179 | 63.0 | 8 | 53.3 | 1 | 33.3 | 4 | 80.0 | 10 | 33.3 | 5 | 62.5 | 7 | 77.8 |
| | In excess | 57 | 20.1 | 1 | 6.7 | 1 | 33.3 | 1 | 20.0 | 0 | 0 | 2 | 25.0 | 1 | 11.1 |
| Vitamin B12 | Deficient | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | In range | 282 | 63.4 | 15 | 93.7 | 2 | 40.0 | 4 | 100.0 | 21 | 50.0 | 10 | 90.9 | 9 | 100.0 |
| | In excess | 163 | 36.6 | 1 | 6.3 | 3 | 60.0 | 0 | 0 | 21 | 50.0 | 1 | 9.1 | 0 | 0 |
| Vitamin D | Deficient | 22 | 5.6 | 1 | 10.0 | 0 | 0 | 0 | 0 | 5 | 12.2 | 0 | 0 | 0 | 0 |
| | Insuff. | 93 | 23.8 | 3 | 30.0 | 0 | 0 | 3 | 60.0 | 10 | 24.4 | 2 | 18.2 | 3 | 30.0 |
| | Adeq. | 119 | 30.4 | 3 | 30.0 | 3 | 100.0 | 1 | 20.0 | 16 | 39.0 | 4 | 36.4 | 6 | 60.0 |
| | Optimal | 157 | 40.2 | 3 | 30.0 | 0 | 0 | 1 | 20.0 | 10 | 24.4 | 5 | 45.4 | 1 | 10.0 |
| Vitamin E | Deficient | 13 | 4.6 | 10 | 66.7 | 0 | 0 | 0 | 0 | 5 | 16.7 | 0 | 0 | 0 | 0 |
| | In range | 263 | 92.9 | 5 | 33.3 | 2 | 66.7 | 4 | 80.0 | 25 | 83.3 | 8 | 100.0 | 8 | 88.9 |
| | In excess | 7 | 2.5 | 0 | 0 | 1 | 33.3 | 1 | 20.0 | 0 | 0 | 0 | 0 | 1 | 11.1 |

## 5.4. DISCUSSION

### 5.4.1. General discussion and main findings

The present study has displayed the relative trends in nutritional abnormalities that affect LT HPN patients. Most notably, deficiencies of selenium and vitamin D as well as excesses of manganese and B-group vitamins. The study consolidates and confirms the occurrence of nutritional abnormalities long suspected by HPN clinicians and experts; previous evidence in the literature has been limited to individual case reports rather than larger scale retrospective population studies (Vanek et al. 2012). Patients are regularly monitored at routine intervals (at least six monthly) and their PN therapy is tailored to their needs, so the prevalence and degree/extent of out-of-range blood tests was somewhat unanticipated.

Respectable and satisfactory participant recruitment (64.5%) was observed in this study considering that LT PN populations are inherently small in size, last officially documented as 10 per million in the UK (Smith et al. 2011). Fortunately for the type of study performed, the relatively small sample size was characterised by offering a rich data set for analysis, resulting in a large amount of blood test result values for inclusion in the study.

In terms of research findings for nutritional abnormalities, a similar project was recently undertaken at an NHS trust in Plymouth reviewing micronutrient status in HPN patients (Murphy and Lewis 2016b). Their key findings were the persistent deficiencies of selenium and vitamin D, concordant with the findings of this study as well. Interestingly, they reported a large percentage of patients (84%) remaining deficient in vitamin D since commencing HPN, although it was noted that there were limited number of patients included in their audit (n=22). Additionally, another project was performed by Conway et al. (2014) and noted similar findings in terms of vitamin D deficiency (56% of patients deficient) and manganese excess (65%), in 63 of 89 patients on HPN. Interestingly they excluded patients showing signs of systemic inflammatory response (as per different white cell count, CRP and albumin), an approach which could be implemented in future studies to exclude the effect of the APR on micronutrient biochemical tests. Besides the above mentioned studies, this

appears to be the one of the first studies of its kind to retrospectively investigate a broad range of ten micronutrients from a sizeable and specific population of HPN patients and document the frequency of their derangement. Since the Cardiff IF clinic caters for the HPN needs of almost all patients in Wales, the findings also accurately characterise the nutritional abnormalities for Wales as a country. As of July 2015, Cardiff IF clinic catered for 93 out of a total of 98 patients in Wales (94.9%), and this number has since grown dramatically again (Hawthorne and Juckes, personal communication, Dec 2016).

The main findings for each group of micronutrients are discussed and reviewed in turn.

#### 5.4.1.1 Trace elements and ferritin

The most remarkable observation was that approximately half of the blood test results for manganese were in excess (50.6%), alongside complete absence of deficiency. As previously mentioned, unmanaged manganese toxicity has the potential to cause irreversible neurological side-effects and parkinsonian-like symptoms. The most accepted dose for manganese supplementation was first stated by Takagi et al. (2002) as 1µmol per day, still supported by ASPEN recommendations (Vanek et al. 2012), and still less than the dose included in Additrace (5µmol) (Fresenius Kabi 2016). The studies by Howard et al. (2007) and Dickerson (2001) suggest that manganese PN requirements are likely to be met by contamination alone and should "possibly" not be intentionally supplemented in PN formulations. Yet, a recent systematic review by Baker et al. (2016) graded evidence on manganese supplementation and surmised that there was limited evidence to support not supplementing manganese in LT HPN. Further intervention studies being necessary, such as an on/off exposure study design over a period of at least six months as proposed by Takagi et al. (2002). A prevailing explanation for the large number of toxic manganese blood test results is that they result from overprovision of manganese in the TE preparation Additrace®, an opinion shared with Conway et al. (2014) after 64% of their patients (n=89) had elevated manganese levels while receiving Additrace® or Decan®. In our study,

it transpired during data collection that over the course of the time period for participants' duration requiring HPN, a large proportion of patients necessitated removal of the preparation from their PN regimen (data not collected), presumably due to the toxic manganese results. This finding is in keeping with views from others that the dose of manganese in TE preparations is excessive, especially alongside unquantified amounts as a ubiquitous contaminant (Hardy 2009; Abdalian et al. 2012; Abdalian et al. 2013). Overall, our findings for the high proportion of manganese blood test results being in excess suggest that dosing in HPN, whether intentional (as within Additrace) or unintentional (as a contaminant) requires further research into quantifying sources of manganese contamination, since evidence-based safe recommendations for its dosing and supplementation in LT PN have now been established. Ultimately evidence from the literature suggests that the PN industry should strongly consider producing manganese-free TE preparations for those with sensitive requirements e.g. those with cholestatic liver disease. The newer TE preparation Nutryelt contains less manganese in line with recommendations by Vanek et al. (2012) yet faces delays in its use for bespoke PN until it has demonstrated physical stability across a range of formulations.

A key finding from the analysis of trace elements was the large number of tests that were deficient for the TE selenium (32.8%), suggesting the presence of deficiency states and unsatisfactory selenium dosing in a large proportion of patients. Selenium appears to be one of the most manipulated components of the patients' PN regimens with 400mmol being provided in each daily vial of Additrace®; alongside any extra selenium to be dosed to patients when deficiency is observed and physical stability permits its inclusion in the PN formulation. However a factor which complicates interpretation of this finding is that selenium is a reverse APR reactant, its representation as deficiency can in some cases be due to the APR where a patient has or recently had illness, inflammation or infection; as is common with GI diagnoses and biochemically shown by a high CRP value (Ringstad et al. 1993; Stefanowicz et al. 2014). There is the general consensus that PN additives should provide more selenium (Vanek et al. 2012), this has in part been accomplished with the composition of the new TE preparation 'Nutryelt'. In light of the large SD value

for selenium, one also recommends that future research should establish which HPN patient groups require more selenium in their PN. Hopefully in time the PN industry will be able to market more PN TE additives to suit variable selenium patient requirements.

Serving as a loose indicator of iron provision, a relative finding was that ferritin showed 7.4% of deficient blood test results; a value that adds to the growing knowledge that iron deficiency anaemia is a common clinical problem in LT HPN patients. A recent study by Hwa et al. (2016) noted iron deficiency in 60 patients (32.4%) while maintained on HPN, as demonstrated by ferritin levels below the lower limit of the reference interval, necessitating replacement therapy with iron dextran, iron sucrose and ferrous gluconate. It is acknowledged that a great proportion of HPN patients at C&V UHB required further iron supplementation (usually as separate iron infusions as physical stability of the PN limits extra provision of iron in formulation), however there was poor documentation of patient records for iron administration. Clearly, there were a high proportion of results in excess of the upper limit for the reference range (20.1%). It is more likely that this result was a complication of concurrent inflammation or illness rather than excess iron provision as serum ferritin rises non-specifically as an inflammatory marker during illness and infection (Koperdanova and Cullis 2015); however it is not known how many patients may have received iron infusions within the time period of which data was collected. Measurement of serum iron as a blood test would have shown a more accurate representation of iron status in the patients but this is not routinely measured by C&V UHB.

Copper and zinc showed more consistent results with that of the general population, each displaying a more symmetrical distribution across the reference interval with less blood test results classified as deficient or in excess. However, the results do suggest that copper deficiency could be a potential problem in this population of patients (12.4%) with deficiency states previously clinically reported (Karpel and Peden 1972; Dembinski et al. 2012; Pramyothin et al. 2013; Frankel 2016), still its role as an acute phase reactant should be noted (Shenkin 2008; King 2015). Conversely, Vanek et al. (2012)

have recommended reduction of parenteral copper doses in PN additives over concerns for toxicity states; hence the results show the difficulty in gauging the optimal dose of copper for HPN patients. Additionally, the results suggest that a possible synergistic relationship may exist between copper and zinc, as excess provision of zinc has been demonstrated to be risk factor for copper deficiency (King 2015). Contrasting with the literature where most concern remains over the potential for zinc deficiency, it displayed a trend for more results in excess, a bizarre finding considering that dosing revisions have recommended increased provisions in PN are necessary (Vanek et al. 2012). Again, the potential for zinc as a reverse acute phase reactant should be noted in its ability to give a false indication of deficiency during the APR (Shenkin 2008).

Regarding the suitability of Additrace® for the population of LT PN patients, in 'Chapter 3' it transpired that only 12 out of 59 participants (20.3%) received Additrace® within their weekly PN regimen. A result which demonstrates its unsuitability for the needs of LT PN patients in terms of total TE dosing, since one would have expected more patients to receive the preparation within their PN regimen as it caters for the daily basal requirements of PN patients. Throughout the process of data collection, it became apparent that excess blood test results for manganese (and more infrequently copper and zinc) necessitated the removal of the multi-TE product Additrace® (the only way to limit manganese exposure to patients is to remove the compound preparation) (Buchman et al. 2009; Hardy et al. 2008; Shenkin 2015; Vanek et al. 2012). This incurs several effects, all the other TE then require manual addition to the PN feed (where singular TE preparations exist, limited availability), a somewhat labour intensive process which has the advantage of giving more precise individualised TE dosing for patients (for Cu, Mn, Se and Zn). An additional complication of this process is that patients then miss out on the other five essential TE included in Additrace® alongside copper, manganese, selenium and zinc. As the UK market leading preparation for TE in PN, it seems long overdue review of its composition in light of key research findings and expert opinion (Vanek et al. 2012). However the financial implications for its reformulation by its distributor 'Fresenius Kabi' may not be in their primary

interests while it is still relied upon as the only TE preparation available for consumer use in the UK. Meanwhile in 2015, Baxter and Laboratoire Aguettant announced the release of a new TE preparation named 'Nutryelt®' (Baxter 2015), its composition including doses in line with current recommendations from both ESPEN and ASPEN (see 'Chapter 4, Section 4.1.3.' for 'Table 4.1.') (Staun et al. 2009; Vanek et al. 2012). The main noteworthy changes in its composition by comparison to Additrace® include less manganese, more selenium, less copper and more zinc. The findings from the present study for excessive results for manganese and deficiencies of selenium corroborate the composition of Nutryelt®. Unfortunately, this new product faces opposition for its inclusion in LT PN as homecare companies are reluctant to incorporate it in PN production until it has satisfied physical stability tests across a range of formulations (as one would expect to find in LT PN patients requiring bespoke PN).

#### 5.4.1.2. Vitamins

The results for folate and vitamin B12 show little (0.7%) to no (0%) deficient test results respectively. A positive finding showing that patients are adequately dosed for these water-soluble vitamins, as provided by either Cernevit® or Solivito N Adult®; in some cases patients may have received intramuscular three monthly injections for diagnosed B12 deficiency anaemia. Although the findings appear alarming for the high number of test results in excess, it is generally considered that excesses of water-soluble vitamins are free from toxic effects because they are readily excreted from the body (Shenkin 2008). The large SD value and high mean for vitamin B12 showed that the data were spread across a wide range of values, again demonstrating ample yet considerably variable vitamin B12 provision in the cohort of patients. Again, similarly, data was not collected on how many patients received vitamin B12 injections during the time-period of which data was collected, which could have skewed the result to greater values for toxicity states. Generally, these results show that the preparations provided more than the necessary amounts for these vitamins, with general agreement for lack of concern when in excess.

Showing less definitive findings, vitamin A demonstrated both large amounts of blood tests as deficient (21.8%) and in excess (17.8%); rather than showing a trend in one particular direction, there was a narrower window for keeping patients in range. It has already been established that vitamin A is subject to photodegradation without light protection, especially in the absence of lipid inclusion in the PN feed (Haas et al. 2002; Ferguson 2014). This process could account for the observed deficiencies in this study considering that PN is batch delivered for patients either weekly or fortnightly. On the other hand, Shenkin (2015b) states that ultraviolet radiation of retinol is unlikely with normal room lighting. Nethertheless the deficiencies were still in keeping with a similar study by Labadarios et al. (1988), although it was noted to be a dated study with a limited sample size (43% deficient, n=22). Generally said, further research regarding the optimal dose of vitamin A is necessary especially under specific storage conditions; one would suggest intervention studies such as lipid vs. no lipid, light vs. no light or high vs. low dose vitamin A.

The results for vitamin E showed a tendency for deficiency over toxicity; however generally vitamin E proved itself as the most controlled and well supplemented micronutrient in the study with 89% of results in range. Its findings in this study are in keeping with its review from the literature which found little published evidence of nutritional abnormalities aside from incidences of inadequate vitamin E supplementation (Thurlow and Grant 1982; Porter et al. 2005; Biesalski 2009).

Patients showed inadequate vitamin D status in 30.1% of blood tests, this being the collective result for both states of deficiency (5.9%) and insufficiency (24.2%). The unquantified effect of this profound 'inadequate' vitamin D status is well known to adversely affect patient bone health (DeLuca 2009; Fessler 2009). A number of factors could contribute to approximately a third of the patient sample having inadequate vitamin D status and include:

- Reduced amounts of sunlight exposure and intradermal vitamin D synthesis by comparison to the general population e.g. more home-bound patients, confined to their residence by long infusion hours.
- Omission of vitamin D from PN regimen (from PN additives Vitlipid N

Adult® or Cernevit®) as some clinical situations necessitate their removal, for more detail see 'Chapter 4, Section 4.1.4.'.

- Inadequacy of vitamin D dose within PN additives (Vitlipid N Adult® or Cernevit®)
- Inadequate further vitamin D supplementation. Most patients require additional vitamin D supplementation outside their PN regimen. The adequacy and degree of this extra supplementation (oral/IV) may play a role in the reported deficiencies; many oral supplements exist with large variation in doses and frequencies from 200IU daily to 50,000IU weekly, depending on whether treatment aims are for deficiency or maintenance. An ergocalciferol intramuscular injection exists providing 300,000 units, usually given once or twice annually depending on serum 25-OH vitamin D levels.
- The possibility that vitamin D instability may play a role in reduced doses being delivered to patients from their PN regimen.

An intrinsic limitation associated with the reporting of vitamin D 'inadequacy' (<50nmol/L) as based on measurement of 25-hydroxyvitamin D is that it does detect the activated forms of vitamin D (alfacalcidol or calcitriol). Some patients with known renal issues could potentially have been receiving preparations containing these forms of activated vitamin D, resulting in potential overestimation of the degree of vitamin D deficiency experienced by the HPN population.

The amount of vitamin D synthesised via sunlight exposure should be considered independent of both vitamin D provision from PN and diet as a stable and consistent factor, an approach also taken by the SACN (Scientific Advisory Committee on Nutrition 2016). This sensible method then excludes the variable and often limited vitamin D provision from other sources. It is clear that this avenue requires further research not only in establishing the true stability of vitamin D in PN but also in establishing the optimal dose for PN patients. In agreement with Fessler (2009), standard PN additives contain significantly reduced doses by comparison to the recommended requirements of adults in the general population; actual maintenance requirements gauged

to be as much as 800IU per day with treatment doses said to be much higher (Holick 2007; Cannell et al. 2008; Holick et al. 2011). In a view to reflect the latest opinion for higher requirements and as previously mentioned, in July 2016 the UK SACN reviewed their daily recommended dose to 10mcg (400IU), previously 5mcg (200IU), as a baseline dose for all adults regardless of age or estimated UV synthesis. Perhaps it is time for PN additives to also reflect the more recent recommendations for daily vitamin D provision. Optimal vitamin D supplementation outside of the dose provided in PN additives is also a key consideration. A recent audit performed by Murphy and Lewis (2016a) from South-west UK based HPN centre found interesting and positive findings from their established vitamin D treatment guideline. They investigated vitamin D status in HPN patients in relation to a treatment intervention with vitamin D, either oral high dose treatment (9600IU/day) where GI absorption was possible or IM injection (300,000IU) for those with inadequate absorptive capacity. They observed vitamin D levels to be significantly improved post-intervention, according to the same vitamin D classification system implemented in the present study. Again however their study was limited by a small sample size (n=13), yet was able to establish a sound and effective protocol for the treatment of vitamin D deficiency in HPN patients.

#### 5.4.1.3. Comparative group analyses

No substantial findings were elucidated from the comparative analyses when the micronutrient blood test results were subcategorised according to 'IF pathophysiological classification', 'underlying disease' and 'indication for HPN'. Once the blood test results had been subcategorised amongst the different groups, there were variable and often insufficient numbers of tests to be able to draw conclusions or findings. Also, no patients were classified as having mechanical obstruction as a pathophysiological cause for their IF and as such no micronutrient blood tests could be categorised.

However this analysis did allow context of the blood test results for short bowel syndrome as a both an underlying disease and in terms of its pathophysiological classification, as this was the category with the largest data set post sub-categorisation. Since most nutrient absorption occurs in the small intestine, it is entirely conceivable that patients with SBS are at risk of nutritional deficiencies. One study has stated that even with as little as a third of remaining small bowel length, the body is still able to maintain adequate vitamin and mineral stores, provided there is a well-balanced diet (Westergaard and Spady 1993). Yet in patients requiring LT PN, it can already be assumed that a regular oral diet is insufficient for their needs.

The small intestine is the predominant site for absorption for nearly all vitamins, minerals, proteins and fats (Bryant and Hampton 1992). The location of their absorption give an idea of the anatomical influence upon their implication in nutritional abnormalities. Iron and zinc are known to be absorbed along its length, folate in the upper third; selenium, vitamins A, D and E in the ileum along with vitamin B12 absorption just before the small intestine joins the large intestine (Gmoshinskii and Mazo 2006; Lambert 2008). As such deficiencies are apparent and can be observed in Tables 5.9-5.11 for selenium, zinc, vitamins A, D and E; greater quantities of deranged blood test results being demonstrated with the greater degree of bowel loss and HPN dependency.

Some other notable features of the comparative analysis for 'IF pathophysiological classification' are as follows:

- High excesses of manganese across all subcategories. However notably less within SBS-JIC, those requiring less intensive HPN therapy than SBS-JC and SBS-J. Leading to a theory that those requiring more HPN (by volume or frequency) potentially receive greater doses of manganese as a contaminant (Hardy 2009).
- More deficiency states for selenium in those with motility disorders, deficiencies have previously been shown to be related to poor absorption of selenium from the GI tract (Rannem et al. 1998).
- More deficiencies of zinc in mucosal disease. Increased prevalence of zinc deficiency (19%) has been observed in other mucosal diseases (Bao et al. 2016).
- Similarly, more deficiencies were observed for vitamin E in those with mucosal disease. The only comparable evidence being that in vitamin E deficient rat models, gastric mucosal injury was greater (Naito et al. 1999).
- Higher degree of derangement of micronutrient results was observed in patients with a greater degree of bowel loss/HPN dependency (SBS J > SBS-JC > SBS-JIC). For instance one expects considerable stomal losses of selenium, zinc and copper in SBS-J patients (see Table 5.9) (Nightingale 2006; King 2015). Yet the results cannot clarify whether the degree of derangement in results is potentially due to the complication of inflammatory disease states on the accuracy of reported BT results; or whether it is simply harder to gauge accurate micronutrient requirements in those with extensive bowel loss/resection.
- More vitamin A deficiencies in motility disorders, concordant with studies implicating the role of vitamin A in the normal functioning of the enteric nervous system (ENS) which governs the function of the gastrointestinal system (Sato and Heuckeroth 2008; Wright-Jin et al. 2013).
- Highest incidences of iron deficiency (as indicated by deficient ferritin)

were in the subcategories for SBS and dysmotility, as paralleled with findings by Hwa et al. (2016).

### 5.4.2. Limitations

The findings of this all-encompassing study have been positive in confirming current issues reported in LT PN patients, yet there are limitations associated with this study. For instance, although excellent participant recruitment rates were achieved for this study, it still amounted to a small number of patients from a single HPN centre. Inclusion of more participants from more HPN centres would have made the findings of nutritional abnormalities more generalisable to the wider population and also in terms of the differing HPN practices between centres across the UK.

For one of the key results, the true degree of overprovision of manganese is complicated by the unquantifiable degree of contamination from an array of potential sources. These include the use of contaminated sterile solutions and/or needle manipulation during PN production, as well as possible contamination from needles when blood samples are taken from patients, since manganese is known to leach from metal needles (Cornelis et al. 1996; Yang and Lewandrowski 2002; Hardy 2009).

A more unavoidable limitation was the degree of variation existing between participants and the data extracted from each participant. For example, some patients may have started PN at an earlier date in time or be maintained on more (or fewer) PN feeds per week, i.e. some patients are entirely dependent on their PN. However, for the research purposes of this study, the degree of interpatient variation cannot be controlled. Prescribers monitor patients' nutritional status as recommended and the biochemical monitoring is still indicative of their nutritional status and our ability to meet their needs via manipulation of their PN regimen, regardless of how many nights per week they feed. Likewise, there were variable durations of time that each participant had spent receiving PN by the point in time of data collection. However the retrospective clinical nature of the study should be noted in that the clinical

treatment decisions were made over the course of each patients history of PN therapy to correct deranged blood test results i.e. patients being given reduced or further supplementation, and not left running LT deficiencies or toxicities.

Although C&V UHB aim to follow ESPEN recommended guidelines for at least six monthly biochemical monitoring, in practice this is not always possible as sometimes it may be a slightly longer interval between monitoring depending on clinic/patient availability or whether patients require more frequent review after deranged test results. In some instances, biochemistry test results are not available for a number of reasons, these include laboratory error, inadvertent omission of blood test request (i.e. forgotten), incorrect sample collection (e.g. wrong sample container) or expired blood samples. Overall the results from this study still display the clinical picture for the degree of deranged results from the population, and should be considered in line with the view of Fragkos et al. (2016) who showed that over the time course of HPN administration that micronutrient deficiencies were maintained regardless of underlying IF aetiology and/or presence of fistula or stoma, considering the limited micronutrient PN preparations available. Both Fragkos et al. (2016) and Forbes and Forbes (1997) were able to show that HPN service management by a specialised IF/HPN NST in an ad hoc fashion was effective and able to cover patients' LT PN requirements, as paralleled by the HPN monitoring performed at C&V UHB. Yet aside from this consideration, it should be noted that the present study did not separate blood tests that could have been associated with potential deficiency states prior to patients starting HPN, as noted in a similar study by Murphy and Lewis (2016b).

Another unavoidable and inherent limitation associated with blood biochemistry is the actual degree of accuracy, reliability and precision of the reported results themselves (see Chapter 4, Section 4.1.5.1). Since these biochemical parameters are used clinically to monitor and guide treatment decision process in practice, they are therefore still the best data parameters to have included in the study. Yet it has been mentioned by some that more accurate biochemical indicators of nutrition states exist which may play roles in future research studies (Daniells and Hardy 2010; Hambidge 2003; He

2011; Hotz et al. 2003; Nève 2000). Likewise, a similar limitation exists for folate and vitamin B12 in that the biochemical tests used to assess their status have biphasic and inverse characteristics, suggesting that the cut-off points for their reference interval are somewhat unreliable and inaccurate. Selhub et al. (2008) suggested a method to establish better cut-off points for assessing nutritional status for these vitamins, by using dose-concentration graphical intersections to guide the assessment of adequacy of vitamin provision.

The effect of the APR on the accuracy of the reported biochemistry results should also be considered as a possible limitation, as concomitant infection decreases intestinal absorption of nutrients and can cause direct loss of micronutrients from the body (see Chapter 4, Section 4.1.5.1.). Traditional biochemical indicators for micronutrients (e.g. iron, zinc, selenium, copper) are altered during the APR, giving inaccurate estimation of nutritional status (Bresnahan and Tanumihardjo 2014). The 'accuracy' of blood test results are clinically interpreted on an individual case basis in the day-to-day practice setting during review of patients' biochemistry and PN regimen, in which case prescribers make an informed decision whether to alter patients' PN prescription based on the reliability of the blood test results. For research purposes, all test data was included regardless of how accurately or reliably it was interpreted at the time. The APR is estimated to account for an overestimation of 16% of diagnoses of vitamin A deficiency and underestimations of 15% for the prevalence of iron-deficiency anaemia (Wieringa et al. 2002). The concise review article by Bresnahan and Tanumihardjo (2014) reports that the effect of the APR on micronutrient status during infection is most notable for retinol, iron, ferritin and zinc (by degrees of up to 25% reduction, 20-50% reduction, 30-1400% increase and 12% reduction, respectively). The underestimation of selenium during the APR has also been well-documented (Maehira et al. 2002). Meanwhile a more concise investigation into the effect of the APR on micronutrient status found that the magnitude of the effect was greatest for selenium and vitamins A, B6, C, and D, for which the median plasma concentrations decreased by more than 40%, although it was noted that there was marked interpatient variation for the effect of the APR on each micronutrient (Duncan et al. 2012). Altogether,

this limitation complicates accurate and reliable interpretation of meaningful blood biochemistry, particularly in situations like the present study. It has been proposed that blood concentrations of acute phase proteins (e.g. CRP, cytokines) can be measured to assess the time scale and severity of infection, allowing corrective interpretation of blood tests for the APR during illness; however there would need to be standardised cut-off points for each nutritional application which do not yet exist (Abraham et al. 2003; Bresnahan et al. 2014). Future studies should aim to incorporate these corrective measures to give more accurate context for findings of the specific nutrients affected by the APR.

### 5.4.3. Future work

The findings from this chapter have revealed some notable more critical areas requiring further research within the scope of this PhD project, they include the following:

- Researching the extent of the issue surrounding inadequate vitamin D provision in HPN patients and whether there is a detrimental effect on patient bone health
- Investigating the stability of vitamin D in the multi-component additives used to formulate HPN, to exclude any unknown potential stability problems which may limit the true dose of vitamin D being delivered to patients.
- Performing a 'gap-analysis' for selenium prescribing in PN, to investigate whether patients are adequately prescribed sufficient doses of selenium in their PN (from both multi-component and singular additives) in line with their blood test results, and whether physical stability regulations (per volume of PN feed) limit the dose some patients require. Initial data collection and project familiarisation showed selenium to be a well-manipulated micronutrient in PN. Livingstone (2016) has explained the difficulty in getting a micronutrient preparation to suit all HPN patient needs; varied patient diagnoses may necessitate different micronutrient dosing requirements e.g. from the degree of remaining bowel or remaining

oral/enteral consumption. In the case of selenium, its absorption occurs in the upper small intestine without homeostatic control where more than 90% of dietary selenium is absorbed. This demonstrates the importance for its correct provision to patients with variable lengths of small intestine and the accurate determination of their requirements, also in patients without SBS requiring HPN for non-SBS diagnoses and indications (Livingstone 2016).

Other recommendations for future work relating to this chapter but not to be covered within the scope of this PhD project include:

- Prescribers should strive to treat out of range biochemistry results wherever possible using up-to-date evidence-based guidelines e.g. correct vitamin D supplementation e.g. make sure clinicians follow the vitamin D deficiency guideline.
- Undertake larger scale studies incorporating patients from more HPN centres to create more generalisable results of a higher grade of research value with the ability to produce well-informed findings. Also, to incorporate more patients from each of the subcategories for 'IF pathophysiological classification', 'underlying disease' and 'indication for HPN'.
- Perform intervention studies to establish the most appropriate doses of micronutrients for inclusion in PN. These would ideally be implemented from standardised protocol driven treatment guidelines e.g. specific high dose vitamin D treatment for all patients found to have 25-hydroxyvitamin D levels below 50nmol/L, or a study comparing the incidence of nutritional abnormalities with Additrace® vs. Nutryelt®. Other ideas for intervention studies would be to further investigate micronutrient stability in PN that is subjected to prolonged storage conditions, as it has been proven that some vitamins degrade over time once formulated in PN (Ferguson 2014).
- Perform a comparison study for nutritional abnormalities in HPN patients by contrast to the general population. To address the question: 'Do nutritional biochemistry test results actually result in the expected

rates of deficiency and toxicity in the general population?' Or could there be unrecognised nutritional abnormalities in the general population as well. For instance, it has been suggested that much of the UK and Scandinavia is deficient in vitamin D in the winter months due to lack of sunlight exposure (Pearce and Cheetham 2010).

- In light of the gross number of manganese test results in excess; further studies investigating the actual amount of manganese present as contaminants in standard solutions, preparation materials and prepared products would help to reveal more appropriate doses for patients, a recommendation shared with Hardy (2009).
- Incorporate the use of more accurate biochemical monitoring techniques for assessment of nutritional status. For example, a variety of different samples can be used to assess selenium status (hair, nail, selenoproteins, etc); at present serum selenium is still the favoured measure but it is not known whether it is the most accurate or reliable (Nève 2000; Thomson 2004). Also, it has recently been shown that protein expression of copper enzymes (caeruloplasmin and superoxide dismutase) are more sensitive than current standard indicators for the evaluation of copper status (Harvey and McArdle 2008; Olivares et al. 2008).
- Propose revision of TE dosing guidelines and current TE formulations so that:
    - Separate products are available each of the individual TE, to allow easier manipulation of individual patient requirements.
    - A variety of fixed dose micronutrient products are available for use, as HPN population has considerably variable requirements. Particularly for selenium and manganese components.
    - PN products are labelled with maximum allowable contaminant levels for TE known for contaminant issues i.e. aluminium, chromium and manganese.
- In the absence of new TE formulations, it would be useful to compare the incidence of nutritional abnormalities in those given solely a fixed dose TE preparation versus those who have already necessitated

removal of the fixed dose compound preparation and require manual manipulation of their TE dosing in PN i.e. demonstrate the clinical impact of the problem with accurate TE dosing.

- Monitoring of nutritional status should encompass up-to-date and best practice biochemical nutritional detection techniques. Buchman et al. (2009) eluded to this topic in relation to vitamins D and K at their workshop. It is well accepted that HPN patient review should consider the full clinical picture, not just review of the reported test results i.e. whether there are concomitant symptoms; especially during situations where the accuracy and reliability of blood test biochemistry may be affected.

## 5.5. CONCLUSION

The current preparation Additrace® does not meet the day-to-day TE requirements for LT use in PN patients; the preparations for vitamins, Cernevit® and Solivito® have also shown themselves as unable to meet the general requirements for these patients. The findings from this study are in line with views held by other researchers in that the composition of the products is responsible; suggesting review of their dosing is necessary (Buchman et al. 2009; Btaiche et al. 2011; Vanek et al. 2012; Núñez-Ramos et al. 2015; Żyła et al. 2015). Adjustments to micronutrient doses in PN should be guided by regular monitoring of micronutrient status. Yet while efforts should be made to keep micronutrient dosing in HPN patients as individualised as possible, the limited existence of compound preparations frustrates this clinical practice. NST and clinicians need to be aware of the findings of this study and its implications for their LT PN patients; particularly the accurate and reliable assessment of micronutrient status and subsequent dosing in PN. For example, PN components should be monitored both on an individual basis as well as for their effect upon each other, especially copper and zinc. In conclusion, the study has helped to verify and validate suspected issues associated with micronutrient dosing in LT HPN patients and outlined areas requiring further research.

# CHAPTER SIX:

## Laboratory investigations into the stability of vitamin D in multi-component additives using High Performance Liquid Chromatography (HPLC)

## 6.1. INTRODUCTION

This chapter details investigations into the stability of vitamin D in multi-component PN additives. The rationale being that problems associated with vitamin D stability within the PN admixture could interfere with provision of the desired dose to patients (potential sub-optimal dosing). Results in Chapter 4 revealed vitamin D as nutrient of notable clinical interest since many patients' blood tests showed deficient and insufficient vitamin D status.

This section of the thesis aimed to:

- Perform a critical analysis of the literature pertaining to vitamin D stability in PN
- Develop a stability-indicating assay using High Performance Liquid Chromatography (HPLC) to determine vitamin D stability in multicomponent additives and 'standard' HPN formations
- Propose evidence-based recommendations for future directions to investigate vitamin D stability in PN

## 6.2. BACKGROUND AND RATIONALE

In recent years there has been growing interest in vitamin D and its relation to bone health, especially with the re-emergence of rickets in some urban areas. This is transferable to the HPN population where MBD is well documented and the numerous contributing factors are still poorly understood (Shike et al. 1981; Pironi 2002; Derepas et al. 2013). It is feasible that the dose and stability of vitamin D included within patients' PN regimens could be a contributory factor. As previously mentioned in Chapter 4, the SACN have recently increased the RNI for vitamin D to 10μg/day and it is possible the PN dose recommendations are lagging behind, with consequential health problems for LT PN patients.

### 6.2.1. General vitamin D stability

All vitamins are diverse compounds varying in their stability and susceptibility to degradation by chemical or physical factors (Combs 2012). e.g. temperature, light, storage. Assessment of stability and degradation usually involves quantification of pure vitamin detection from formulation samples at various time points. Stability testing aims to ensure and provide a guideline for reassured product stability, bioavailability for the individual components and final total formulation within a given time interval (Bakshi and Singh 2002).

Vitamin D has been reported to be slightly sensitive to temperature, humidity, light and acidic conditions as well as being very sensitive to oxygen and stable to alkaline conditions (Shurson et al. 1996). The degree of sensitivity depends on the final product form, conditions of manufacturing and storage (Frye 1994). Findings have been corroborated by Mahmoodani et al. (2017) in which degradation studies were performed using HPLC methods. Overall, vitamin D is stated to be less susceptible to oxidative losses than vitamin A, carotenoids and vitamin E (Eitenmiller and Landen 2008). From a stability standpoint in food/nutrition, vitamin D has been shown to be stable in fortified milk with only slight losses when subject to light exposure, a loose indicator of stability in PN admixtures (Renken and Warthensen 1993). With regards to bioavailability, in a regular GI diet, absorbed vitamin D is incorporated into

chylomicrons following enterocyte uptake and transported by the lymphatic system (Van den Berg 1997). It is assumed that during IV provision of vitamin D in PN, it is also incorporated into chylomicrons and delivered to fatty tissues for storage. Less is known about the extent of bioavailability from individual food sources other than greater uptake and efficacy of increasing serum vitamin D levels is observed with the activated form of vitamin D over the prohormone vitamin D (Van den Berg 1993). Perhaps PN patients' vitamin D status would respond better to PN supplemented with additives containing activated vitamin D rather than the current prohormone forms of vitamin D.

### 6.2.2. Vitamin D stability in PN

Few studies have investigated the stability of vitamin D in PN admixtures; of those that have, none are recent. Allwood and Kearney (1998) acknowledged the sparsity of knowledge surrounding the stability of vitamin D in PN admixtures during storage. A study by Gillis et al. (1983) documented a 32% loss of vitamin D following a 24-hour infusion of PN. Comparison of sample concentrations at various sites within the infusion set-up suggested that vitamin D may bind to plastic found in bags and administration sets. However, another study by Koo et al. (1986) reported no significant differences among PN samples obtained immediately on preparation, before, and after the use of an in-line filter at the end of a 24 hour infusion period. This finding goes against those of Gillis et al. (1983); suggested reasons for the opposing results are thought to relate to differences between detection of vitamin D via a radiolabelled trace and standard vitamin D recovery methods, as well as variable amounts of PN solution in contact with plastic tubing according to variable infusion rates. Additionally, glass bottles were used for PN storage in the study by Koo et al. (1986), whereas Gillis et al. (1983) used polyvinyl chloride bags which may have had greater adsorptive potential for vitamin D on their surface. Similarly to the findings of Koo et al. (1986), Dahl et al. (1986) reported no bioavailable losses during simulated delivery within a fat emulsion in an ethylene vinyl acetate (EVA) bag. A more recent study which can help demonstrate the degradative effect of light and oxygen on vitamin D storage in soybean oil (a component of fat emulsions) found that vitamin D losses were 68% and 44% in light and semi-dark conditions respectively. This

study by Hemery et al. (2015) also suggested that the natural antioxidant effect of vitamin E influenced the stability of the reported stability findings for vitamin D.

A study by Blanco et al. (1994) determined an assay investigating vitamin D amongst other fat-soluble vitamins in paediatric PN solutions. They successfully separated all vitamins on a $C_{18}$ bonded phase column using methanol as an eluent and UV detection at 265nm. The use of a narrow bore column alongside a lower solvent flow rate (0.2mL/min) achieved lower detection limits than ordinary HPLC columns. However, the publication did note the requirement of a pre-concentration step to determine vitamin D with average recoveries stated as 91-110%, as well as the implementation of a complex sample clean-up process involving centrifugation with hexane, organic extraction, filtration and evaporation. Ultimately their study found the degradation of vitamin D during light exposure to decrease from 90.2% to 64.7% between 10 and 24 hours post-preparation. This demonstrates the fundamental sensitivity of vitamin D to light and other potential factors e.g. other PN components, composition of admixtures, container and administration materials.

The inconclusive findings from these limited studies and paucity of research findings do not exclude stability issues and/or degradation issues relating to vitamin D as a potential occurrence in compounded PN admixtures.

## 6.3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) INVESTIGATIONS INTO VITAMIN D STABILITY

### 6.3.1. HPLC system

HPLC is a powerful separation method used to separate compounds in solution. Compounds from an analytical sample partition between mobile and stationary phase at different rates, eluting at different time points. Each resolved compound is subsequently detected by a variety of detectors (Snyder et al. 1997); both the area and height of the signal being proportional to the quantity of analyte for detection (Meyer 2010).

Reverse phase HPLC (RP-HPLC) is a specific form of HPLC in which a polar aqueous phase is passed through a packed bed of hydrophobic stationary phase (i.e. a column) under pressure (Snyder et al. 1997). The more hydrophobic compounds adsorb to the stationary phase and are eluted slower than hydrophilic compounds (Ettre 1993).

#### 6.3.1.1. Stability indicating HPLC

HPLC is used to indicate stability of compounds over various time points by detecting deterioration in peak size and/or identification of degradation compounds and impurities (Shah et al. 2012). RP-HPLC with UV detection is routinely used as the analytical method of choice for stability assessment for specific analytes and drug compounds (Qiu and Norwood 2007).

Drug regulatory and approval processes require full validation of stability indicating methods to ensure reproducible monitoring of degradation products (Smela 2005; Maggio et al. 2013). Broadly speaking, it encompasses the following steps: i. sample generation, ii. method development & optimisation, and iii. method validation (Blessy et al. 2014):

RP-HPLC determines the detection of fat-soluble vitamins by employing organic mobile phase compositions to ensure they are solvent throughout analysis. Organic solvents are often used in combination, their differing

strengths of polarity optimise selectivity and control the separation of fat-soluble compounds (Dionex 2010).

HPLC methods employing UV detection are common and particularly useful for analytes containing unsaturated bonds or aromatic groups such as with structurally complex fat-soluble vitamins (Nollet 2000; Dionex 2010; Cosmosil 2017).

Charged aerosol detection (CAD), is a relatively recent approach as a method of HPLC analyte detection. It has established itself for the detection of fat-soluble vitamins, lipids and lipid peroxidation products which lack a specific chromophore and responsivity to UV absorption (Cascone et al. 2006; Moreau 2006; Plante et al. 2011; Acworth and Kopaciewicz 2017). CAD detection functions via nebulisation of non-volatile compounds and application of charge to the analyte. An electrometer then generates a signal correlating to the concentration of analyte present (Almeling et al. 2012).

CAD is known for its highly sensitive quantitative detection of compounds over a broad dynamic range, covering at least four orders of magnitude with lower limits of detection down to pA (pico-ampere) and fA (femto-ampere) (Plante et al. 2011). CAD detection has proven itself as a reliable alternative to UV detection for weakly UV active compounds (e.g. vitamin D). Its advantages include the universal detection of non-volatile analytes, responses independent of chemical properties, alongside simple and reliable means of use (Gamache et al. 2005; Vehovec and Obreza 2010).

#### 6.3.1.2. HPLC detection of vitamin D

Many HPLC assays are capable of detecting and assessing vitamin D in non-complex samples (Kumar et al. 2015; Sigma Aldrich 2016). Its detection in serum plasma was commonplace with UV detection as the gold standard of choice for activated vitamin D detection in adults for assessment of vitamin D status (Jones 1978; Hollis and Frank 1985). Nowadays it is considered a cumbersome assay and recent advances in liquid chromatography mass spectrometry (LC-MS) has facilitated easier and less time-consuming

detection of vitamin D without detection of complicating metabolites (Holick 2005; Guo et al. 2006).

HPLC methods have also been used to detect vitamin D from complex multi-component sample mixtures, usually samples of fat and water-soluble vitamins, down to sample concentrations of 0.25μg/mL (Dionex 2010; Cosmosil 2017; Kucukkolbasi et al. 2013; Xinlei et al. 2015).

Fewer assays have been published which accurately and consistently document the detection of vitamin D from samples of PN admixtures (Allwood and Martin 2000; Skouroliakou et al. 2008; Ferguson 2014). Specific difficulties include the development of a balanced assay that sufficiently detects vitamin D amongst other nutritional components or that are capable to detect vitamin D at its low RDA/RNI dose.

The literature has shown UV detection to be the most frequently employed detection method to resolve vitamin D with the UV range 250-280nm, alongside variable polar solvent aqueous mobile phase compositions. HPLC LC-MS was also demonstrated as a frequent method of detection but was outside the scope of available equipment in our laboratory (Szczesniewski and George 2009; Duan et al. 2010; Aurand and Cramer 2017).

HPLC has been used to detect vitamin D with CAD detection (Plante et al. 2010). It is primarily marketed for the analysis of lipids and lipid components (fatty acids, glycerides etc) but has also been shown to detect vitamin D from complex nutrition lipid admixtures, yet not strictly PN (Plante et al. 2009). As such CAD detection of vitamin D from PN admixtures has yet to be fully established.

#### 6.3.1.3. In-house HPLC systems

Laboratory investigations involving UV detection were carried out using a Spectra System® from Thermo Finnigan (Thermo Scientific, West Palm Beach, United States). The system included an SCM1000 vacuum membrane degasser, P2000 gradient pump, AS3000 autosampler and UV1000 UV detector.

Subsequent laboratory investigation involving CAD detection were carried out on an Ultimate 3000 RS system with Corona® Veo RS CAD detector (Thermo Scientific, West Palm Beach, United States).

#### 6.3.1.4. Multi-component vitamin preparations

Each vial of Cernevit® contains an orange-yellow caked powder for reconstitution to 5mL with water for injection (WFI). It contains cholecalciferol 5.5mcg (220IU) along with other water and fat-soluble vitamins. Whereas each vial of Vitlipid N Adult® holds 10mL of a milky emulsion containing just fat-soluble vitamins, of interest, ergocalciferol 5mcg (200IU). See Appendix VIII for the full formulations for each preparation.

These multi-component vitamin preparations are intended for daily dose administration directly into the PN formulation to meet the basal daily requirements of LT PN patients.

#### 6.3.1.5. Reference standards

Analytical grade standards of vitamin D were sourced from Sigma-Aldrich Co Ltd (Heatherhouse Industrial Estate, Irvine, UK, KA12 8NB).

- Cholecalciferol
    - Powder (≥98% HPLC grade)
    - Solution 1mg/mL (HPLC grade, in ethanol)
- Ergocalciferol
    - Powder (>98% HPLC grade)

6.4. Methods

### 6.4.1. Development of HPLC assay using UV detection to detect vitamin D in multicomponent preparations

To begin with, HPLC with UV detection was chosen to build upon existing methods to develop an assay for the detection of vitamin D in the multicomponent preparations Vitlipid N Adult® and Cernevit®.

It is worth noting that HPLC assay methods usually specify the form of vitamin D for investigation (either ergocalciferol or cholecalciferol). However, experts have noted the difficulty in separating retention peaks for the two forms of vitamin D because the compounds are so structurally similar, as shown in Figure 6.1 (Henderson and Berry 2009; Plante et al. 2009). However, this was not an issue in these investigations as each multi-component preparation only contained a single form of vitamin D respectively; cholecalciferol (vitamin $D_3$) 1µg/mL in Vitlipid N Adult® and ergocalciferol (vitamin $D_2$) 1.1µg/mL in Cernevit®.

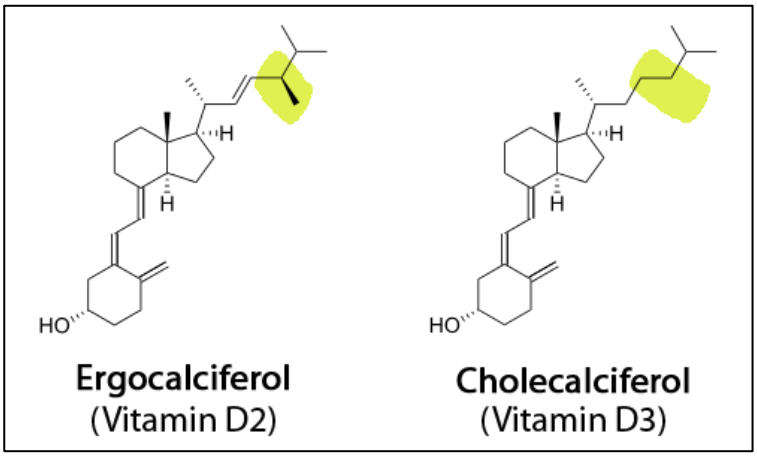


**Figure 6.1:** Chemical structures for Vitamin $D_2$ and $D_3$.

Various assay methods were initially trialled to optimise vitamin D selectivity and replicate the degree of detection stated (Dionex 2010; Xinlei et al. 2015; Sigma Aldrich 2016; Cosmosil 2017). Finally, the isocratic elution method used in two application notes by Phenomenex was chosen for assay development to identify vitamin D from the samples Cernevit® and Vitlipid N Adult® as they

displayed the greatest detection of vitamin D from the sample solutions (Phenomenex 2016a; Phenomenex 2016b).

Initial assay conditions were as follows:

**Column**: Chrompack OmniSphere 5 C18 150x3mm, 5µm particle size (Varian, Palo Alto, USA).

**Mobile phase composition**:

**A:** Acetonitrile 75%

**B:** Methanol 25%

**Flow Rate**:1.3 mL/min

**Column temperature**: ambient room temperature

**UV detection**: 280 nm

**Run-time:** 15 minutes

**Injection volume:** 10µL

The method was adapted as follows:

- Establishing a run-time of 20 minutes to ensure all compounds eluted from column.
- Increasing the injection volume to 20µL and relative column load of vitamin D (0.02µg Cernevit®, 0.011µg Vitlipid N Adult®).

### 6.4.2. Development of HPLC assay using charged aerosol detection (CAD) to detect vitamin D in multicomponent preparations

The introduction of the novel, more sensitive method of CAD detection within the laboratory permitted a different avenue to assay and detect vitamin D in PN additives.

The method by Plante et al. (2009) was chosen for assay development to undertake analysis of vitamin D in Cernevit® and Vitlipid N Adult®. Their method demonstrated good resolution of vitamin D at concentrations of 30ppm in ethanol/BHA (butylated hydroxyanisole) for a mixed fat-soluble vitamin standard solution.

Initial assay development commenced under the following parameter conditions:

*CAD Corona® parameters:*
**Gas**: 35 psi via nitrogen generator
**Filter**: Corona
**Range**: 500 pA
**Nebulizer heater**: 30 °C

*HPLC Parameters*
**Mobile phase composition:**
**A:** Methanol/water/acetic acid (750:250:4)
**B:** Acetonitrile/methanol/tetrahydrofuran/acetic acid (500:375:125:4)
**Gradient**: 0–70% B to 46 min; 70–90% B to 60 min; 90% B to 65 min; 0% B from 65.1 to 72 min
**Flow rate**: 0.8 mL/min
**Run time**: 72 min
**HPLC Column**: Chrompack OmniSphere 5 C18 150x3mm, 5µm particle size (Varian, Palo Alto, USA)
**Column Temperature**: 40 °C
**Sample Temperature**: 10 °C
**Injection Volume**: 10 µL
The assay method was adapted as follows:

- Injection volume was increased to 40 µL to increase the relative column load of vitamin D (4.4ng Vitlipid N Adult, 8ng Cernevit).
- Sample temperature was increased to room temperature to avoid potential precipitation, sedimentation or dissolution of sample components within the formulation.
- Gradient: 0–70% B to 20 min; 70–100% B to 65 min; 100% B 65-70 min; down to 0% B from 70.1-75 min. Gradient changes shown in Table 6.1.
- Decreasing the time to get to 70% B, thereby increasing the gradient at the start of the run so that the more soluble components of the sample eluted quicker, minimising their co-elution on top of vitamin D.
- Reducing the gradient and increasing the time to get from 70% B to 100% B, to space out all the resolved peaks within the area/region where vitamin D was known to resolve.
- Adding final 'wash' stages with 100% A and B to ensure all components of the sample mixture had eluted from the column, particularly the lipid components as these have been demonstrated to contaminate the column and exhaust its analytical integrity (Majors 2003).

**Table 6.1:** The developed gradient elution method to resolve vitamin D using CAD.

| Time (minutes) | A (%) | B (%) |
|---|---|---|
| 0 | 100 | 0 |
| 20 | 30 | 70 |
| 65 | 0 | 100 |
| 70 | 0 | 100 |
| 70.1 | 100 | 0 |
| 75 | 100 | 0 |

### 6.4.3. Preparation of multi-component preparations

Each day, a new vial of Cernevit® was reconstituted with 5mL WFI. Vitlipid N Adult® is a ready-made oil-in-water emulsion of 10mL in volume.

HPLC samples were prepared by filling light-protective amber HPLC vials with samples of Cernevit® or Vitlipid N Adult®. The samples were kept away from sunlight to prevent the potential effect of photo-degradation of the vitamins.

## 6.5. RESULTS

### 6.5.1. UV detection - Cernevit® and Vitlipid N Adult®

Despite various efforts for method optimisation and successful identification of vitamin D as a pharmaceutical standard, vitamin D could not be identified from the compound preparations Cernevit® and Vitlipid N Adult® using UV detection. Figure 6.2 gives an example, showing the total absence of a detectable vitamin D peak by comparison to a spiked sample.

The main limitation associated with UV detection of vitamin D from the samples was the co-elution of other components in the sample. Efforts to spread out their elution times and reduce their subsequent effects on the assumed vitamin D peak were unsuccessful. These efforts included reducing the flow rate, increasing the run time and addition of a phosphate buffer to help reduce tailing of the peak. Also, the isocratic mobile phase composition was manipulated across various degrees of polarity with methanol, tetrahydrofuran and acetonitrile.

Approximation of the point for vitamin D elution was performed by using varying concentrations of vitamin D standards that were stronger than the sample concentrations. However, the peak could not be consistently identified and appeared to 'move' between subsequent runs which should not have been affected by the concentration, suggesting other factors interfered with consistency of vitamin D elution during repeat and successive runs. It was ultimately concluded that the concentrations of vitamin D in the samples of Cernevit® and Vitlipid N Adult® (5-5.5mcg/mL) were not strong enough to be reproducibly detected by the UV detector, especially amongst other components of the PN additives.

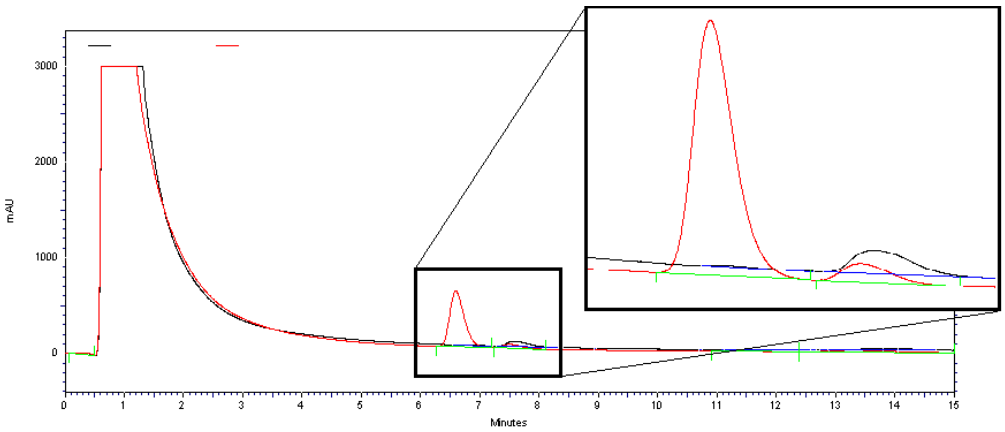


**Figure 6.2**: A chromatogram of a pure sample of Cernevit® (black line) overlaid with a chromatogram of Cernevit® spiked with extra vitamin D (red line). The figure displays the absence of a detectable peak for vitamin D from the pure Cernevit® sample using UV detection (no corresponding black vitamin D peak beneath the large red spiked vitamin D peak).

### 6.5.2. CAD detection - Cernevit®

Disappointingly, the adapted method described by Plante et al. (2009) could not detect vitamin D from reconstituted samples of Cernevit®.

The peak corresponding to vitamin D could not be identified from the chromatogram despite spiking samples of Cernevit® with up to ten times the quantity of vitamin D contained in the multicomponent preparations. It was thought to be due to vitamin D coming out of solution or partitioning into ethanol. The potential resultant effect being that vitamin D could have been missed when the injection volume was taken from the sample vial. Further attempts to spike Cernevit® with vitamin D without using an ethanol-based vitamin D standard were unsuccessful and resulted in immediate precipitation of vitamin D. This identified a need for further sample preparation for Cernevit® before any further investigative HPLC work and attention was turned towards Vitlipid N Adult® instead.

### 6.5.3. CAD detection - Vitlipid N Adult®

6.5.3.1. Identification of vitamin D within assay

Vitamin D was successfully identified from samples of Vitlipid N Adult®, see below Figure 6.3.

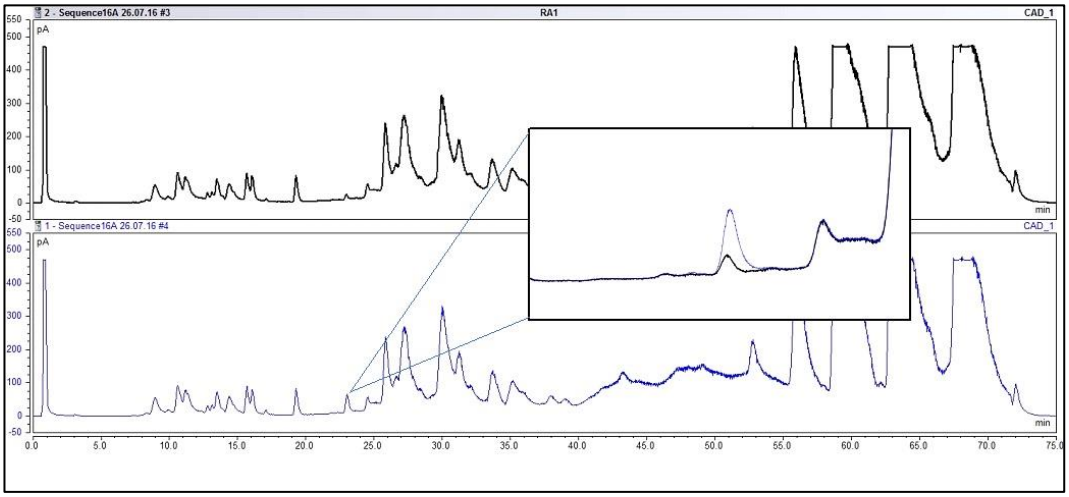


**Figure 6.3**: Two chromatograms displayed on top of each other, the top and bottom chromatograms represent the unspiked and spiked samples of Vitlipid respectively. The zoom frame shows the chromatograms overlaid upon each other.

6.5.3.2. Quantification of vitamin D

The limit of detection (LOD) and limit of quantification (LOQ) have been defined by Snyder et al. (1997) as:

- LOD: Signal to noise (S/N) ratio of 3:1 or 2:1, being the smallest level of analyte to give a measurable response (i.e. the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value).
- LOQ: A S/N ratio of 10:1, being the smallest concentration of analyte that gives a response that can be accurately quantified.

Repeat HPLC runs gave reproducible peaks for vitamin D from Vitlipid N Adult® with detection signals of 15pA and baseline noise ∽1-1.5pA.

The assay demonstrated that vitamin D could be repeatedly detected from the multi-component preparation Vitlipid N Adult® but that it was already at its LOQ; the concentration at which vitamin D could be accurately quantified from the sample. An obvious issue since any further degradation studies would not be accurately quantifiable beyond this limit. Furthermore, these results are from concentrated samples of Vitlipid N Adult®, prior to its dilution in large volume PN feeds. Increased dilution to clinically relevant levels would make it impossible to detect vitamin D.

## 6.6. DISCUSSION

### 6.6.1. General discussion

Vitamin D was only able to be identified and quantified from Cernevit® when using CAD detection, a method renowned for its sensitive detection capabilities. This demonstrates the difficulty in detecting vitamin D across both additives, especially as they have practically the same dose (5-5.5µg/vial). The fact that the LOQ is equal to the undiluted clinical dose shows that quantification in PN will not be possible with this assay and detection systems described. Further modification of mobile phase composition, column type or other minor method specifics are unlikely to result in improved detection of vitamin D for this application. The study shows that present methods have exhausted UV and CAD detection with the two most commonly used compound PN vitamin preparations. CAD was supposed to have been a more promising avenue due to its increased sensitivity, but was still unable to achieve suitable detection.

Where the PN additives are complex mixtures of fat and water-soluble vitamins, each compound exhibits different physicochemical characteristics and retention times. Developing an assay to detect a single component amongst other compounds is a challenging process, particularly in the present instance where the low concentration of vitamin D amongst the other components complicates its detection. Similarly, the low concentration of vitamin D in the samples of Cernevit® and Vitlipid N Adult® required larger than usual injection volumes to be put on the column in attempts to increase its relative detection. However this has the result effect of increasing the relative loads of other components in the preparation samples. The elution of large compounds or compounds present at a higher concentration is known to damage column integrity, potentially detrimental for repeat stability assessments and the data repeatability (Sigma Aldrich 1999).

### 6.6.2. Limitations and future recommendations

Previous studies assessing vitamin stability in PN have often excluded vitamin D from their analysis, presumably due to its low concentration and poor applicability to methods with reduced sensitivity, especially if its concentration and detection is expected to decrease in stability assessment studies (Henton and Merritt 1990; Billion-Rey et al. 1993; Blanco et al. 1994). The rational next steps for this area of research include the use of sample clean-up methods, such as solid phase extraction to reduce interference from other components in the additives e.g. from fatty acids resulting from the lipid emulsion, or other water/fat soluble vitamins; or so that samples spiked with solvents do not have immiscibility problems. Another advantage of using solid phase extraction is that it creates a more concentrated sample for analysis once calculation of percentage analyte recovery has been performed.

Although CAD is considered a sensitive detection method, other detection methods such as diode array or mass-spectroscopy are more sensitive because they incorporate spectral information in peak identification (Vervoort et al. 2008; Vehovec and Obreza 2010). Similarly, fluorescence detection is also able to detect analytes with high sensitivity and a broad dynamic range, up to 100 times more than UV detection (Swartz 2010). While CAD and MS detection are known to be more sensitive than UV, they depend highly on the nature of the analyte in question. These avenues should be considered for future studies alongside other recommendations to produce a more concentrated initial sample of the additive or PN containing vitamin D. They may be more likely to result in successful degradation studies.

However, one wonders whether these proposed additional methods would have a realistic positive benefit towards the research aims. The more concentrated samples from the PN additives may result in a more identifiable peak for quantifiable stability studies, but still may not result in sufficient detection if the methods are used with realistic samples of large volume PN admixtures containing the PN additives, particularly in the face of dilution factors ranging from 200-600 fold (e.g. 1-3L PN volumes).

Still in light of the scarcity of research in this area, this project has confirmed the difficulty in 'finding' the low dose of vitamin D in compound IV injectables, even when using a newer and more sensitive detection method (CAD).

## 6.7. CONCLUSION

In response to few research findings relating to vitamin D stability in PN, the present study design proved itself as a pertinent area for investigation. However little has been elucidated other than further establishing the difficulty in detecting vitamin D within compound PN additives using HPLC methods coupled with UV and CAD detection. As such, vitamin D stability studies under the stated methods cannot be performed on the preparations Cernevit® and Vitlipid N Adult®.

Further studies investigating this area of research need to consider alternative means of sample clean-up/preparation including purification, solid phase extraction alongside more accurate and sensitive means of HPLC detection e.g. LCMS or diode array HPLC.

# CHAPTER SEVEN:

## Bone health and metabolic bone disease (MBD) in LT HPN patients

## 7.1. INTRODUCTION

The extent of vitamin D deficiency and insufficiency was demonstrated in Chapter 5 and subsequently this chapter concerns the assessment of metabolic bone disease (MBD) in patients receiving LT PN. This will allow appreciation and evaluation of the effect of inadequate vitamin D status on LT PN patients' bone health.

### 7.1.1. Chapter aims

The specific aims relating to this chapter are as follows:

- To discover the number of patients from a HPN cohort categorised as having 'normal', 'osteopenic' and 'osteoporotic' bone status, thereby estimating the prevalence of bone disease in HPN patients
- To investigate whether there is correlation between worsening of bone health and duration of time receiving HPN
- To explore whether there are any trends relating to the different subtypes of patients' IF classification and their potential effect on the degree of bone disease classification in LT PN patients

### 7.1.2. Background

7.1.2.1. Metabolic bone disease (MBD)

Patients with intestinal diseases are at risk of developing biochemical disturbance and osteoporosis due to GI malabsorption and malnutrition (Nygaard et al. 2016). Added to this, patients with severe intestinal failure often require PN and there is believed to be a correlation between patients who receive PN and worsening of their bone health (Shike et al. 1980). This is based on the documentation of increased risk of developing MBD in populations of IF patients receiving HPN; secondary osteoporosis being frequently reported (Seidner and Licata 2000; Pironi 2002; Haderslev et al. 2004).

Osteoporosis has been succinctly described by the WHO as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures" (WHO 1994, p. 3), while MBD is defined as a series of bone disorders that can present as osteomalacia, osteopenia, or osteoporosis. Osteomalacia is characterised by softening of bones from defective mineralisation of calcium and phosphorus. Osteopenia and osteoporosis are characterised by a porous trabecular bone network resulting in a reduction in bone density and strength along with an increased risk of fracture (Seidner 2002; Pironi and Agostini 2015a). Osteoporosis is characterised by a greater loss of bone mineral density (BMD) than osteopenia, as reflected in bone density measurements.

*7.1.2.1.1. Pathogenesis of MBD in LT HPN patients*

Epidemiological studies have shown that the pathogenesis for MBD in HPN patients is multifactorial, a combination of both patient specific factors (e.g. age or post-menopausal status, underlying illness) and factors relating to HPN therapy (Pironi and Agostini 2015a). A patient-specific example may be the patient's underlying disease state of SBS resulting in poor absorption of fat soluble vitamins (in particular vitamin D), or a PN-related factor could be an

under-effective PN regimen not providing enough calcium, phosphate or vitamin D (Raman et al. 2006; Hamilton and Seidner 2008).

The underlying disease state has been shown to be the predominant pathogenic role contributing to MBD and factors relating to HPN therapy have been associated with both deterioration and improvement in bone health (Foldes et al. 1990; Klein and Coburn 1991; Saitta et al. 1993; Cohen-Solal et al. 2003; Haderslev et al. 2004; Pironi et al. 2004). The results from these longitudinal studies suggest that the variations in MBD of patients on LT HPN are associated with the patient's sex and age at starting HPN or at developing IF. Other factors associated with PN provision are known to influence the development of MBD; they include deficiencies of calcium, vitamin D and phosphate as well as aluminium toxicity, acidosis, excess vitamin D and amino acid solutions (Raman et al. 2006). The multifactorial nature of MBD in LT PN patients is acknowledged, yet accelerated bone loss has been reported during HPN and raises concerns about the specific PN-related factors which may contribute to the disease (Foldes et al. 1990; Klein and Coburn 1991; Verhage et al. 1995).

In regard to the association between aluminium toxicity and MBD, Kruger et al. (2013) demonstrated that there was higher aluminium content in the bones of LT adult PN patients versus control patients ($P<0.0001$). It was suggested that aluminium acts against bone formation by interfering with osteoblast activity and reduces PTH secretion; thereby increasing the patients' risk for bone disorders such as osteomalacia, osteoporosis and fractures (Dunstan et al. 1984). Aluminium contamination of PN has been noted as a concern (Hernandez-Sanchez et al. 2013), yet cannot be investigated in this project as aluminium levels are not recorded at C&V UHB nor are contaminant levels recorded in PN components or admixtures in the UK, a hotly disputed topic of late (Gura 2010).

*7.1.2.1.2. Diagnosis and monitoring of MBD*

Monitoring of MBD relies on assessment of bone mineral density (BMD) using Dual Energy X-ray Absorptiometry (DEXA) scanning. This technology ascertains the degree of bone demineralisation when patients start HPN and its progression over the course of HPN therapy. It is regarded as the gold-standard for diagnosis of osteoporosis, and over the years this technique has been paid the most attention in terms of technical development and biological validation (Kanis and Gluer 2000). DEXA scanning also demonstrates good long-term precision from stable calibration and manufacturer set quality control procedures (Blake and Fogelman 2007).

In general, diagnosis and monitoring of MBD in HPN patients relies upon:

- Assessment of BMD performed at various bone sites in the body (primarily the lumbar spine and/or femoral neck). It is usually expressed in three ways:
    - Bone density ($g/cm^2$)
    - T-score (the number of standard deviations above or below the mean BMD value for a healthy 30-year-old adult of the same sex and ethnicity as the patient)
    - Z-score (the number of standard deviations above or below the mean BMD value for the patient's age, sex and ethnicity)
- Measurement and interpretation of:
    - blood serum concentrations and urinary excretion of minerals
    - blood serum concentrations of vitamin D and parathyroid hormone
    - biochemical markers of bone turnover

The results generated from DEXA scanning are presented in the format of T and Z-scores because the normal values of adult BMD are higher in men than in women and because BMD decreases with age. Various studies have shown that the risk of patients developing fractures increases with reduced BMD (Marshall et al. 1996).

Classification of BMD was first based upon a report published by the WHO which classified the severity of MBD based on the T-score value at the spine,

hip or fore-arm, see Table 7.1 (WHO 1994). Application of operational ranges (categorised intervals) were proposed by WHO to categorise the degree of loss of bone density because BMD values are seen as a continuous risk factor where no fracture threshold exists.

**Table 7.1:** The WHO classification system for diagnosing osteoporosis using bone density measurements (WHO 1994).

| Classification | T-Score * |
|---|---|
| Normal | -1.0 or greater |
| Low bone mass (osteopenia) | Between -1.0 and -2.5 |
| Osteoporosis | -2.5 or less |
| Severe osteoporosis (established osteoporosis) | -2.5 or less, and a fragility fracture |

[* Units are standard deviations above (positive) or below (negative) the young adult mean value]

Although the WHO classification was originally only intended for use in white (caucasian) post-menopausal women; since its introduction, its use has been universally extended to all individuals in general practice for assessment of fracture risk, diagnostic classification, and initiation of treatment. Over the years, this has been regarded as an unsubstantiated leap in its application. Recently, Leslie et al. (2006) discussed the limitations for reporting BMD in groups other than white post-menopausal females and proposed recommendations to further validate BMD reporting in these groups i.e. the use of population-specific adjustments where differences in fracture risk are not explained by the risk prediction model developed for white post-menopausal female populations. An example being the use of the Z-score rather than the T-score for BMD reporting in females prior to the menopause and in males younger than fifty. In the context of clinical practice, the 1994 WHO diagnostic criteria is still routinely used for BMD reporting in all individuals despite only being intended for post-menopausal women.

In terms of sites at which diagnosis of osteoporosis should be made using DEXA, Kanis (2002, p. 1931) stated that "measurement at the hip is the gold standard in terms of site, since it has the highest predictive value for hip

fracture". Hip fracture is the most severe complication of osteoporosis and its interpretation using DEXA scanning helps to predict the risk of all fractures just as well as other techniques e.g. fracture risk assessment tool (FRAX). Kanis et al. (1997) explained that DEXA accuracy at the hip exceeds 90%, with residual errors arising for a variety of reasons such as overlying metal objects, obesity, previous fracture, concurrent osteomalacia or osteoarthritis. Blake and Fogelman (2007) also agree that measurement at the hip is the most reliable site for predicting hip fracture risk. More attention is given to hip fractures over other fractures because they incur the greatest morbidity and associated medical costs for health services (Dolan and Torgerson 1998). Both the review by Blake and Fogelman (2007) and a recent American position statement by Siris et al. (2014) have utilised the same classification criterion as the WHO for diagnosing osteoporosis using T-score of -2.5 or less from DEXA scanning technology; they explained that measurement can be taken at any of the following three sites, lumbar spine, femoral neck or total hip (Kanis and Gluer 2000). Additionally, a concise publication by Maghraoui (2012) which explains how to clinically interpret a DEXA scan, suggests that the femur (neck or total hip) is the optimum site for predicting risk of fracture, while the spine is best reserved for instances when assessing response to treatment. After review of these sources, it is considered most appropriate to use the DEXA scan results at the body sites of the hip (both femoral neck and total hip) for our interpretation of research findings in this study in relation to prevalence of MBD and the potential longitudinal effect of PN on MBD. Data from other body sites will also be analysed and discussed where appropriate. For instance, Blake and Fogelman (2007) explained how the spine is considered the optimum site for follow up assessment because the treatment changes are usually largest and the precision error is as good or better than that at most other sites (Blake et al. 1996; Faulkner 1998).

#### *7.1.2.1.3. Guidelines for measurement of bone disease*

The majority of patients who are deemed to be at risk of MBD are recommended in the ESPEN guidelines to have their BMD measured at annual intervals (Staun et al. 2009). In some instances, the small proportion of patients who present with low BMD when starting HPN may require more

frequent monitoring of their BMD (six monthly intervals). This frequent need for monitoring in the early stages of HPN therapy is demonstrated in a questionnaire-based study by Wengler et al. (2006) where 64% of European HPN centres measured BMD for all patients at least once or twice a year, they concurred with Haderslev et al. (2004) that BMD monitoring should occur at yearly intervals. Aside from the annual BMD monitoring recommendation to use DEXA scanning for the assessment of the risk of fracture, diagnosis of MBD and determination of treatment necessity, formal guidelines are lacking for how to interpret change in BMD from DEXA scans over time, particularly with reference to therapeutic treatments for bone disease.

#### *7.1.2.1.4. Treatment of bone disease*

Treatments aim to improve BMD and reduce the risk of fracture through lifestyle and dietary modifications, treatment of underlying disease and optimisation of patient's vitamin D status (in both medication and PN therapy). It is also important to ensure patients receive adequate calcium in their diet or via supplementation. Sunyecz (2008) explained how the maintenance of correct balance of calcium and vitamin D is the basis upon which other osteoporosis treatments are commenced. With regards to PN, formulations should at least maintain a positive calcium balance in the patient to slow any further bone loss (Hamilton and Seidner 2008).

Medications used to treat MBD include (Pironi and Agostini 2015b; Compston et al. 2017):

- Oral calcium supplementation (carbonate/citrate), e.g. 500-1000mg twice daily (e.g. Adcal, Calcichew)
- Oral vitamin D supplementation, either as combination with calcium (e.g. Adcal D3) or alone (e.g. Fultium D3)
- Anti-resorptive medications:
    - Bisphosphonates, either IV (e.g. yearly zoledronate or 3-6 monthly pamidronate) or oral (e.g. weekly alendronate or monthly ibandronate).

  - Selective oestrogen receptor modulators e.g. raloxifene, which activate estrogenic receptors in bone, mimicking their bone protective effects.
  - Hormone replacement therapy e.g. oestrogen with/without progestogens.
  - Calcitonin, which inhibits osteoclast function thereby slowing bone resorption.
- Parathyroid hormone (PTH) analogues (e.g. teriparatide), which act as anabolic agents on bone, indicated for post-menopausal women at high risk of fracture.

When absorption of oral vitamin D from the GI tract is insufficient as with SBS patients, an intramuscular (IM) vitamin D injection (ergocalciferol) can be given to maintain normal serum 25-hydroxyvitamin D concentrations (up to every three months). Likewise, if a patient's blood biochemistry reveals a deficiency in calcium despite having maximal calcium allowance in their PN regimen (4.5-11 mmol), a calcium infusion can be arranged.

7.1.2.2. Vitamin D and calcium

Vitamin D is first introduced and discussed within 'Chapter 4, Section 4.2.2.4'. This includes its physiological role, optimal dosing as well as current opinion and recommendations, especially in relation to bone health.

Ensuring adequate provision of calcium and vitamin D is of paramount importance to ensure optimal patient nutritional status, reduce bone loss and decrease the risk of bone fracture (Rosen 2017a), see below Table 7.2 for dose guidelines for prevention of osteoporosis.

**Table 7.2:** Recommended doses of calcium and vitamin D for the prevention of osteoporosis (NIH Consensus Development Panel on Optimal Calcium Intake 1994; Rosen 2017a)

| | Recommended dose of calcium and vitamin D per adult category in the prevention of osteoporosis | |
|---|---|---|
| Calcium | Men and pre-menopausal women | 1000mg/day |
| | Post-menopausal women | 1200mg/day |
| Vitamin D | Men < 70 years of age and pre-menopausal women | 20µg (800IU)/day |
| | Men > 70 years of age and post-menopausal women | 15µg (600IU)/day |

Dosing recommendations for treatment of osteoporosis differ to that of prevention; although calcium recommendations remain the same, the vitamin D recommendations depend on the classification of vitamin D status. See Table 7.3 for example treatment recommendations proposed by UHW. Differences in treatment recommendations can depend on local reference intervals and opinion for vitamin D status as well as local formulation considerations.

**Table 7.3:** Vitamin D dosing recommendations for the treatment of osteoporosis as per C&V UHB (Datta and Stone 2016).

| Vitamin D status | Initial treatment dose | Maintenance dose |
|---|---|---|
| Deficiency (<30 nmol/L) | 50,000 units once weekly for six weeks.<br>OR<br>4,000 units daily for ten weeks.<br>OR<br>300,000 units intramuscularly, single dose. | 25,000 units every month<br>OR<br>1,000 units daily.<br>OR<br>300,000 units intramuscularly once or twice per year. |
| Insufficiency (30-50 nmol/L) | Once deficiency state corrected, patients start from maintenance doses. | 25,000 units every month long-term.<br>OR<br>1,000 units daily long term.<br>OR<br>300,000 units intramuscularly once or twice per year. |

### 7.1.3. Rationale

The prevalence of bone disease in patients receiving LT PN is an evident problem. More information can be elucidated to the contribution of optimal care for these patients by investigating the prevalence and degree to which HPN populations are affected by MBD, particularly with reference to the duration of time patients receive PN. For instance, there may be critical time points for which patients require therapeutic intervention.

## 7.2. METHODS

### 7.2.1. Research permissions

This study was conducted using the research permissions as described in Chapter 2. All sixty participants recruited and maintained on LT HPN were eligible for this section of research.

### 7.2.2. Study design

This study was performed as a retrospective cross-sectional database analysis of data from patients' most recent bone DEXA scans, and also employed retrospective longitudinal methods for those patients with data from more than one point in time.

### 7.2.3. Data collection and sample population

Data were collected from the medical records of consenting participants recruited from the outpatient clinic at C&V UHB. Specifically, this was achieved via manual data transcription of the results from bone DEXA scans from the online 'Clinical Portal' system which stores patients' medical records.

Data for each investigation parameter were collected as follows:

- Prevalence of patient bone classification status: data were collected from each participants' most recent bone DEXA scan as of 01 September 2016.
- Longitudinal assessment of patients' bone status: data were collected from the date each participant was commenced on HPN up until 01 September 2016.

Data collection was limited to the available medical records for the participants. As an extra precaution to ensure all data was transcribed, data collection required close collaboration with the Medical Physics department at C&V UHB who held additional records of patients' DEXA scan results, unavailable through the online Clinical Portal system.

As already mentioned, assessment of bone health using DEXA scans is recommended at annual intervals for HPN patients, however in practice it can be more irregular, often performed every couple of years (Staun et al. 2009). However, bone health review still occurs at relatively regular clinical intervals, justifying its use as a parameter for investigation. Also, Blake and Fogelman (2007) explained that clinical monitoring should not be more frequent than 1-2 years due to the limited repeat sensitivity of DEXA scanning, unnecessary radiation exposure and to allow sufficient time between scans for their accurate clinical interpretation.

### 7.2.4. Data handling, storage and analysis

Relevant data were manually transferred into a Microsoft Access database for storage and handling, while data analysis was undertaken using Microsoft Excel. Participants were anonymised and coded to maintain their confidentiality throughout.

The results from the patients' DEXA scans were categorised as 'normal', 'osteopenic' or 'osteoporotic' according to the 1994 WHO classification for BMD (WHO 1994)

#### 7.2.4.1. Data parameters

Results from DEXA scans at C&V UHB give three results (BMD value, T score, Z-score) for each body site scanned (AP spine, femoral neck, total hip). While results from this study show the DEXA scores across all three body sites, the results from the femoral neck and total hip have been chosen to indicate overall opinion for prevalence of MBD, bone health findings and bone status. Rationale being that results from the hip are considered optimal for predicting risk of fracture. Results from the AP spine (anterior-posterior spine) are also shown for interpretation of bone health in relation to patients' duration of receiving PN.

Since DEXA T-scores are the results used for diagnosis of MBD, they were chosen in this study to demonstrate the prevalence of bone disease in the PN

cohort according to their most recent DEXA scan. Whereas, the DEXA Z-score results were used for data analysis involving longitudinal assessment of bone disease over time because it is relative to the individual patients' sex, age and ethnicity. Also clinical interpretation of Z-scores are often used for identifying secondary causes of osteoporosis i.e. secondary to LT PN provision (Swaminathan et al. 2009; Sheu and Diamond 2016). Similarly, other longitudinal bone health studies have chosen the Z-score over the T-score, the rationale being that it acts as a relative indicator of the score in relation to the individual over time, irrespective of the score for a healthy young adult (Cohen-Solal et al. 2003; Wren et al. 2014; Poinsot et al. 2017).

Data was included for analysis in longitudinal assessment if patients had been receiving PN for at least three months, a reasonable clinical interval in LT PN (Parrish 2014). This ensured a time interval beyond which PN could be considered to contribute to the state of patients BMD and bone health, amongst the other factors which are known to affect bone health e.g. age and sex.

#### 7.2.4.2. Data analysis

Descriptive statistics were used to analyse the data:

- Prevalence of patient bone status:
    - Number and percentage of patients with applicable data, categorised according to most recent T-score WHO classification.
- Longitudinal assessment of bone status:
    - Number and percentage of patients with applicable data from multiple points in time, categorised according to Z-score WHO classification.

Prevalence data were also cross-classified with individual patient data relating to their pathophysiological classification for IF according to the ESPEN classification system (Pironi et al. 2015). Findings are presented as the number of patients with applicable data, categorised according to most recent T-score WHO classification.

## 7.3. RESULTS

Of the sixty participants eligible for inclusion within this section of research, fifty-three patients had recorded DEXA scans performed by C&V UHB.

### 7.3.1. Prevalence of bone disease

The following results in Figure 7.1 present the bone status of all patients with applicable data on LT PN according to their most recent DEXA scan.

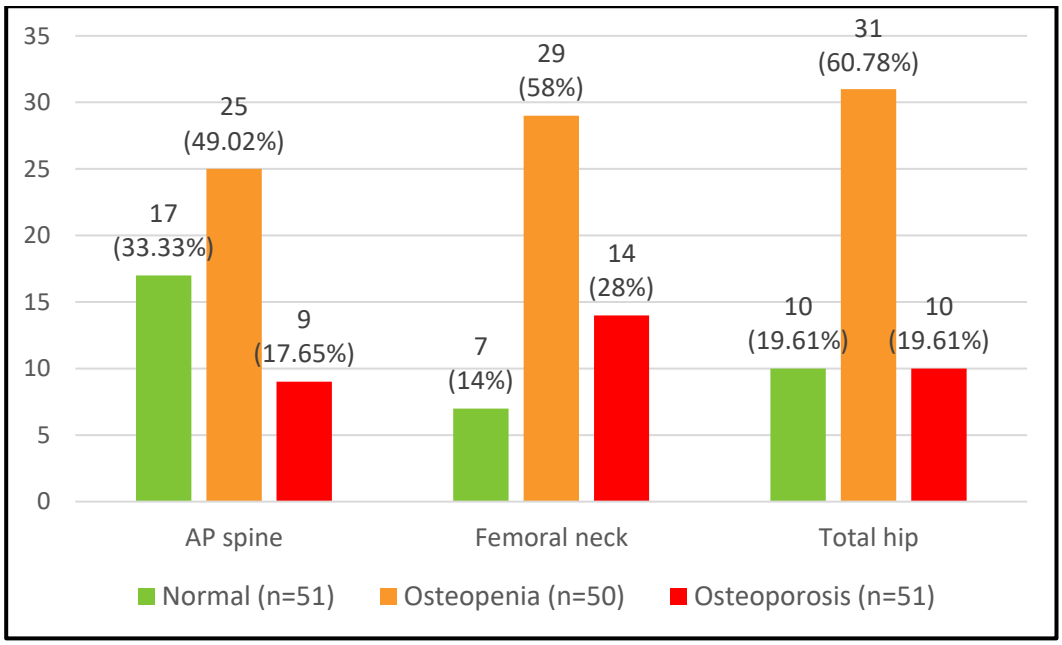


**Figure 7.1:** A clustered column chart to show the number (and %) of patients' most recent DEXA scan results (T-score) at three sites (AP spine, femoral neck and total hip), as classified by the WHO definition for osteopenia and osteoporosis.

### 7.3.2. Longitudinal progression of bone disease

Below are the results to show the progression of patients' bone health as displayed by their DEXA scan results at three different sites (AP spine, femoral neck, total hip).

The net difference between first and second DEXA scans (where data exists per patient, since starting HPN) is shown in Table 7.4. Then for those patients with additional applicable data, the net difference between the second and third DEXA scans are shown in Table 7.5.

Loss of BMD is indicated in DEXA scan results by a reduction in the BMD value between successive DEXA scans. Correspondingly T and Z-scores will also reduce, or get more negative between DEXA scans when there is loss of BMD. T and Z-scores span both positive and negative decimal numbers, usually between the range +3 to -3. When the net difference between successive DEXA scans is calculated, a net negative value indicates an improvement in BMD and a net positive indicates a worsening of BMD. As such, the range shows the extremes observed between improvement (a negative value) and worsening (a positive value) of BMD and Z-scores.

Standard deviation values have not been included because technically the scores produced from BMD DEXA scan results are standard deviation values themselves.

**Table 7.4:** Net difference between DEXA 1 and DEXA 2 (since starting HPN), (n=30).

| | Net difference between 1st and 2nd DEXA scores | | | | | |
|---|---|---|---|---|---|---|
| | AP spine | | Femoral neck | | Total hip | |
| | BMD | Z score | BMD | Z score | BMD | Z score |
| Range | -0.136 – 0.121 | -1.3 – 1 | -0.112 – 0.171 | -1 – 1.5 | -0.170 – 0.163 | -1.2 – 1.4 |
| Mean | -0.0072 | -0.133 | 0.0168 | 0.0367 | 0.0205 | 0.0733 |
| Median | 0.0015 | -0.1 | 0.007 | 0 | 0.0195 | 0 |

**Table 7.5:** Net difference between DEXA 2 and DEXA 3 (since starting HPN), (n=14).

| | Net difference between 2nd and 3rd DEXA scores | | | | | |
|---|---|---|---|---|---|---|
| | AP spine | | Femoral neck | | Total hip | |
| | BMD | Z score | BMD | Z score | BMD | Z score |
| Range | -0.076 – 0.109 | -1.5 – 0.8 | -0.041 – 0.135 | -0.4 – 1 | -0.056 – 0.163 | -0.5 – 1.2 |
| Mean | -0.00086 | -0.157 | 0.0265 | 0.157 | 0.0249 | 0.15 |
| Median | -0.0085 | -0.15 | 0.016 | 0.05 | 0.0085 | 0 |

The net difference between first and last recorded DEXA scan results (where data exists per patient, since starting HPN) are presented to show longitudinal progression of bone disease from a different standpoint, see Table 7.6 below.

**Table 7.6:** Net difference between the first DEXA (since starting HPN) and latest recorded DEXA (at point of data collection), (n=30).

| | Net difference 1st DEXA scan (since starting HPN) and latest recorded DEXA scan (at point of data collection) | | | | | |
|---|---|---|---|---|---|---|
| | AP spine | | Femoral neck | | Total hip | |
| | BMD | Z score | BMD | Z score | BMD | Z score |
| Range | -0.207 – 0.121 | -2 – 1 | -0.115 – 0.232 | -1 – 1.8 | -0.181 – 0.274 | -1.3 – 2 |
| Mean | -0.0102 | -0.297 | 0.0308 | 0.06 | 0.0367 | 0.123 |
| Median | 0.003 | -0.3 | 0.023 | -0.05 | 0.022 | 0 |

### 7.3.3. Bone status classification according to IF disease classification

**Table 7.7**: Number of patients classified according to bone status (T-score) at each bone site (AP spine, femoral neck, total hip) and further sub-categorised to IF pathophysiological classification.

| IF pathophysiological classification | Num. of patients | WHO bone health classification | Bone site (T-score) | | |
|---|---|---|---|---|---|
| | | | AP spine (n=51) | Fem. neck (n=50) | Total hip (n=51) |
| Extensive small bowel disease | 3 | Normal | 1 | 1 | 1 |
| | | Osteopenic | 0 | 1 | 1 |
| | | Osteoporotic | 2 | 1 | 1 |
| Intestinal fistula | 4 | Normal | 1 | 1 | 4 |
| | | Osteopenic | 2 | 2 | 0 |
| | | Osteoporotic | 1 | 1 | 0 |
| Intestinal dysmotility | 6 | Normal | 2 | 0 | 0 |
| | | Osteopenic | 4 | 6 | 6 |
| | | Osteoporotic | 0 | 0 | 0 |
| Mechanical obstruction | 3 | Normal | 1 | 1 | 1 |
| | | Osteopenic | 2 | 0 | 1 |
| | | Osteoporotic | 0 | 2 | 1 |
| Short bowel | 35 | Normal | 12 | 4 | 5 |
| | | Osteopenic | 17 | 20 | 22 |
| | | Osteoporotic | 6 | 10 | 8 |

## 7.4. DISCUSSION

### 7.4.1. General discussion

Figure 7.1 shows that 58% and 60.78% of patients have osteopenia characterised by the onset of bone disease at the sites of the femoral neck and total hip, respectively; alongside the presentation of osteoporosis in 28% and 19.61% of patients at the same respective sites. Thus constituting a concrete finding that patients with IF receiving PN have bone health co-morbidities. These findings are in keeping with the study performed by Pironi (2002) which demonstrated similar distributions of MBD from T-scores in a large cohort of 284 participants; 43% of osteopenia and 41% of osteoporosis. However, these results were interpreted from an osteopenic or osteoporotic result at any of three sites (femoral neck, lumbar spine and total body); fortunately the results can still be paralleled to our findings as the authors stated that the T-score values did not differ between sites of DEXA measurement.

However the extent to which duration of time receiving PN contributes to adverse bone health is demonstrated separately. In Table 7.4, the Z-scores show an initial average loss of 0.0367 (femoral neck) and 0.0733 (total hip) between the first and second DEXA scans since starting PN (n=30) i.e. a worsening of bone health. For those patients with applicable data for a third DEXA scan, this value then increases between the subsequent DEXA scans (0.157, 0.15, femoral neck and total hip, respectively), demonstrating a marginally greater reduction in bone score and loss of BMD.

Table 7.6 shows the longitudinal effect of PN administration on bone health from a different perspective, the net difference between first and last DEXA scans (n=30). One would have expected to observe a greater loss in net Z-score here, yet unexpectedly the losses are less than those seen in Tables 7.4 and 7.5. A potential reason for this finding is that although the intention of this table was to show greater bone loss over longer periods of time, the actual data set still included data from those with a limited number of DEXA scan results over smaller time periods i.e. some patients still only had a couple of applicable DEXA scan results. Interestingly, whereas the net difference in Z-scores across

Tables 7.4-7.6 at sites corresponding to the hip (femoral neck, total hip) demonstrate worsening of BMD, the data demonstrates an improvement in BMD at the site of the spine, the site considered optimal for follow-up assessment of bone health in relation to treatment (Faulkner 1998).

A demonstrable finding across Tables 7.4-7.6 is the wide range between the minimum and maximum values for net difference between DEXA scans, showing that there were both dramatic improvements and deteriorations in bone health between successive scans in some patients. Perhaps a reflection of opposing core factors, the successful pharmacologic treatment of osteoporosis with both medicines such as bisphosphonates or the provision of optimal supplementation with calcium and vitamin D; while an opposing factor would be the potential harmful effect of IF and LT PN on bone health. Another observation from these tables is the variable differences between mean and median values for the net differences in DEXA Z-scores; demonstrating an asymmetrical data set where large differences exist in the longitudinal degree of loss of bone density i.e. the middle data value is at times further away from the mean value.

Generally our findings from the longitudinal analyses are in agreement with Raman et al. (2006) who noted a negative association between BMD and duration of HPN. However other longitudinal studies have showed that this is not always the case (Foldes et al. 1990; Klein and Coburn 1991; Saitta et al. 1993; Cohen-Solal et al. 2003; Haderslev et al. 2004; Pironi et al. 2004); providing further support to the variable nature of contributing factors to MBD (e.g. age and sex at starting HPN) and the small extent/degree of net change in bone scores observed in our results. The median values of zero for femoral neck and total hip in Table 7.4 also demonstrate the mid-range 'averaged' nature of the results. It is possible that bone loss may not be as pronounced as once thought for patients on LT nutritive support, Seidner (2002) suggested that the underlying illness itself may be the main driving factor contributing to MBD. In any case, the onset and progression of MBD in LT PN patients should still be considered multi-factorial, not limited to the impact of LT PN administration. The results from Tables 7.4 and 7.5 also

suggest that there was negligible difference between successive DEXA scans (and hence longer time periods receiving PN) as demonstrated by similar mean values for net difference in Z-scores at the hip i.e. bone health consistently worsened over time without dramatic differences in loss of BMD between scans.

The results are not profound and reflect the multitude of factors involved in the homeostasis of bone health in LT PN patients. For example, the efforts to pharmacologically improve bone health, supplement patients with calcium/vitamin D (oral/PN), negative implications of PN on bone health as well as established patient factors known to result in bone loss such as advanced age, sex and menopausal status. For example, Chapter 2 presented the average age of HPN patients to be 58.10 (±13.78) which is the likely age for onset of bone health problems, along with an average age of 57.18 (±14.72) in women alone during which time the likelihood of being peri or post-menopausal is known to affect their BMD. Therefore our findings are conflicted in the same way as Haderslev et al. (2003) in that the true extent of MBD during HPN is unknown because it can present or develop before patients commence HPN.

Regarding the DEXA scan results once they had been further sub-categorised according to the participants' underlying pathophysiological classification for IF; it was difficult to gauge meaningful findings from the results displayed in Table 7.7. A greater quantity of data would be required to assemble conclusions from all the sub-categories. However, the sub-category 'short bowel' included data from 35 cases and one observes that a greater degree of scan results were osteopenic and osteoporotic at the hip (femoral neck/total hip) by comparison to the AP spine which showed a greater proportion of 'normal' bone density scan results. This finding may add to the theory that loss of bowel length results in loss of absorption and physiological function of the gut, especially in its regulation of factors relating to bone homeostasis e.g. calcium and vitamin D absorption. The pathogenesis is thought to relate to a chronic inflammatory condition characterised by increasing concentrations of pro-inflammatory cytokines (e.g. TNF-α, IL-6), which stimulate osteoclast

activity to contribute to low BMD (Hise et al. 2008). Similarly n-6 fatty acids within lipid emulsions could initiate the inflammatory response.

The effectiveness of bisphosphonate treatment on improving bone health is well established for the secondary prevention of osteoporotic fractures and the improvement of BMD (NICE 2012; SIGN 2015). Successful treatment response is signified by a BMD value that is stable or improves (Rosen 2017b). Deemed a positive health outcome measure for patient bone health, but a complication for research investigating the adverse effect of LT PN on bone health when nearly all patients receive treatments to improve their bone health/BMD.

A relatively recent clinical consideration has been the continuous overtreatment with bisphosphonates in general practice (Adler et al. 2016). It is now recommended that those receiving alendronate or risedronate for five years, or zoledronate (once yearly IV) for three years, with 'stable' BMD and no previous vertebral fractures or low risk of future fractures, should discontinue bisphosphonate therapy (Rosen 2017b); otherwise known as a 'bisphosphonate holiday'. Rationale being that residual benefit is observed beyond these time scales and patients are subjected to more risk than benefit. Perhaps this should be a consideration for longstanding LT PN patients on bisphosphonate therapy who display worsening of bone DEXA scores. It has been noted however that there are few data to guide decisions regarding bisphosphonate treatment duration and the subsequent bisphosphonate holiday, basis is deemed to rely on clinical interpretation of BMD results and individual risk factors. Two years is usually seen as sufficient for the break period when patients demonstrate $\backsim$5% bone loss over this time (Watts and Diab 2010).

An easily overlooked yet potential contributory factor to worsening of bone density scores is the intense drive to supplement patients with high doses of vitamin D, which may have resulted in the net resorption of bone if patients showed biochemical signs of hypercalcaemia (Jones 2008; Tebben et al. 2016).

### 7.4.2. Limitations

There were some elementary limitations associated with this study. For example, the limited quantity of data from the C&V UHB cohort meant that it was difficult to design a study which incorporated all of the desired data parameters e.g. the effect of vitamin D on DEXA results, or a year on year observation of effect of LT PN on bone health. These factors would have required consistent and regular intervals for patients to have started PN, had each of their DEXA scans performed and vitamin D status measured; hence the methods employed in this chapter involved calculation of the net difference from preceding DEXA scans for each patient regardless of year or point in time. Similarly, more participants were expected to have had DEXA scans performed, showing a potential decline from clinical practice recommendations as some had no BMD results. Other than unintentional omission, reasons for their oversight could relate to the unnecessary clinical need for some patients to have scans performed e.g. young age, absence of risk factors or a projected limited duration of time on PN (post-surgery, bowel recovery period).

With hindsight, this was a difficult study to pursue with such a limited population and quantity of data from variable dates in time. Hence the decision to undertake the longitudinal analyses in the manner observed with relative difference from the first DEXA score. For instance, initially a five-year time interval was proposed but only a limited number of patients' bone scores fitted the five-year criterion. Another consideration is that DEXA scans performed shortly after the 3-month window of patients being on PN may not show as great a reflection for the contribution of the effect of PN administration on bone health by comparison to longer time scales since commencement of PN.

Although DEXA scanning is well-established in the diagnosis and treatment of osteoporosis, Blake and Fogelman (2007) have explained that its use in monitoring BMD over time is more controversial due to its limited repeat sensitivity, a consideration to bear in mind with the findings from our longitudinal analyses. The DEXA technique itself has its own limitations

associated with its measurement of BMD. The scores produced do not give an absolute risk of fracture, but a relative risk of fracture; providing information only on the quantity of bone, not its quality (Maghraoui 2012). Other limitations in its measurement include the sensitivity to error with variable soft tissue composition (e.g. fat vs. lean tissue) and inherent limitations in longitudinal reassessment of bone density (Wells 2009). However, DEXA scanning is still considered the current best indicative method for measurement of bone density and diagnosis of osteoporosis. Lu et al. (2001) performed a study examining differing criteria for osteoporosis and application the WHO criteria. They found that only 25% of patients were consistently diagnosed across all the BMD variables and recommended the inclusion of risk based information in diagnostic criteria because its inclusion resulted in consistent diagnoses in 68% of cases. The present study has shown that interpretation of DEXA scans themselves can be somewhat inconsistent with potential under or over diagnosis; future studies should attempt to include further risk assessments across multiple body sites to give more accurate diagnoses of MBD.

Another complication owing to data collection was that some patients had their DEXA scans performed with different health boards and the results not recorded on the C&V UHB system. Although attempts were made to collect all data from known sites, some may have evaded collection.

### 7.4.3. Recommendations and future work

Future studies should include a design format that will allow clear interpretation of the contribution of LT PN to the adverse onset and development of MBD. For instance, studies incorporating greater numbers of patients from more HPN centres would contribute more data for analysis with more generalisable findings. Particularly alongside more control over data parameters such as DEXA scan time points in relation to the year performed or the date of PN commencement. Or further still, controlled intervention or comparison studies involving segregation or sub-classification of patients according to their sex, age and pharmacological interventions e.g. long-term bisphosphonate or steroid treatments which are known to contribute to

adverse bone health. However, a foreseeable complication is the vastly differential practices in relation to treatment and management of patients with MBD, as evidenced by a questionnaire based study across multiple HPN centres (Pironi et al. 2017).

Further studies in this area may benefit from the implementation of fracture risk assessment tools to help diagnosis of osteoporosis, identify risk of fracture, treat secondary prevention of bone fractures and aid treatment decisions, validated measures include FRAX or QFracture® (NICE 2012; SIGN 2015).

It would be of benefit in future studies to incorporate evidence of vitamin D deficiency in relation to DEXA results, the exact design of this sort of study would be complicated, relying on DEXA scans being taken within timely relation to tests for vitamin D status. Perhaps a more beneficial study would be the comparison of those patients with longstanding inadequate vitamin D status (despite efforts to optimise their status) against those patients with adequate vitamin D status. Furthermore, inclusion of other factors known to play a role in skeletal remodelling e.g. PTH, magnesium, phosphorus, may be of similar research benefit. A study performed by Wozniak et al. (2015) implemented a design to correlate vitamin D status and bone health in patients on PN for longer than six months and a vitamin D level performed with this time. They noted a trend for greater risk of osteopenia in children with sub-optimal vitamin D status; similar studies of this design may be of value in future research.

A study that would be of considerable interest would be a direct PN intervention study involving comparison of vitamin D and bone health DEXA data from patients receiving LT PN, against those in the general population (not receiving PN). Full appreciation for the effect of LT PN could then be gleaned against the multitude of factors which contribute to the development of MBD affecting both populations.

## 7.5. CONCLUSION

MBD is an apparent and challenging complication observed in LT PN patients. Our study has further demonstrated the prevalence of MBD and loss of bone density in HPN patients from both a cross-sectional and longitudinal perspective. Nevertheless, there is still great scope for further research to clearly clarify the factors which contribute to its onset and development in LT PN patients, particularly in the context of its pathogenesis over time and the effects of successful treatment on BMD. Future studies should incorporate case-control and intervention style study design to establish and quantify the contribution of factors known to influence MBD e.g. age, sex, menopausal status, micronutrient status and any concomitant treatment e.g. bisphosphonates, corticosteroids, diuretics, anticoagulants as well as initial diagnoses/conditions/treatment factors (such as bone status prior to initiation of HPN).

# CHAPTER EIGHT:

## Evaluation of trace element (TE) provision from Additrace® in LT PN

## 8.1. INTRODUCTION

This chapter follows on from Chapter 5 after the extent of abnormal blood test results were demonstrated for TE in LT HPN patients, in particular, excesses of manganese and deficiencies of selenium. This section of research investigates the suitability of the compound TE preparation Additrace® for LT HPN patients and attempts to ascertain further reasons for the occurrence of out-of-range blood tests. Additionally, this section also intends to identify situations where the stability of the PN formulation may limit the provision of TE to patients, so as to be able to investigate whether stability dosing limits could potentially result in nutritional deficiency states. The chapter aims to explore optimal dosing requirements for micronutrients in LT PN, using correlation of previous micronutrient doses in PN with subsequent biochemistry blood test results.

### 8.1.1. Chapter objectives

- To determine and assess the suitability of Additrace® for the population of patients maintained on LT HPN
- To correlate/consider patients PN prescription data with their subsequent blood test results
- To quantify the extent of supplemental TE dosing in LT HPN patients
- To suggest/consider the implication of PN physical stability dosing limitations as a contributing factor to the occurrence of deficient blood test results
- To perform comparative analyses (in terms of TE dosing and blood test results) on the basis of whether patients had been receiving Additrace® and/or extra TE supplementation

### 8.1.2. Rationale

The findings of the study performed in Chapter 5 showed a notable skew of TE blood test results away from the 'normal' reference range values, particularly for deficiencies of selenium and excesses of manganese.

The following study was proposed as a way of evaluating the suitability of the compound preparation Additrace® in meeting PN patients' TE requirements whilst also evaluating the service provided by C&V UHB in terms of meeting patients TE dosing needs, by reference to patients' health outcomes in terms of nutritional status (See Chapter 4, section 4.1. for Additrace composition).

During instances when Additrace® is judged to not be clinically suitable, or if patients require additional TE supplementation, there exists individual TE preparations to aseptic manipulation in PN for copper, selenium, iron and zinc. Iron is not monitored directly from blood serum samples at C&V UHB because assessment of iron status relies upon the more complex interpretation of haemoglobin and blood/cell stores alongside the presence of any symptoms relating to iron deficiency or toxicity. As such, the present study can only evaluate doses of copper, manganese, selenium and zinc in relation to blood test results, since the other TE in Additrace® (chromium, fluoride, iodine, molybdenum) are not assayed in blood tests by C&V UHB, nor do separate individualised preparations exist. Patients' blood tests are also monitored for manganese status, but no separate preparation exists for supplementation.

Similarly, vitamins were not chosen for evaluation in this chapter because their findings in Chapter 5 did not display trends in nutritional abnormalities, also no separate products exist for their individualised supplementation outside of the compound preparations Cernevit® or Vitlipid N Adult®, (aside from folic acid injection). Another factor worth noting is that some vitamins e.g. vitamins A, D (oral/IM) and E are extensively supplemented outside of PN in an effort to further supplement patients presenting with nutritional deficiencies who are already receiving the compound vitamin preparations in their PN.

As one may observe, the well-documented PN prescription data best serves itself towards evaluation of the TEs copper, selenium and zinc (via Additrace® and/or extra supplemental) which is not feasible nor reliable for the other vitamin components of PN. These also happen to be the more clinically relevant TE for consideration when monitoring patients in clinic.

## 8.2 METHODS

### 8.2.1. Research ethics, permissions and approvals

For this final research chapter, ethical approval was sought and granted by Cardiff School of Pharmacy and Pharmaceutical Sciences. NHS Research and Development (R&D) permission was granted by C&V UHB as a service evaluation as of 18/10/2016 (see appendix VII for signed approval of SE application).

### 8.2.2. Study design

8.2.2.1. Data collection and study population

The study was performed as a retrospective longitudinal database analysis to investigate the occurrence of micronutrient abnormalities experienced by the population of patients maintained on LT HPN (in relation to their PN prescription) from data held with C&V UHB between May 2014 and May 2017. Specifically, in relation to the doses of TE in patients' PN prescriptions leading up to the blood test events, and whether the results were classified as deficient, in range or in excess (toxic) as depicted by the local C&V UHB reference limits.

8.2.2.2. Data handling, storage and analysis

Data security and patient confidentiality was maintained using the same processes of anonymization and data handling as outlined in Chapter 2 (Section 2.4.).

During data collection, the anonymised and coded data were transferred into an excel database.

*8.2.2.2.1. Data parameters*

Data was collected on the following:

- PN prescription records in use as of 01 May 2014, through to the end of May 2017. The data included the prescription dates, volume of feeds, inclusion of Additrace® (Y/N) and TE doses (Cu, Se, Zn).
- Micronutrient blood test results recorded between May 2014 and May 2017

The doses of micronutrients included in patients PN were correlated with the resultant micronutrient biochemistry blood tests (which give an indication of patient micronutrient status). Prescription data was matched with blood test data providing that patients had been receiving the PN prescription for at least three months. This time interval was chosen to allow sufficient time for the dose of micronutrient in patients PN to accurately correspond to total body stores and representation of nutritional status, in light of any recent micronutrient or dose-volume adjustments. The three-month window is standardly used as a suitable monitoring time window to check patients response to TE dosing revisions, particularly after dose increases in light of deficient test results (Parrish 2014).

#### *8.2.2.2.2. Method of analysis*

Successfully matched data were separated into four categories as shown in Table 8.1. The categories were based upon whether the micronutrient preparation Additrace was included and/or extra selenium was supplemented into the PN.

**Table 8.1:** Categories 1-4 to which the matched paired data were assigned.

| Category | Additrace | Extra TE |
|---|---|---|
| C1 | Yes | Yes |
| C2 | Yes | No |
| C3 | No | Yes |
| C4 | No | No |

The blood tests results arising from the data pairing in each of the four categories were analysed using descriptive statistics as follows:

- To show the total number (and percentage) of recorded micronutrient blood tests that were deficient, in range and in excess. In so doing, identify trends or themes in micronutrient abnormalities and dosing correlations occurring for each category.
- The average dose for TE provided in PN per data category (C1-C4)

- The dose of TE included within the PN admixture as a percentage of the maximum permitted for the volume of the feed. To allow interpretation of whether volume of the PN feed limits the dose of selenium given to patients.

The collected data was used to calculate the extra doses of TE given outside of Additrace®, where applicable; and also to calculate the maximum dose of TE per volume of feed, allowing the actual total TE dose as a percentage of the maximum allowance. The maximum dose of micronutrients permitted per volume of PN feed are given in Table 8.2. Where a patients PN regimen instructed a combination of both aqueous and lipid feeds or a combination of two different feeds (e.g. of different volumes or containing different TE doses), the TE doses and % of maximum permitted doses across the feeds were averaged according to the ratios of different feeds. For instance, 3 aqueous and 2 lipid feeds per week >>> [dose A x (3/5) + dose B x (2/5)]/5.

**Table 8.2**: Maximum dose of micronutrients (Cu/Se/Zn) permitted per litre volume of PN feed.

| TE | Max. dose of TE per vol. of PN |
|---|---|
| Copper | 20µmol/L |
| Selenium | 1200nmol/L |
| Zinc | 200µmol/L |

The accuracy of data transcription was verified by manually checking 5% of all prescriptions and 5% of all micronutrient blood test results. No trends in error for data transcription were identified.

## 8.3. RESULTS

One hundred and fifty-eight (158) patients were registered on the IF clinic list between the dates of 01/05/2014 - 01/05/17 as of May 2017. Of these patients, one hundred and sixteen (116) had recorded prescriptions to show they were receiving PN during this time period; forty-two (42) patients were excluded on the basis of receiving other long-term IV infusions e.g. magnesium. Finally, of these 116 patients, ninety-eight (98) patients had data to meet the inclusion criteria and allow pairing of their PN prescription data to blood test results owing to the three-month rule.

### 8.3.1. Evaluation of micronutrient provision in PN (from paired data)

On the next page, Table 8.3 displays the results for the micronutrient blood tests (copper, selenium and zinc) which were matched with patient prescription data (categories 1-4 depending on micronutrient inclusion and formulation in PN), providing patients had been receiving PN from the prescription for at least three months. There were no results for manganese as it cannot be manually manipulated/supplemented in PN other than being given as part of the preparation Additrace®.

Table 8.4 shows the average doses for TE for all paired data that resulted in blood tests results that were 'in range', thereby giving an indication of optimal dose requirements for the LT PN population.

**Table 8.3**: Results from categories 1-4 of paired prescription and blood test data for each of copper, selenium and zinc.

| | | Number (and percentage) of blood tests (paired with prescription data) per micronutrient (Cu/Se/Zn) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Copper** | | **Selenium** | | **Zinc** | |
| | | Num. | % | Num. | % | Num. | % |
| Category 1. Additrace® (Yes), extra micronut (Yes) | Deficient | 0 | 0 | 13 | 35.14 | 1 | 4.55 |
| | In range | 7 | 100 | 23 | 62.16 | 21 | 95.45 |
| | In excess | 0 | 0 | 1 | 2.70 | 0 | 0 |
| | Total | 7 | 100 | 37 | 100 | 22 | 100 |
| Category 2. Additrace® (Yes), extra micronut (No) | Deficient | 9 | 11.84 | 16 | 40.00 | 0 | 0 |
| | In range | 59 | 77.63 | 24 | 60.00 | 56 | 94.92 |
| | In excess | 8 | 10.53 | 0 | 0 | 3 | 5.08 |
| | Total | 76 | 100 | 40 | 100 | 59 | 100 |
| Category 3. Additrace® (No), extra micronut (Yes) | Deficient | 12 | 4.98 | 26 | 10.00 | 15 | 6.22 |
| | In range | 205 | 85.06 | 216 | 83.08 | 200 | 82.99 |
| | In excess | 24 | 9.96 | 18 | 6.92 | 26 | 10.79 |
| | Total | 241 | 100 | 260 | 100 | 241 | 100 |
| Category 4. Additrace® (No), extra micronut (No) | Deficient | 1 | 6.25 | 0 | 0 | 0 | 0 |
| | In range | 14 | 87.5 | 0 | 0 | 10 | 100 |
| | In excess | 1 | 6.25 | 0 | 0 | 0 | 0 |
| | Total | 16 | 100 | 0 | 0 | 10 | 100 |

**Table 8.4**: Average doses of TE (Cu, Se, Zn) required by PN patients which resulted in *'in range'* blood test results, from all paired data (C1-C4).

| | Average dose of TE resulting in 'in range' blood test results from all paired data (C1-C4) | | |
|---|---|---|---|
| | Number of paired data | Average dose | Dose range (±SD) |
| **Copper** (µmol) | 285 | 10.94 | 0-25 (±6.52) |
| **Selenium** (nmol) | 266 | 829.20 | 400-1550 (±276.08) |
| **Zinc** (µmol) | 290 | 110.70 | 0-200 (±43.74) |

### 8.3.2. Average micronutrient doses and stability considerations

The average doses of TE given per data category (inclusive and exclusive of Additrace) as well as the percentage of maximum TE provision per volume of PN are given in Table 8.5.

**Table 8.5**: Results for average TE doses and stability considerations for each matched data category.

| | | **TE dose considerations per data category** | | | |
|---|---|---|---|---|---|
| | | C1. Additrace® (Yes), extra TE (Yes) | C2. Additrace® (Yes), extra TE (No) | C3. Additrace® (No), extra TE (Yes) | C4. Additrace® (No), extra TE (No) |
| Average (range ±SD) of the total micronut. provision from PN, inclusive of Additrace® | **Cu** (µmol) | 19.70 (10.63-24.17) (±6.21) | 20.00 | 8.73 (1.43-25.00) (±4.41) | NA |
| | **Se** (nmol) | 1057.54 (400.00-2200.00) (±452.66) | 400.00 | 862.81 (400.00-1550.00) (±260.12) | NA |
| | **Zn** (µmol) | 159.00 (100.00-200.00) (±27.94) | 100.00 | 115.64 (1.00-200.00) (±42.02) | NA |
| Average (range ±SD) of the total micronut. provision from PN, exclusive of Additrace® (nmol) | **Se** (nmol) | 657.54 (0.00-1800.00) (±452.66) | NA | 862.81 (400.00-1550.00) (±260.12) | NA |
| | **Cu** (µmol) | 5.06 (3.13-10.63) (±2.70) | NA | 8.73 (1.43-25.00) (±4.41) | NA |
| | **Zn** (µmol) | 73.15 (0.00-112.50) (±32.76) | NA | 115.64 (1.00-200.00) (±42.02) | NA |
| Average (range ±SD) of the percentage of max. micronut. provision per volume of PN (per patient) | **Se** (nmol) | 37.11 (10.78-66.77) (±16.70) | 14.75 (6.79-22.22) (±3.49) | 32.47 (8.33-106.94) (±16.50) | NA |
| | **Cu** (µmol) | 73.05 (26.56-96.15) (±31.85) | 41.60 (30.39-66.67) (±9.73) | 18.96 (1.79-83.33) (±11.63) | NA |
| | **Zn** (µmol) | 35.82 (18.68-64.10) (±16.31) | 21.82 (10.19-33.33) (±4.75) | 25.76 (2.22-66.67) (±13.22) | NA |

### 8.3.3. Manganese (Additrace® vs. no Additrace®)

Since manganese cannot be (intentionally) supplemented in PN outside of the compound preparation Additrace®, comparisons were made from the findings of blood test data (from paired PN prescription data) on the basis of whether Additrace® was incorporated in the PN. Results are shown below in Table 8.6.

**Table 8.6**: Results for all manganese blood tests from paired prescription and blood test data based on inclusion on Additrace®.

| | **Number (and percentage) of manganese blood test results (paired with prescription data)** | | | |
|---|---|---|---|---|
| | Additrace® (Yes) | | Additrace® (No) | |
| | Number | % | Number | % |
| Deficient | 0 | 0 | 2 | 0.80 |
| In range | 26 | 31.71 | 137 | 54.80 |
| In excess | 56 | 68.29 | 113 | 45.20 |
| Total | 82 | 100 | 250 | 100 |

## 8.4. DISCUSSION

### 8.4.1. General discussion and main findings

The findings from this chapter have followed on from the findings of Chapter 4 in a concise and satisfactory manner. Following successful approval for the study as a service evaluation, more patients were eligible for inclusion in the study by comparison to Chapter 5 offering a greater representation of the LT PN population and more valid context for data findings. The study successfully incorporated the use of a data pairing model to correlate PN prescription TE dose data with blood test results from PN patients. As before, blood tests and prescription records constitute a secondary data source, they offer an accurate, reliable and plentiful/rich data source from which to derive new knowledge.

In general, the results show that categories 1 (C1) and 4 (C4) had the smallest number of applicable paired data results, presumably because of the inadequacy of Additrace® (C1) and the unlikelihood of providing PN without any micronutrient supplementation to patients (C4).

As expected, categories 2 (C2) and 3 (C3) contained more data fitting their criteria, since they depicted manipulation of micronutrient dosing in PN whether inclusive (C2) or exclusive (C3) of Additrace®, befitting the sensitive and bespoke micronutrient needs of LT PN patients.

As a whole, clinician-directed TE dose manipulation in PN was associated with more blood test results in range than when PN was supplemented with Additrace® alone, as evidenced by results for C1 and C3. Although it can be considered a more time-consuming exercise it is associated with better health outcomes. One wonders whether all TE should be manually supplemented in this manner, or whether the use of a compound preparation with a better pharmacological dose profile would suffice.

#### 8.4.1.1. Copper

Results from C1 suggest that the dose of copper provided by Additrace® is sufficient for LT patient needs since 100% of results (n=7) were in range. However, the limited number of inclusive data should be noted, especially in light of recent dosing revisions to other micronutrient formulations in which the copper content has been reduced i.e. 20μmol in Additrace®, down to 4.7μmol in Nutryelt® and 6.3μmol in Addeven® (for further reference, see Chapter 4, Table 4.1. Trace element product compositions). Similarly, 77.63% of blood test results were within range for C2 with modest excesses, indicating suitable/appropriate copper provision from just the inclusion of Additrace®. Yet a greater percentage of blood test results were in range (85.06%, n=241) when the copper dose was directly manipulated by prescribers without the addition of Additrace® (C3), in which case the average dose of copper given in this category was 8.73μmol, considerably lower than the Additrace® dose (20μmol) and close to the doses of Addeven® and Nutryelt®. Disputedly/confoundingly, the results from C4 (n=16) show that 87.5% of results were within reference range for PN prescriptions without any copper provision at all. This finding should be take into account the small number of paired data and the more probable reflection of the increased copper status of those with impaired liver excretory function (Staun et al. 2009).

#### 8.4.1.2. Selenium

Results from C1 and C2 both indicate similar degrees of deficiency (C1, 35.14% vs. C2, 40%). One would expect a greater degree of in range BT results in C1 after allowing for more dose manipulation. A possible explanation could be the selenium doses already being at the maximum limits per volume of PN for some patients (C1, 37.11% vs. C2, 14.75%), meaning that it was not possible to supplement greater doses for some patients without compromising the physical stability of PN. C3 results showed more promising findings in that 83.08% (n=260) were in range when practitioners directly manipulated selenium doses themselves; greater control over selenium dosing resulting in more selenium blood test results within range (see Figure 8.1). No results were applicable for C4, in part demonstrating the essential nature of selenium as a

micronutrient for PN patients. In following on from the findings of Chapter 4, selenium deficiency still presents itself as an issue and yet the tendency for selenium to act as a reverse acute phase reactant should still be taken into account, potentially presenting greater levels of deficiency.

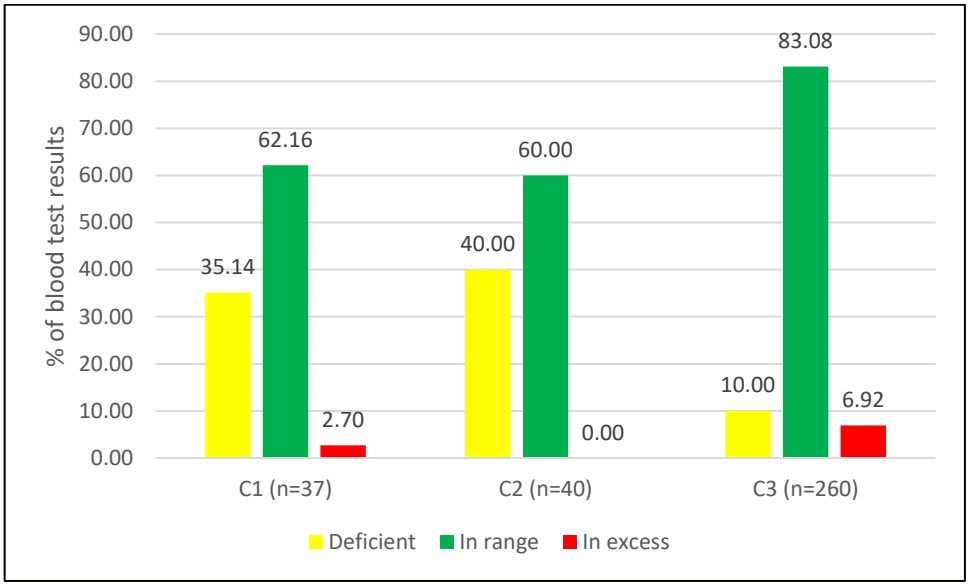


**Figure 8.1**: A clustered column chart to show the percentage of selenium blood test results (deficient/in range/in excess) per data category type.

8.4.1.3. Zinc

Findings from C1 and C2 were comparable, both giving ∽95% of BT results in range, demonstrating the ability of Additrace® to provide the zinc requirements for LT PN patients (alone or with extra supplementation). An interesting/intriguing finding was that less blood test results were within reference limits for C3 where zinc dosing relied exclusively on direct clinician/prescriber supplementation (82.99%, n=241). The average dose given for this category was 115.64µmol, more than the dose in Additrace® (100µmol), yet less than the doses in Addeven® and Nutryelt® (153µmol).

8.4.1.4. Average micronutrient doses and stability considerations

*8.4.1.4.1. Copper*

Distinctly far-ranging average doses of 19.70μmol and 8.73μmol gave rise to high proportions of 'in range' copper results. Given the greater number of included data for C3, the average dose of 8.73μmol is more likely to be representative of LT PN patient copper requirements; a dose closer to that of the newer preparations. In terms of stability restrictions, the results show that copper had the highest/greatest limitations in terms of maximum permitted dose of copper per volume (C1, 73.05%), yet in reality was less of an issue as all tests for this category were in range.

*8.4.1.4.2. Selenium*

In C1 and C3 when extra selenium supplementation was given, average total selenium doses of 1057.54nmol and 862.81nmol are noted, respectively. These doses are more than double the dose in Additrace® (400nmol), a finding which exemplifies the difficulty in getting/giving Additrace® to suit patients' selenium needs. It is comforting that this finding is in keeping with the revisions of the formulations of the preparations Addeven® and Nutryelt® (887nmol and 900 nmol respectively). One may notice the large range demonstrated for selenium doses across categories C1 and C3 (400-2200nmol), typically demonstrating the very variable requirements of selenium for the complex LT PN cohort, this however does not undermine the greater adequacy of doses greater than 800nmol resulting in 'in range' blood test results. Prior to data collection it was anticipated that stability dosing restrictions for selenium (based on volume of PN) may have prevented patients from receiving their optimal dose of selenium. However the low percentages for 'average percentage of maximum selenium provision per volume of PN' show that this is not the case and suggests that there is ample room/scope for extra supplementation; however it is more likely just to be an issue for the small number of fluid sensitive or restricted patients, or those who are critically ill, those with ongoing diarrhoea or increased fistula/stoma outputs (higher selenium requirements).

#### 8.4.1.4.3. Zinc

Average doses for zinc (C1, 159μmol; C2, 115.64μmol) were slightly greater than the dose included in Additrace® (100μmol). A reassuring finding in line with the increased dosing provisions in newer preparations. The low percentage values for the maximum provision of zinc per volume of PN shows the stability restrictions not to be a limiting factor towards achieving adequate zinc status for LT PN patients.

### 8.4.1.5. Manganese (Additrace® vs. no Additrace®)

Results from Table 8.6 show that there was less applicable data for prescriptions including Additrace® than those without (n=82 cf. n=250); in part, this demonstrates its unsuitability for the general needs of patients as much of data did not include the preparation. As expected, more manganese blood test results were in range when the preparation was not included in patients' PN (54.80% cf. 31.71%). Yet intriguingly in a converse outlook, 45.20% of blood tests were still in excess when no Additrace® (and therefore no manganese) was supplemented in patients' PN (vs 68.29%, see Figure 8.2). This finding supports the views by Howard et al. (2007) and Dickerson (2001) that patients are likely to meet their manganese requirements by its presence in PN as a ubiquitous contaminant alone, since it is known to leach from metal needles used in the aseptic production of PN (Cornelis et al. 1996; Yang and Lewandrowski 2002; Hardy 2009). Overall, the implication of the excessive manganese dose and its problematic complications has been demonstrated; most notably in necessitating removal of the compound preparation from PN and as such patients either miss out on the other key TEs or require separate TE additions to PN (where alternative preparations exist).

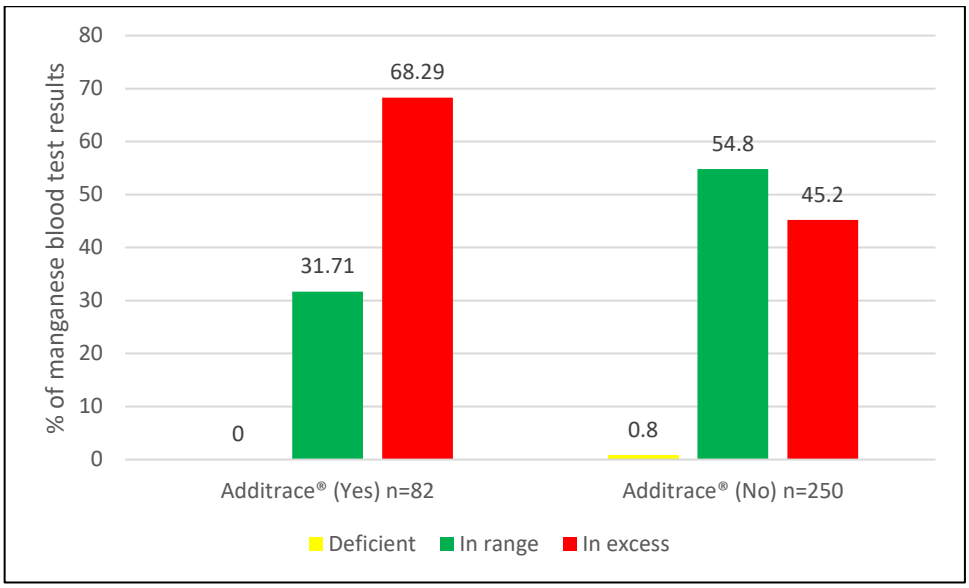


**Figure 8.2**: A clustered column chart to show the percentage of manganese blood test results (deficient/in range/in excess) according to Additrace inclusion in PN.

### 8.4.2. Limitations

This chapter followed on from Chapter 5 with core objectives and has shed light on further accurate knowledge of TE requirements in LT PN, yet the following limitations of the study should be taken into account:

- The same general limitations apply to this study as seen in Chapter 4 due to the nature and style of the study design. For example, the fact that data was included from a single HPN centre means that the results are less generalisable to the HPN network than if more HPN centres were included. Similarly, inherent limitations associated with the precision, accuracy and bias of the reporting of blood tests are still applicable to this chapter. Also, the effect of the APR could still have influenced blood test results to the same unquantifiable degree as in Chapter 4. For consideration of the blood test outcomes in the context of this study, the results are explicitly interpreted as they are reported (deficient/in range/in excess). The consideration of the APR is better used in a clinical decision-making scenario when taking into account all clinical factors which contribute to a patients' clinical scenario. If studies were to take into account the effect of the APR when reporting

of blood test outcomes, the research is best suited to quantifying its effect on the validity of blood test results as a primary aim, rather than over complicating data analysis as in this study where a general awareness and appreciation for its effect is more suitable.

- The disparate number of paired data between the four paired data categories; some categories have a smaller number of applicable data and hence their representative findings are weaker. However, this can be considered a finding within itself since less data matched some category criteria and showed the extent of general Additrace usage in PN e.g. C1-2 vs. C3-4. During the set dates of data inclusion (01/05/14-01/05/17), it would transpire that it had already become routine practice to remove Additrace from PN or withhold it if patients already demonstrated high levels in their blood tests.
- The basis for the rationale of this study hinges on the theory/assumption that PN TE dosing directly and exclusively correlates to micronutrient status in blood, the present study does not account for differences in oral TE consumption, EN nutrition, TE contaminants or the variability/differences in number of days administered, which could have influenced the blood test results.
- Similarly, the findings were based on the assumption that all PN was administered to patients and as such did not take in account missed days, illness, holiday prescriptions, poor compliance or any potential delays between effective prescription date and PN delivery.
- Some academics have expressed views that dose-concentration relationships are not accurate pharmacokinetic models to predict serum TE concentrations in relation to TE doses (Harraki et al. 1995; Hambidge 2003; Hotz et al. 2003). Additionally, since serum TE concentrations do not determine the chemical form of a particular element, biologic activity, or availability in the body as a whole and may not represent actual body stores.
- The decision to allow three months between the date of the signed PN prescription and the TE blood test results could be seen as too long a time period, resulting in the omitted inclusion of potentially applicable

data had a shorter time window been implemented (a lot of collected data was not applicable for inclusion in data analysis). Especially since sometimes in clinical practice 4-6 weeks is used as a clinical judgement point to spot trends from clinical PN prescription/regimen amendments. However, the three-month time window can equally be viewed as a way of completely ensuring that blood test results were indicative of TE doses in PN.

- Similarly, some data from sensitive LT PN patients may not have made the three-month data window if they required more frequently changes to their PN regimen and PN prescription, affecting the representation of data findings to reflect LT PN patients. Yet conversely, this could be interpreted as a positive remark in that the data findings reflect TE requirements in more stable LT patients.
- This chapter did not consider the clinical documentation of signs or symptoms of TE abnormalities (deficiencies/toxicities) and so cannot be linked to the clinical severity of the consequences of TE abnormalities.
- Within the data time window (2014-2017), there was a small team of pharmacists in control of micronutrient dosing decisions; this can be seen in a positive light that there was less scope for random variability in action and more familiarity with prescription handling based upon evidence based best practice. Yet the findings from this service evaluation can also be seen as informative of the actions of a small number of people in which actions relating to implicit bias could have resulted e.g. unfounded beliefs about PN dosing without conscious realisation. Still, the reporting of this service evaluation ultimately acts to ensure improvements in service provision.
- A dated study by Pluhator-Murton et al. (1999) stated that the effects of storing PN at increased temperatures can significantly decrease zinc, copper, and manganese availability in home PN solutions that are typically compounded and delivered in batches of 7–10 days for home supply, similar to the PN produced for C&V UHB patients by the homecare company, Calea. This finding could potentially have

influenced the results from this study but also could be considered as a constant and unavoidable factor. HPN is always aimed to be kept cold chain during delivery but slight fluctuations in temperature can occur.

### 8.4.3. Future work and recommendations

The undertaking of this service evaluation has helped to elucidate and identify areas requiring further investigation relating to TE dosing, compound micronutrient preparations and correlation with blood test biochemistry. It would be useful for future studies in this area to incorporate the following:

- Efforts should be made to ensure homecare services provide the newer TE preparations Addeven® and Nutryelt® across a range of bespoke PN formulations; there still appears to be resistance against their use despite their existence and evidence of revised dosing adjustments. Better still, provide a range of TE preparations to cater for the variable needs of the LT population e.g. a preparation with reduced copper and manganese would be useful for patients with evidence of cholestatic liver disease. Similarly, for instances when compound preparations are still unsuitable, the availability of more individual/singular TE preparations (not just copper, iron, selenium and zinc) would be useful to offer individualised TE supplementation in PN; especially since our results showed that clinician directed TE dose manipulation in PN was associated with more in range blood test results.
- Findings from the current chapter alongside further studies incorporating more HPN centres could inform the composition of a 'better' ideal TE preparation to suit the basal requirements of LT PN patients' needs i.e. the inclusion of more data from more HPN centres would generate more credible and generalisable findings. Especially if other HPN centres are using different compound TE preparations and/or dosing and monitoring practices.
- The current study investigated paired data separated into four categories depending on whether Additrace® and/or extra TE

supplementation was given. A further double cohort comparison study in which patients who had received Additrace® then required its removal from PN (after blood test review), or those who were not receiving Additrace® and then had it added to their PN, would be useful to quantify the relative effect of Additrace upon blood test results for each participant.

- Further studies should attempt to incorporate the reporting of symptoms associated with TE nutritional abnormalities to correlate the clinical significance with the extremes of out of range blood test results.
- It would be worthwhile investigating the lesser known TE and their clinical significance e.g. iodine, cobalt, chromium etc. As yet not much is known of the clinical impact for their derangement, nor their optimal dosage.

From the outcomes of PN prescription doses that were associated with in range blood test results. Based on data shown in Table 8.4, the findings from this study would suggest/recommend the optimal doses of TE for the basal/general needs of LT PN patients as 10μmol copper, 800nmol selenium and 100μmol zinc. The suggested dose for selenium accurately reflects the revised dose in newer preparations, however the doses for copper and zinc are more mid placed between the doses of older and newer preparations (Additrace® and Decan® vs. Addeven® and Nutryelt®). In this light, further studies of the similar design in this chapter which incorporate the newer preparations would shed more accurate findings of TE dosing and suggestions towards optimal TE requirements in LT PN patients.

## 8.5. CONCLUSION

This chapter has successfully evaluated the use of Additrace® in terms of resultant biochemical test outcomes. Above all, the findings have demonstrated clinician-directed manipulation of TE in PN to be optimal in resulting in a greater proportion of TE blood test results within range; in so doing has allowed successful estimation of optimal doses for copper, selenium and zinc. The problem of manganese overprovision in PN from the compound preparation Additrace® has also been further quantified. Its lack of inclusion in PN should in itself serve a persuasive motion to urge homecare companies to consider stocking, trialling and implementing the use of the newer compound TE preparations across a range of LT PN formulations. For the majority of patients, stability dosage restrictions have been discounted as a factor resulting in deficient blood test results, except for the smaller sub-population of patients with extreme volume restrictions for their PN. Overall, this chapter has further demonstrated the pressing clinical need of TE preparations to cater for the general needs of LT PN patients, or for those with more individualised needs. Alongside an actual need for homecare companies and PN production units to implement the use of the newer TE preparations in routine practice and establish their intended use for LT PN patients; further studies will then be able to assess their clinical suitability against the outcomes observed in this study investigating Additrace®.

# CHAPTER NINE

## General discussion

## 9.1. GENERAL DISCUSSION

The field of PN covers a vast body of knowledge in terms of meeting patient requirements, PN stability and manipulation to optimise nutritive support; as well as more clinical research parameters such as management of complications and multidisciplinary team review. This research project has covered extensive and variable grounds in terms of research findings relating to the provision of LT PN.

While the knowledge-base surrounding PN is well-established, there is still plenty more to clarify and consolidate; the broad-ranging title of 'nutritional deficiencies/abnormalities in LT PN' allowed initial research findings to guide and develop further research avenues. The PhD project commenced with a characteristic survey of the C&V UHB HPN population followed by an in-depth literature review of documented micronutrient deficiencies and excesses; and an assessment of the extent of nutritional abnormalities demonstrated in patients' blood test monitoring. Findings from this assessment directed subsequent stages of research navigation; in particular, the high proportion of patients with inadequate vitamin D status focused research laboratory efforts towards determining/confirming the stability of vitamin D in PN formulations as well as investigating the extent of MBD and bone health problems experienced by HPN patients. Similarly, TE findings for evident deficiencies of selenium and excesses of manganese in LT PN patients prompted an evaluation of the provision of TE from the first-line preparation Additrace® through comparison to patients' nutritional status as evidenced by routine biochemistry monitoring.

Overall, each chapter of this research project has been able to shed new light on research findings and contribute to different areas within the field of PN (e.g. PN population characteristics, micronutrient status, bone health/MBD and the adequacy of PN preparation formulations).

### 9.1.1. Overview and impression of research journey

The focus of the PhD was to explore the extent of nutritional abnormalities (deficiencies/excesses) experienced by LT PN patients and their resultant biochemical effects (e.g. vitamin D deficiency leading to bone health problems). This aim has been methodically achieved for the micronutrient components of PN and the results presented throughout; one would argue that it is harder to gauge optimal patient requirements for micronutrients as they are needed in far less quantities than macro-components (e.g. lipid, glucose, amino acids) which are easily correlated and manipulated in relation to patient weight.

Key research findings have already been discussed within each chapter. A recurring theme documented throughout the literature review and from the results/findings of this project is the issue of pronounced vitamin D deficiency in LT PN populations. The critical extent and effect of this deficiency is not just limited to bone health complications, as vitamin D is continually being shown to be a critical health factor in many diseases (e.g. autoimmune diseases, cancer, cardiovascular disease, metabolic diseases). However some correlations are considered more tenuous opinions rather than evidence based (Theodoratou et al. 2014). Our finding that 30.1% of blood test results were inadequate (states of deficiency and insufficiency) for vitamin D (Chapter 5) is paralleled by findings of similar studies (Thomson and Duerksen 2011; Kumar et al. 2012; Ellegerd et al. 2013; Murphy and Lewis 2016a; Nygaard et al. 2016); yet further studies would benefit from a comparator cohort such as the general population with similar baseline characteristics (age, sex etc) and absence of IF/disease. Nevertheless, the importance of the results demonstrated in this thesis in relation to vitamin D deficiency and the prevalence of MBD from a single cohort of patients speak volumes for long suspected issues observed in the PN field, now officially documented for a population of PN patients, rather than on a case by case basis.

Where such pronounced widespread deficiency is observed in the PN population for vitamin D, there needs to be greater research efforts to confirm its stability in PN additives. This is in addition to the fact that a more sensible

and optimal dose needs to be included in PN additives, particularly since PN is the primary point for nutritional supplementation in this population. And especially still considering the current dose in PN additives is less than half the recommended daily dose for all adults in the general population (Scientific Advisory Committee on Nutrition 2016).

It was a disappointment that research efforts to establish the stability of vitamin D in PN additives and PN formulations were unsuccessful. If stability could have been proven, one could have excluded instability of vitamin D within PN admixtures as a contributory factor towards the development of MBD in PN populations. However the likelihood is that patients receive suboptimal dosing from PN additives, and/or concomitant oral vitamin D supplementation is ineffective with patients' reduced bowel length for absorption (Buchman et al. 2009; Vanek et al. 2012; Massironi et al. 2013). From a side-line view, it has been discovered that the two forms of vitamin D used for supplementation in PN have differing efficacy in raising 25-OH vitamin D levels and should not be regarded as equipotent or interchangeable. Ergocalciferol ($D_2$) being significantly less efficacious than cholecalciferol ($D_3$) (Houghton and Vieth 2006; Tripkovic et al. 2012). Perhaps the routine supplementation of Vitlipid N Adult® in lipid (3-in-1) bags is less effective in augmenting vitamin D levels than the use of Cernevit® in aqueous (2-in-1) bags.

Research findings for TE have been particularly insightful throughout the project. More information has been elucidated for TE that are required in greater quantities (e.g. selenium) or in lesser quantities (e.g. manganese); whilst similarly demonstrating the inadequacy of the first-line UK licensed TE additive Additrace® for LT PN needs. These beliefs have been suggested in other publications (Van Rij et al. 1979; Abrams et al. 1992; Hardy et al. 2008; Hardy 2009; Shenkin 2009); now that the present study has demonstrated these findings in a HPN cohort, the findings are finally substantiated. Certain obstacles prevent optimal TE dose manipulation in PN. For instance, the refusal of homecare PN companies to start using newer or reformulated TE

additives (e.g. Nutryelt®, Addeven®), or the fact that manganese-free TE additives do not exist.

A notable matter presented and discussed throughout the thesis is the variable nature of the HPN population, especially in terms of their patient and PN related factors. The inherent variability of numerous factors has presented itself as a finding in itself. For example, the variety and distribution of underlying disease leading to commencement of PN therapy, the variable lengths of remaining short bowel for SBS patients, their variable PN requirements (fluid, calories, electrolytes and micronutrients), variable degree of external oral nutritional consumption or the variable susceptibility and extent of nutritional abnormalities. However these considerations complicated research efforts for the smaller subset of a sample HPN population in this research. Future studies would benefit from greater patient numbers to allow sub-categorisation within each of these considerations and in turn produce specific findings related to patient sub-classes e.g. micronutrient dosing in patients with certain stoma types, PN requirements in patients with gastroparesis, or prevalence of vitamin D deficiency and/or bone disease in patients with GI obstruction. Recruitment would have to take place from multiple HPN centres in a UK-wide study to achieve greater participant numbers, in which case the study protocol and accompanying documentation would need to satisfy each individual NHS R&D department involved. While this study design would capture more meaningful data from many patients in terms of their disease classification, indication for HPN and the specifics of their PN formulation (e.g. the use of different PN additives); the practices relating to PN prescribing, monitoring and review may differ between the centres.

### 9.1.2. Study design

The study designs and methodological rationale chosen throughout the thesis befit the research aims; yet as with most projects, they would have benefited from a larger cohort of participants (and more data) for inclusion. The approach taken for the types of study designs in this thesis took into account all the factors mentioned in the following limitations section and represent the

best possible way of utilising, analysing and presenting the data (and data findings) to best effect for the aims of the PhD project.

For future studies assessing micronutrient status with the intention of correlating patient disease states and micronutrient doses to states of deficiency or excess, a different statistical approach may be sought. By using greater participant numbers and HPN centres, patients might fit into better categorical time points (in relation to time point starting PN and duration receiving PN) and methods employing a greater statistical emphasis may be used. For instance, the use of Wilcoxon's test to compare blood test results pre- and post-treatment intervention (e.g. vitamin D or bisphosphonate); or Pearson's correlation between blood test results (e.g. calcium, phosphate, vitamin D), bone scores (BMD, T, Z) and/or PN parameters (doses of calcium, phosphate, vitamin D).

### 9.1.3. Study implications

Overall, our research findings have generalisable benefits and implications for the field of PN going forward in light of the developments made from this project. The extent to which micronutrient abnormalities occur in LT PN patients is now known, and practical implications as to how to manage them have been documented, with suggestions to future research and PN additive compositions.

The evaluation of the suitability of Additrace® and its correlation to patient micronutrient status showed that prescriber directed manipulation of micronutrient dosing in PN was the most effective, resulting in micronutrient tests within the reference range. Now, with the confirmed awareness of specific themes for nutritional abnormalities, other centres can adopt similar prescribing approaches to those of C&V UHB if they notice similar nutritional abnormalities occurring during routine monitoring.

Early research steps have been undertaken regarding assessment of vitamin D stability in PN additives, its extent for deficiency and associated documented MBD in HPN populations. Yet there is still scope for substantial further

research efforts in these areas, particularly in correlating patient vitamin D status with MBD. Focus can be aimed at ensuring manufacturing companies and home PN companies license and provide PN additives of more rational and sensible composition.

While the various studies in this project have contributed to the body of knowledge surrounding PN, they have also highlighted areas for further research and improvements.

### 9.1.4. Critical appraisal of research findings

The successive research findings from the present PhD project took a natural flow in terms of research journey. The potential inadequacy of compound micronutrient preparations for patients' LT PN requirements was first realised during the cross-sectional study in Chapter 3 when it discovered that only 20.3% of patients received the TE preparation Additrace® in their PN regimens; similarly the other preparations were used in PN to a less than expected degree (89.8%, Cernevit®; 39.0%, Solivito N®; 37.3%, Vitlipid N Adult®). These simple yet notable research findings set the scene for subsequent studies investigating micronutrient provision in LT PN. The literature further revealed both expert opinion and documented evidence of concern regarding the under-provision (selenium, vitamin D) and over-provision (manganese) of micronutrients, alongside publication of nutritional derangement for other micronutrients. Research aims for the PhD were set to explore the adequacy of provision of micronutrients in LT PN.

A key publication by Vanek et al. (2012) accurately describes the current issues and considered opinion amongst PN clinicians regarding with micronutrient provision in LT PN. It describes micronutrient requirements according to recommendations and describes how some requirements were based on oral requirements without consideration for the differences specific to the intended population (LT PN patients) e.g. increased micronutrient requirements in critical or chronic illness. Ultimately, recommendations are given for pressing areas of further research and reformulation of PN micronutrient additives. The present PhD project has been able to further

substantiate links between nutritional abnormalities and clinical complications as well as document the degree of inadequacy of the micronutrient preparation Additrace®.

#### 9.1.4.1. Manganese

A preliminary finding from earlier stages in the PhD was that Additrace® was only included in 20.3% of patients' PN regimens, suggesting that there were problems with its suitability for the LT PN patient cohort to require its exclusion from PN. Chapter 5 revealed that 50.6% of patients' blood test results were in excess (i.e. states of toxicity); presumably the primary reason for the preparation's removal from patients' PN regimens since long-standing manganese excesses are associated with potentially irreversible parkinsonian-like effects, confusion, seizures and deposition in brain and neural tissues (Dickerson 2001). This avenue was then further followed up in Chapter 8 which assessed the adequacy of micronutrient provision from Additrace® via correlation with patients' blood test data (according to the prescription and blood test data pairing model). Firstly, less data was applicable to "C1: Additrace® (Yes) Extra TE (No)' according to the data model; again due to the known unsuitability of Additrace® for LT PN requirements. Secondly and as expected, the data-pairing model was able to show that more manganese blood test results were in range without the inclusion of Additrace® (or any supplemental manganese, since no singular preparation exists) in patients' PN (54.8% cf. 31.71%). Although a successful research finding which affirms that the excessive manganese dose in Additrace®, there is still scope to ascertain the optimal requirements for both LT PN patients and the general population. It is troubling that 45.2% of blood test results should still be in excess without any known supplementation of manganese in patients' PN, suggesting TE contamination of PN may still be responsible, a research area which has waned in recent years (Pluhator-Murton et al. 1999; Hardy et al. 2008)

9.1.4.2. Copper, zinc and selenium

Again, starting with the initial finding that Additrace® was only included in 20.3% of patients' PN regimens, the preparation was perceived as unsuitable for LT PN patients' needs. The assessment of micronutrient status in Chapter 5 then demonstrated a greater number of deficient blood test results for selenium (32.8%); alongside more modest derangement of results for copper and zinc, although still somewhat unexpectedly out of range [copper (def, 12.4%; in excess 7.7%), zinc (def, 5.8%; in excess, 11.6)]. These findings were then followed up in the penultimate chapter which correlated the doses of TE in patients' PN regimens (from both Additrace® and/or additional supplementation) with their corresponding micronutrient status. It was further corroborated that TE preparations do not satisfy patients' selenium requirements after it was found that 40.0% of patients' blood test results were deficient just from the provision of Additrace® (without supplemental additions of selenium) in their PN. However, the difficulty in meeting patient requirements was also demonstrated, since 35.14% of blood test results were deficient even when there was direct manipulation of the selenium doses in patients' PN regimens. Selenium deficiency is well-referenced in the literature (Levander 1984; Abrams et al. 1992; Shenkin 2009; Etani et al. 2014; Chen et al. 2016). Other studies have noted deficiency using Additrace® with/without supplemental dosing (Fuhrman 2006; Btaiche et al. 2011; Parrish 2014; Murphy and Lewis 2016b), yet the findings from the present study are the first to implement productive analysis using a data-correlation model based on the presence on the PN additive Additrace® (with or without extra TE provision). The data-correlation model was able to accurately define the general selenium requirements of the LT PN population at C&V UHB as 800μmol/day.

Regarding copper, Chapter 5 showed that 12.4% and 7.7% of results were deficient and in excess, respectively. While less tangibly in extreme states of derangement (by comparison to selenium and manganese), further analyses in Chapter 8 demonstrated that Additrace® catered well for patients' copper requirements (deficient, 11.8%; in range, 77.6%; in excess, 10.5%) with similar incidences for states of deficiency and excess. It is difficult to gauge explanations for these findings, yet it is reassuring that a large proportion of

data were in range. Further still, a greater proportion of paired data were in range when TE in patients' PN were manually manipulated by clinicians, rather than sole use of a compound TE preparation (Chapter 8: C1, 100%; C3, 85.1%). Despite our findings, evidence from the literature suggests that copper toxicity is still the prevailing problem, since the copper dose in both Nutryelt® (4.7μmol) and Addeven® (6.3μmol) has been decreased from the 20μmol in Additrace® (ASPEN Board of Directors and the Guidelines Clinical Task Force 2002; Blaszyk et al. 2005; Howard et al. 2007; Shike 2009; Vanek et al. 2012; Gaetke et al. 2014). It is difficult to interpret the context of findings for results relating to copper provision since it is a known TE contaminant of PN, increased serum representation during the APR and late presentation in clinical deficiency.

Similar to copper, Chapter 5 showed no substantial findings to be elucidated for the TE zinc (deficient, 5.8%; in range, 82.6%; in excess, 11.6%), a slight trend for toxicity being observed. The analyses in Chapter 8 then showed that Additrace® alone catered well for the general needs of the LT PN population (C2, 94.9%). When Additrace® was given with extra supplemental zinc for a smaller number of data (n=22 pairs), 95.5% of zinc blood tests were in range. Again, showing that clinician-directed TE manipulation in PN was associated with more blood tests within reference range. These findings probably explain why the zinc doses in Nutryelt® and Addeven® were increased to 153μmol (cf. 100 μmol in Additrace®), in line with documentation in the literature of zinc deficiency in PN patients (Yanagisawa 2004; Daniells and Hardy 2010; Duncan et al. 2012; Vanek et al. 2012).

Through the correlation of patients' PN prescription TE doses and blood test results, it was possible to recommend optimal TE doses for copper, selenium and zinc to maintain TE status within range, based on the general requirements of the population of LT PN patients at C&V UHB (10μmol copper, 800nmol selenium, 100μmol zinc;/day).

9.1.4.3. Vitamin D

A notable theme in the literature is the concern regarding vitamin D deficiency in the PN population and its association with adverse bone health (Raman et al. 2006; Hamilton and Seidner 2008; DeLuca 2009; Nygaard et al. 2016). The key finding from Chapter 5 found that for the patients registered with C&V UHB, 30.1% of their blood tests demonstrated inadequate vitamin D status (states of both deficiency and insufficiency). This finding links in with findings of later sections where it was shown that increasing duration of time requiring LT PN was associated with worsening bone health (Chapter 7). The theoretical link being that longstanding inadequate vitamin D status contributed to the worsening of patients' bone health. The main finding to demonstrate this link was the longitudinal net loss in patients' Z-scores between their 1st and 2nd DEXA scans since receiving LT PN (0.0367, femoral neck; 0.0733, total hip). Similarly, another key finding from Chapter 7 was the cross-sectional presentation of 58% and 60.78% of patients having osteopenia at sites of the femoral neck and total hip, alongside osteoporosis in 28% and 19.61% at the same respective sites; thus demonstrating the prevalence of bone disease in the consenting sample of C&V UHB patients receiving LT PN. These findings are supported by Raman et al. (2006) in which 33% had MBD at the spine and hip, and 50% at the femoral neck. Similarly, they noted a negative correlation between the duration of HPN and BMD results (r= -0.40), in that bone health deteriorated with increasing time receiving PN. However, it should be noted that these findings were from less patients (n=25) and collective states of osteopenia and osteoporosis; nether the less, they are in agreement with the findings from this PhD.

Factors contributing to patients' sub-optimal vitamin D status (30.1% of blood tests, Chapter 5) were further explored in Chapter 6, in which the stability of vitamin D was investigated within compound PN preparations which are used in the formulation of patients' PN admixtures. This chapter yielded disappointing results by reference to the proposed chapter aims. There were limitations with the laboratory equipment and methods to feasibly detect vitamin D, and unfortunately no time-duration stability tests (e.g. 24/48 hours, 7 days) were able to be performed. Despite this, vitamin D was still

successfully identified from samples of Vitlipid N Adult®. However, this was at its full concentration (5µg/10mL) prior to dilution in large volume PN admixtures and it could only be reproduced at its LOQ, meaning that no further stability studies were possible. This finding can still be interpreted to show the difficulty in even assessing the presence (let alone stability) of vitamin D in PN additives. Significant further research is required to ascertain whether patients are receiving the full intended dose of vitamin D from these additives i.e. ruling out whether vitamin D could degrade over within the admixture, or upon exposure to external factors such as heat or light, or whether vitamin D interacts with other components in the PN admixture (e.g. a chemical reaction or cohesion to other components).

Although it is hard to quantify the amount of vitamin D patients receive outside their PN regimens (e.g. it is also synthesised cutaneously from light exposure) and the absorption of vitamin D on an individual patient basis cannot not be determined, the cross-sectional survey in Chapter 3 noted 61% of patients received concurrent vitamin D supplementation outside of their PN within the preceding year. It can be thought that the low dose of vitamin D in PN additives (by comparison to updated guidance on dietary reference intakes) alongside its unproven stability in PN and the inherent nature of on-going LT PN therapy, exposes/ or is associated with risk for the development of bone disease in these patients (e.g. MBD) (Scientific Advisory Committee on Nutrition 2016).

## 9.2. LIMITATIONS AND CHALLENGES

A particular challenge in the earlier stages of the PhD was applying for access to NHS patient data with the intention of undertaking research. Ideally more HPN centres would have been recruited and included in data analysis but the application process for even a single centre was laborious in terms of satisfying both NHS ethics and R&D approval requirements, especially as a research student from outside the NHS organisation. Eventually permissions were approved and satisfied amongst all parties, allowing the research project to proceed and resulting in a remarkably successful recruitment rate of 64.5%. Fortunately, in the later stages of the PhD, R&D permitted the service evaluation approval for a final study section for the PhD, this allowed inclusion of all patients registered on the IF clinic list register receiving LT PN, rather than a consenting sub sample of the patient cohort; ultimately permitting total data capture within the time period for the study in Chapter 8.

It became apparent during data collection that prescription changes and monitoring decisions resulted from the intervention of a small number of staff members. There is the chance that the findings from this PhD are representative of the actions of a few professionals. On the other hand, this could be considered a positive finding in that the extracted data was not affected by variable healthcare practices.

Another challenge presented itself in being able to satisfy study designs to feasibly achieve the research aims in light of the numerous confounding factors inherent to the PN population. Examples of these variable factors include: disease state, indication for PN, co-morbidities, PN requirements, oral intake outside of the PN regimen, duration receiving PN, blood test monitoring intervals and time points for PN prescription changes and review.

In addition, there was the realisation during planning of the studies of the difficulty in attributing and correlating patients test results for micronutrient status to their micronutrient provision in PN. For instance, the fact that vitamin D is also synthesised from UV exposure, or that patients may get variable intake of micronutrients from their oral diet, or that patient review

and monitoring occurs are irregular time points, that patients were initiated, maintained and weaned off PN across different time point, or that their PN regimen may have undergone manipulation during this time (calorie, fluid, micronutrient dose changes).

The micronutrient data capture from this PhD relates solely to the use of Additrace® and/or manual supplementation using singular TE preparations (where preparations exist and are permitted by PN stability); a considered limitation since newer preparations do exist. However there appears to be a barrier in the form of getting homecare PN companies to incorporate these preparations in their manufacture of PN. For the companies to permit their addition to PN, they have to assess and determine the stability of overall PN formulation across a range of different PN formulations (as observed with HPN patients). There is also the business angle, in that homecare companies would rather use a product they own/market/distribute themselves over a competing product from a different supplier. One wonders whether nutrition teams should lobby for the implementation of these newer preparations.

## 9.3. FUTURE WORK AND RECOMMENDATIONS

While the present project has delivered respectable research findings, there exist opportunities for improvement in further studies in applicable areas of PN research.

A well mentioned limitation throughout the thesis is the desire to have had greater participant numbers in order to produce more generalisable findings. Yet, in actuality, the recruitment rate for the HPN population in this project should still be considered respectable since C&V UHB caters for the LT PN needs of almost all patients in Wales, in what is otherwise a naturally small population of affected patients in the UK. Also, characteristically the data gleaned from each participant can be considered 'data-rich' as it included all relevant data over the time they received PN. Hopefully future studies will be able to recruit more patients across a selection of HPN centres.

Successful research findings were discovered in Chapter 8 when correlating micronutrient dosing to blood test results. Future use of this comparative cross-sectional methodology in PN studies would be beneficial to contrast and compare the nutritional effectiveness of different PN additives, or to explore the effectiveness of different interventions in optimising patient micronutrient status (e.g. two vitamin D preparations/doses).

The incorporation of secondary health-related outcome measures (e.g. QOL questionnaires) would prove useful for assessing patient response to PN therapy. Particularly if patients had demonstrated nutritional deficiencies or excesses (perhaps symptomatic) over time and the QOL instruments could detect sensitivity/responsiveness to change.

There should be greater effort to optimise the nutritional benefit of PN wherever possible, rather than incurring successive costs for other IV infusions (e.g. vitamin D, iron). Or similarly with heavy duty medication needs such as high dose oral vitamin D, where the aim is to get as great a dose as possible absorbed in patients with a short bowel. By supplementing PN in the more rational first instance it would ensure that a known fixed dose of

micronutrient bypasses absorption straight into the bloodstream, without incurring secondary administration costs or medicines wastage. However this recommendation relies entirely on developing and proving stability PN formulations with the presence of extra and/or greater quantities of micronutrients.

While the nutritional benefit of PN should be optimised in the first instance for all patients, alternative strategies to optimise patient micronutrient status should be considered. For example, should vitamin D supplementation in PN continue to be a problem, perhaps delivery methods other than IV/oral should be considered. An example being an intranasal salmon calcitonin spray (delivering 200IU vitamin D) being associated with a significant reduction in the risk of new vertebral fractures in postmenopausal women with osteoporosis (Chesnut et al. 2000).

### 9.3.1. Key recommendations

In light of the research findings elucidated from the outcomes of this PhD project and in order to improve/progress within the field of micronutrient dosing in LT PN, the following key recommendations are proposed:

- The newer TE preparations must be used in clinical practice (e.g. Addeven® and Nutryelt®). Their composition has been based on expert feedback and evidence from the literature. It is only through their clinical use in LT PN that evidence of their clinical effectiveness can be proven.
- Pharmaceutical companies must reformulate their compound TE preparations so that they are more suitable for the general needs of the population. Where possible and feasible, this includes:
    - Ideally, a range of PN preparations to cater for the variable needs of the LT PN population need to be available, i.e. for patients with greater/reduced TE requirements. Or, for patients with particular clinical co-morbidities e.g. impaired liver excretory function/cholestasis, in which case preparations with less copper and manganese would be beneficial (Staun et al.

2009)

  - Similarly, singular TE products should be available for all the essential TE (not just copper, iron, selenium and zinc) to permit more precise TE manipulation for more sensitive patients/patient requirements or when requirements are beyond the remit of compound TE preparations.
  - Preferably, when new micronutrient preparations are introduced to the market, they should already satisfy a range of physical PN stability limits. As one would expect for the variable PN requirements in LT PN patients (i.e. extremes of calorie and fluid requirements per PN formulation, which can significantly impact the stability/expiry of the PN formulation). Current 'newer' preparations have faced opposition to routine inclusion in LT PN because they do not have supportive data for the extremes of bespoke PN.

- The area of TE contamination requires significant further research; of late, research has dwindled or is lacking. Dated research states that aluminium, chromium, copper and manganese are particularly problematic contaminants of PN (during its aseptic manufacture) (Hardy 2009; Moukarzel 2009; Lima-Rogel et al. 2014); yet since publication, little has been researched or implemented as corrective actions or as recommendations. Future research should:
  - Accurately define the TE which are problematic contaminants of PN admixtures.
  - Quantify the extent of possible contamination. Would be useful to gauge extent of possible contamination beyond intentional supplementation e.g. for copper and iron.
  - Suggest recommendations to avoid possible contamination.
  - Define cut-off limits for the presence of ubiquitous TE contaminants in PN e.g. concentration of x per mL of PN.
  - HPN councils/groups should state guidance for how to manage possible PN contamination e.g. ESPEN, ASPEN.

- HPN centres should monitor (or find a way to monitor) other important micronutrients provided during LT PN. For instance, iron and vitamin K are not monitored by C&V UHB yet evidence in the literature suggests that there are current issues associated with their optimal provision during LT PN (iron-deficiency anaemia and bleeding syndromes, respectively). Particularly for vitamin K which is only provided in one vitamin preparation (Vitlipid N Adult®) and naturally present in lipid emulsions.

Further studies investigating micronutrient dosing (with both vitamin and TE compound PN additives) and nutritional status in LT PN should include:

- Investigation into the provision of LT PN being performed on a larger-scale basis with the inclusion of greater participant numbers from multiple HPN centres; thereby consolidating stronger research findings resulting from variable PN practices.
    - Greater participant numbers would ensure that research findings for the assessment of micronutrient status across different diagnoses, indications (for HPN) and disease states would incur greater validity and context.
- Future work investigating micronutrient status in PN should quantify the degree of the effect of the APR on the accuracy of reported micronutrient blood test results.
- Studies investigating the degree of TE contamination during PN compounding (aluminium, chromium and manganese).
- Comparison studies:
    - Comparison study of incidence of nutrition abnormalities in those given solely a fixed dose compound micronutrient preparation versus those who had necessitated removal of the preparation and subsequently require manual micronutrient manipulation in their PN (after evidence of nutritional derangement e.g. manganese)
    - Comparison between patients' micronutrient status between

the use of different TE PN preparations in their PN regimens.

#### 9.3.1.1. Vitamin D

In relation to vitamin D, the following recommendations are proposed for future research:

- Future work that correlates patient vitamin D status with bone health measurements. The findings observed in Chapter 7 of the present PhD project confirmed patient bone health deteriorated with increasing duration of time receiving LT PN.
- The implementation of cohort intervention studies to investigate the effectiveness of treatment/supplementation strategies in LT PN patients to aim to increase vitamin D status. For example, the use of particular strength vitamin D preparations in SBS patients requiring LT PN e.g. 4000IU daily for ten weeks for patients with x length of bowel remaining.
- The use of cohort comparison studies to compare vitamin D status in LT PN patients against the general population. Or between different groups of LT PN patients. For instance, by underlying disease classification or indication for PN, or by grouped age categories e.g. pre and post-menopausal. Or further still, by patients grouped according to the concurrent treatments they receive, e.g. steroids or bisphosphonates. The rationale being that medication and patient age have effects on bone health, and research aims would aim to establish the effect of LT PN on bone health.
- The use of further laboratory techniques to establish the stability of vitamin D in PN (e.g. MS-HPLC) by excluding its instability within the PN admixture as a potential reason for inadequate vitamin D status in patients. This avenue would include contacting the authors of published assays to clarify their methods for the detection of vitamin D, since repeated methods during this project were unsuccessful. Nether the less, the low dose of vitamin D in large volume PN admixtures may require future work to include sample clean-up preparation such as

solid phase extraction and calculation of percentage recovery.

## 9.4. CONCLUSION

PN acts as a vital therapy for patients with long-standing and life-threatening conditions; as its continued use and reliance continues to gather pace in the progressive modern healthcare setting (e.g. in conditions such as cancer or in areas where there is now greater access to PN services). In its entirety, this PhD project has covered considerable ground within the area of LT PN and meeting patients' LT nutritional requirements. Further research should follow up the research findings and recommendations that have been proposed in each study subsection. In doing so, ensuring that the provision of PN keeps up with standards of quality practice and improvement, ultimately helping to make sure the nutritional benefit of PN is continually optimised for all patients.

Key findings relate to the characteristic description of HPN patients from a cross-sectional perspective, the clarification of long suspected and now confirmed micronutrient dosing issues for LT PN patients. In particular, the inadequacy of PN additives to provide optimal doses of vitamin D, selenium and manganese. Within each chapter, pertinent issues have been discussed in great depth alongside evidence from published literature. While substantial leaps in research findings have been made, there is still great scope for further research in each of the areas studied within this project; especially for efforts to establish stability of vitamin D in PN and to cross-correlate patient vitamin D status to the development of MBD while receiving LT PN.

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# APPENDICES

# APPENDIX I – CONFIRMATION OF ETHICAL APPROVAL

Part of the research infrastructure for Wales funded by the National Institute for Social Care and Health Research, Welsh Government
Yn rhan o seilwaith ymchwil Cymru a ariannir gan y Sefydliad Cenedlaethol ar gyfer Ymchwil Gofal Cymdeithasol ac Iechyd, Llywodraeth Cymru

NISCHR
Gwasanaeth Moeseg Ymchwil | RES | Research Ethics Service

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Mr Sean Rhys Dodington
PhD Student
Cardiff School of Pharmacy and Pharmaceutical Sciences
Room 1.27
Redwood Building
Cardiff University
Cardiff
CF10 3NB

8 April 2015

Dear Mr Dodington

| | |
|---|---|
| **Study title:** | **Evaluation and Assessment of Nutritional Status in Home Parenteral Nutrition (HPN) Patients** |
| **REC reference:** | **15/WA/0128** |
| **IRAS project ID:** | **169411** |

The Proportionate Review Sub-committee of the Wales REC 7 reviewed the above application on 08 April 2015.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact detail's. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Ms Sue Byng. . Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

**Ethical opinion**

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

GIG NHS | Bwrdd Iechyd Addysgu Powys | Powys Teaching Health Board

Cynhelir Cydweithrediad Gwyddor Iechyd Academaidd y Sefydliad Cenedlaethol ar gyfer Ymchwil Gofal Cymdeithasol ac Iechyd gan Fwrdd Addysgu Iechyd Powys

The National Institute for Social Care and Health Research Academic Health Science Collaboration is hosted by Powys Teaching Health Board

Ariennir gan Lywodraeth Cymru Funded by Welsh Government

1) The Participant Information Sheet should be amended to make the term 'nutritional abnormalities' more user friendly.
2) The researcher should not approach non-responders in clinic but could make one follow-up telephone call to potential participants who had not returned the consent form within a designated period of time and before they attended clinic. This should be outlined in the Research Protocol under the recruitment section.

**You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.**

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

*Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.*

*Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.*

*Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.*

*For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.*

*Sponsors are not required to notify the Committee of approvals from host organisations.*

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

**It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**

**Ethical review of research sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

**Approved documents**

The documents reviewed and approved were:

| Document | Version | Date |
|---|---|---|
| Evidence of Sponsor insurance or indemnity | | 01 August 2014 |
| IRAS Checklist XML | | 24 March 2015 |
| Letter from sponsor | | 12 March 2015 |
| Letters of invitation to participant | 6 | 24 February 2015 |
| Other | 1 | 16 March 2015 |
| Participant consent form | 4 | 24 February 2015 |
| Participant information sheet | 4 | 24 February 2015 |
| REC Application Form | 1 | 24 March 2015 |
| Referee's report or other scientific critique report (Paul Spark) | | |
| Research protocol or project proposal | 6 | 24 February 2015 |
| Summary CV for Chief Investigator [CV Sean Dodington] | | 11 March 2015 |
| Summary CV for supervisor [CV Rebecca Price-Davies] | | 23 May 2014 |
| Summary CV for supervisor [CV Allan Cosslett] | | 15 March 2015 |

**Membership of the Proportionate Review Sub-Committee**

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

**User Feedback**

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

**HRA Training**

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

| **15/WA/0128** | **Please quote this number on all correspondence** |
|---|---|

Yours sincerely

Sue Byng

pp

**Dr John Buchan**
**Vice-Chair**

Email: sue.byng@wales.nhs.uk

*Enclosures:* *List of names and professions of members who took part in the review*

*"After ethical review – guidance for researchers"*

*Copy to:* *Miss Helen Falconer*
*Prof Chris Fegan, Director for Research Development*
*Dr Rebecca Price-Davies*
*Dr Allan Cosslett*

**APPENDIX II – STUDY PROTOCOL**


# Study Protocol

Version 8 – 17/04/2015

# Evaluation and Assessment of Nutritional Status in Long-term Home Parenteral Nutrition (HPN) Patients

| | |
|---|---|
| Sponsor: | Cardiff University |
| Co-ordinating Centre: | Cardiff School of Pharmacy and Pharmaceutical Sciences |
| Supervisors: | Dr Allan Cosslett<br>Dr Rebecca Price-Davies |
| Principle Investigator: | Susanna Harwood |
| PhD Student: | Sean Rhys Dodington |

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## CLINICAL RELEVANCE STATEMENT

Intestinal failure and other various disease states necessitate the need for patients to be maintained on parenteral nutrition (PN). When patients have received this therapy over long periods of time there have been incidences of nutritional abnormalities(1–3). This study aims to investigate how nutritional deficiencies come to occur in patients who are maintained on long-term home parenteral nutrition (HPN). By undertaking this research, we hope to increase our knowledge of what factors contribute to the incidence of nutritional abnormalities; and in doing so, recommend improvements to the provision of HPN services in Wales.

## INTRODUCTION AND BACKGROUND

Parenteral nutrition (PN) is a mode of therapy established for patients who are intolerant of, or those who cannot receive adequate nutrition via the oral or enteral route. Over the last 40 years, it has allowed patients to lead as normal a life as possible, free to continue with their day-to-day activities. It is an admixture which can have in excess of 60 individual chemical entities that must be chemically, physically and microbiologically robust in order to be safely administered to the patient (4). HPN refers to PN given as nutritional therapy to patients in their domiciliary environment.

"Total" PN aims to provide all the nutritional needs of the patient without any significant enteral intake. PN can also be given supplementary to nutrition consumed via the GI tract in those patients still capable of enteral feeding (5). The basic formula for PN is as follows:

- Lipid: as fatty acid emulsions
- Carbohydrate: in the form of glucose solution
- Protein: solutions provide a mix of essential and non-essential amino acids
- Water
- Electrolytes
- Vitamins: available as multi-vitamin preparations
- Trace elements (TE)

Lipid, glucose and amino acids comprise the main components of PN. Vitamins fall into two broad categories, either fat-soluble or water-soluble vitamins (6). TE are inorganic elements included as integral parts of metabolically active organic complexes (e.g. enzymes). The PN formulation should be adapted to suit the individual needs of the patient.

PN is used in the treatment of patients with long-term (LT) chronic conditions. For the purposes of this report, the abbreviation 'LT PN' is used to refer to patients who have received PN for a period of at least 6 months.

NICE have clearly defined candidates for PN as being those who are malnourished or at risk of malnutrition as well as being either unsafe for oral nutrition or having functional problems associated with the GI tract (7). The most common reason for commencing HPN is short bowel syndrome (SBS) and other indications include Crohn's disease, fistulae, small bowel ischaemia and pseudo-obstruction, alongside complex surgical interventions which precipitate the need for HPN. Short bowel syndrome (SBS) is a state of malabsorption following intestinal resection where there is less than 200 cm of intestinal length remaining (8). Cancer patients who experience severe malnutrition and weight loss are also candidates for PN to improve their nutritional status before surgery (9). The chronic nature of all these conditions mean that people often require PN for long periods of time, which makes home administration very beneficial.

PN is also needed for a shorter duration of time by patients in hospital who have more self-limiting conditions or are at risk of malnutrition as it has long been acknowledged that maintaining adequate nutrition is associated with better clinical outcomes (7,10). A recent national enquiry into perioperative deaths identified that 93% of patients receiving PN in hospital required PN therapy for less than 30 days (11). This mainly resulted from post-surgical complications requiring the need for nutritional support.

Complete PN was first realised by Arvid Wretlind in the early 1960's after he developed modern amino acid solutions and human compatible lipid emulsions (12). And over the last 40-50 years, developments within this field have helped in saving many lives of those who would have previously been lost to an untimely death. Berger concluded that there still appears to be a lot to learn about PN, particularly in accurately defining patients' energy, protein and micronutrient requirements; and in monitoring these effectively over time (12).

There has been a steady increase in the number of people receiving HPN in recent years. The most recent British Artificial Nutrition Survey (BANS) report, which details issues surrounding nutritional care in the UK, detailed an increase in new patient registrations from 148 to 228 over the period 2009-2010. After overcoming challenges in reporting rates across HPN centres in the UK, the most up-to-date reported point prevalence for patients receiving HPN in 2011 rested at 8.40 per million (13). This is the most recent statistic detailing the amount of patients maintained on LT HPN and there is no specific reference as to how many of these are Welsh patients. It is clear to see that the provision of HPN is a deep-seated feature of current healthcare and nutrition practice.

One of the more recent endeavours of the NHS has been to treat patients at home to help to reduce the burden placed on hospitals, reduce associated costs and improve clinical outcomes (14). It has become good practice to involve commercial homecare companies in this service (15). These companies provided HPN services for all new patient registrations in 2010 and they now supply up to 94% of the patients maintained on LT HPN, which has risen from 70.6% over a ten year period (2000-2010) (13). This demonstrates the dependency of HPN patients on these services. Howard explained that although providing PN in a home setting has proven expensive, it appears to cut the total management costs by around a half, in comparison to hospital-based management (16).

In an ideal world, the goal would be to come off PN and tolerate enteral nutrition. We should remember that PN is an artificial source of nutrition aiming to nutritionally mimic a healthy, well-balanced oral diet. Unfortunately its use carries certain risks and complications; and thus, abnormalities of nutritional balance are not the only complication of LT PN provision. Patients are susceptible to other serious complications, these include: line infections, sepsis, central line occlusion, risk of pulmonary embolism, metabolic complications, cholestatic liver disease, fatty liver and exacerbation of inflammation (12,17).

## RESEARCH QUESTION

"What is known about the effect of the long-term provision of parenteral nutrition on a patient's nutritional status?"

## STUDY AIM

- To evaluate the extent of nutritional deficiency and accumulation in patients receiving long-term HPN in Wales.

## OBJECTIVES

- To review medical notes and tests from HPN patients in Wales to ascertain reason for PN use, blood nutrient levels and specific details of PN formulas administered.
- To identify which nutrient levels are most commonly out of range (deficient/in excess), as indicated by the standard reference ranges implemented at the University Hospital of Wales (UHW).
- To compare the levels of individual nutrients administered to patients to the recorded blood levels.
- To consider whether deficiencies/excess may be due to interactions or instability occurring in the HPN admixture during compounding or storage.
- To evaluate the clinical implications of any instability or incompatibilities discovered and make recommendations to improve HPN received by patients.

## PROJECT RATIONALE

Patients receiving long-term PN are at risk of developing nutritional deficiencies and clinicians involved in the care of these patients need to be aware of these risks and how to manage them. In 1998, it was stated that there was a lack of investigation into how these deficiencies may occur or into the clinical significance of the extent of the deficiencies (18). Much of the research that has been performed over subsequent years has been difficult to place in clinical context. It is generally accepted that the longer a patient receives a set PN formula, the higher the risk that the formula will not match the nutritional needs of the patient (19,20). This is the first project aiming to analyse clinically documented data collected in a large cohort of patients receiving LT PN (subject to finalised inclusion criteria). There is also the opportunity for the inclusion of data from more HPN centres, subject to initial data handling at UHW.

In earlier years, much of the knowledge gleaned regarding nutritional deficiencies came from the inadvertent omission of vitamins or TE. This, along with guidance on oral intake helped to shape recommendations for parenteral guidelines. However, metabolism of IV nutrients differs to oral administration, bypassing the enterohepatic circulation which can be a large factor in the metabolism and distribution of nutrients. This helps to justify the need for precise dosing when giving PN (2).

As part of their care, patients receiving long-term PN have regular monitoring, this includes anthropometry (body size measurements), biochemical measurements and micronutrient testing every 6 months (21,22). Over time, the results from these tests have revealed that some patients are acquiring nutrient abnormalities. These have been most notably demonstrated for the vitamin, TE and electrolyte components of PN. The cause of these nutritional abnormalities is unclear and difficult to comprehend since HPN is tailored to the individual needs of the patient, thus constituting an area that requires further research. Most reports of deficiencies in patients on HPN relate to the omission of a particular nutrient from the admixture, which in turn results in that particular deficiency. In previous years it has even been known for shortages of injectable multivitamin preparations to be responsible for nutritional abnormalities from the rationing of supplies of vitamins in IV preparations (23–25).

There would appear to be a few possible notions that may help to explain how nutritional abnormalities come to occur. It is even possible that interplay of some or all of the following explanations may be responsible for their development:

1. Instability or compatibility issues arising during compounding or storage may be responsible for abnormal nutrient doses being delivered to patients (6).
2. Contamination of materials used during the manufacture of the PN admixture (e.g. aluminium) could increase the content of certain components (26).
3. Interactions arising during the administration of the PN admixture to the patient (e.g. during line administration, flushing).
4. Errors or mistakes associated with the PN composition and regime could be responsible for nutritional abnormalities e.g. nutrient doses not altered correctly, or inadvertent omission of changes to PN composition.
5. Long-term administration of set PN regimes (of the same composition) may result in patient's nutrient levels gradually going out of range. For instance, even doses that are only slightly high or low could result in nutrient levels going out of range when given over a long period of time (20).
6. Patient monitoring (tests/clinic visits) not occurring as frequently as clinically necessary. Individual patient nutritional requirements may change over time and the PN formulation may gradually become less appropriate for the patient. The PN is not changed in time with the patients nutritional needs (27).
7. Patient-specific factors may influence the distribution and utilisation of the PN components within the body (22,27,28).

By using patient data that is accurately known in detail (e.g. medical history/notes, biochemical blood tests and PN prescription/formulation), an attempt can be made to find out reasons for how the nutritional abnormalities come to occur.

Data from Hope Hospital in Salford has indicated that two-thirds of all readmissions are for complications of HPN rather than the underlying condition or surgery. This led to a backlog effect with resultant delays on waiting lists for treatment of intestinal failure and subsequent increases in mortality rates for patients on the waiting lists; demonstrating both the unmet

needs for patients on LT PN and also the heavy burden that complications of HPN places on secondary care services (15).

## STUDY PLAN AND PROPOSED METHODS

Firstly, approval will be sought from NHS Research Ethics Committees and the relevant Research and Development Departments.

An honorary contract has already been obtained with University Hospital of Wales (UHW) that currently allows attendance of the researcher (Sean Dodington) at HPN clinics. Once the study receives ethical approval, the researcher will then have approved access to all data from the patients registered with UHW. UHW manages HPN for the majority of patients in Wales. There are two other minor HPN centres in Swansea and Wrexham. Depending on the initial data findings from UHW, there is the potential scope for inclusion of more HPN centres in the UK to increase data findings and validity.

### Study population

#### *Sampling*

The sample population of patients will be all the adult patients listed on the HPN outpatient clinic list at UHW. They will be recruited by a postal invitation and one follow-up telephone call three weeks later.

#### *Inclusion criteria*

Patients will be included if they satisfy the following inclusion criteria:

- Attending HPN clinic and have been receiving PN for at least six months
- Able to understand and read English
- Able to give written informed consent
- Are of adult age (at least 18 years of age)

#### *Exclusion criteria*

Likewise, patients will be excluded according to the following exclusion criteria:

- Children (under the age of 18)
- Patients with present cancer/malignancies
- Patients unable to understand and read English
- Patients unable to give consent

By ensuring that the patients who meet the inclusion criteria have attended the clinic for at least six months, it guarantees that there will be a minimum of six months worth of hospital

data and information for inclusion in data analysis. Also, in similar studies assessing the effect of long-term PN on patients' health, six months has been used as an acceptable limit for the inclusion of patients in the study and to represent LT use of PN (29,30). Likewise, in the ESPEN guidelines on HPN in adult patients, 'long-term PN' is categorised as referring to patients who have been receiving PN in excess of at least six months (22).

By comparison to adults, children can have more complex and variable PN requirements as they develop. This would complicate data analysis if this patient group were to be included in the study population. Also, the reasoning behind the cut-off point of participants to be at least 18 years of age is that it helps to decide an appropriate point where for research purposes the participants are assumed to have adult physiological body systems; in that they respond to the nutrients provided in their PN in a similar way. In this way, any conflicting factors associated with childhood or adolescence will be avoided.

Although patients who have cancer can receive PN therapy, it is not considered routine practice to support this patient group with artificial nutrition in the UK (by comparison to Europe) and there may be differing nutritional practices between hospitals in their approach to treating this patient group. Thus, patients who have present cancer or malignancies will be excluded from the study. Also, the unknown effect of cancer on a patient's nutritional status justifies exclusion of this patient group because the cancer or its treatment may result in more widely deranged blood tests, or increased electrolyte abnormalities. For patients who develop cancer during the course of the study, their data will not be used from the point at which their cancer diagnosis was made. Patients who have had cancer in the past but do not have active cancer in present time will still be included but the data from the period of time with active cancer will not be included. Exclusions on this basis will be made by screening the participants' medical history and notes.

***Patient recruitment and consent***

All adult patients who routinely attend the HPN outpatient clinic at UHW will be sent a postal invitation to participate in the study. It will contain an introductory letter of invitation, a participant information sheet, two consent forms (one to keep, one to return) and a pre-paid return envelope. The postal invitation will be sent by Susanna Harwood, a specialist PN pharmacist independent prescriber at UHW and member of the clinical team, it will introduce the study and the researcher (Sean Dodington).

The postal invitation will contain a letter to invite the patient to participate in the study, it will direct them to read the participant information sheet and make an informed decision about whether to participate. Should the patient decide to participate, they will need to sign the consent form and send it on to the researcher (Sean Dodington) in the enclosed envelope. If they agree to participate, they thereby permit the use of their hospital medical notes and test results to be used for the purposes of the study

One follow-up telephone call will be made to potential participants who have not responded within three weeks. This is in case those who wish to participate but had forgotten to return the consent form can be given a reminder. Three weeks was chosen as a time period before the follow-up telephone call because it allows reasonable time for receipt of the postal invitation by the potential participant and also plentiful time to their consideration to take part in the study. This follow-up telephone call will be made before the potential participants are due in clinic.

The population of patients who consent to participate will then be assessed to see if they meet the inclusion criteria. It is important to note that no patient identifiable information will be transferred for use in the study. This will be done in cooperation with the consultant practitioners and medical professionals responsible for the patients care.

The researcher (Sean Dodington) is already familiar with issues surrounding confidentiality and the use of patients' medical data for research purposes. He also practices as a pharmacist and deals with patients' personal medical records within his everyday duties as a healthcare professional. This will assure confidence to the participants of his ability to safeguard their personal information.

### Data collection

After consent has been obtained from the patients willing to participate, those who meet the inclusion criteria will be identified as being eligible for the study. Only data from the eligible patients will be used in the study.

The project aims to use patient data for research. This data includes all data relating to patients PN prescription formulas (past and current), biochemical and blood test data. This information and data are stored on the UHW hospital computer system in an electronic format and approval (ethics + R&D) is required to access and use this data. Other relevant

data including past medical history and drug history will also be reviewed. These comprise a secondary data source as the data has already been collected by other healthcare professionals involved in the care of these patients; not the researcher. The databases from which the data will be extracted offer a large amount of data for analysis and appear abundant for possible research findings.

The data will be collected from the networked computer system at St Mary's Pharmaceutical Unit. It will be anonymised and coded, then put in to an Excel spreadsheet to collate the data and permit analysis at Cardiff School of Pharmacy and Pharmaceutical Sciences. The anonymised data will be transferred between sites on a USB device in which the files are password protected. This will be performed by the lead researcher (Sean Dodington) under the conditions of his honorary contract and will be undertaken in a retrospective manner with limits set for the period of data analysis. Once the researcher has approval for access to the data, he will be able to see the quantity, extent and context of the data. This will allow him to set specific and practical time limits for the inclusion of data.

### Sample size

The sample size of the population of patients receiving HPN needs to be large enough to detect a meaningful, statistical clinical difference in the data.

- Null hypothesis ($H_0$): Patients on long-term HPN are experiencing nutritional abnormalities
- Alternative hypothesis ($H_A$): Patients on long-term HPN are not experiencing nutritional abnormalities

It is estimated that there are around 800 patients currently maintained on HPN in the UK (13). The sample size calculation below uses this statistic based on UK population as a whole. In terms of demographics, the characteristics of the Welsh population is broadly similar to that of the UK population.

Assuming the following:

- Confidence level: 95%
- Confidence Interval: 10
- Population size: 800

A minimum sample size of 86 will be necessary to be able to support statistical findings.

There are approximately 100 patients maintained on HPN in Wales and the mainstay of these patients are managed at UHW. Assuming that a good proportion of these patients meet the inclusion criteria and that more patients can be recruited from other HPN centres in Wales if necessary (Swansea, Wrexham); sufficient patients will be able to be recruited for the results from the research to accurately represent the population of HPN patients as a whole. It should be emphasised that there will be ample data available per patient included in the study, consequently there will be great research prospects.

### Data analysis

The following statistical methods will be applied to the data:

- Data will be organised according to disease types to allow consideration for the effect disease has on a patient's nutrient status.
- Data will be analysed using the software package, SPSS® (IBM). One-way ANOVA tests and student t-tests will be used to analyse differences between disease groups and to compare results with reference standards.
- Deficient nutrients will be identified in the patient groups by seeing which nutrients are out of range by comparison to reference standards. These will then be cross-referenced against the doses of nutrients in the patients PN regime.

The researcher has recently completed a course on 'Research, Statistics and Evidence Based Practice' with Cardiff University.

## RECORDING OF DATA AND RETENTION OF DOCUMENTS

All documents containing data relating to the non-identifiable participants will be stored in a locked and secure location within Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University. This location requires personal key-card access from the researchers. All electronic data will be password protected and only the investigators directly involved in the research will have access to it (Sean Dodington, Rebecca Price-Davies, Allan Cosslett). All computers will be locked when unattended, requiring a password for access. This includes the data spreadsheets containing patient medical notes/history, biochemical blood test results and PN prescriptions/formulations. The non-identifiable patient data will be stored on the university server for a minimum of 15 years.

## ETHICAL AND LEGAL CONSIDERATIONS

Before the study commences, approval will be requested from

- Relevant NHS Research Ethics Committees
- Relevant Research and Development (R&D) Departments at hospital sites participating in the study

All patients maintained on LT PN in Wales are managed at UHW. Yet some of these patients may have aspects of their conditions cared for outside of Cardiff (e.g. Swansea and Wrexham). For this reason, approval may need to be sought from the R&D departments at these sites as well and not just UHW. This will allow approved access to all the data from each of the hospital sites, should it be required (e.g. medical notes). The extent of the patients who are managed in this way will not be known until the researcher has access to the data.

Depending on the initial data findings, there may be scope for inclusion of other HPN centres into the study from the UK to increase the validity of data findings.

### Consent

Consent will be obtained from each patient via the postal invitation as detailed above.

The consent will be:

- Written: obtained from standard consent forms requiring signatures
- Informed: taken after reading an information sheet, the participants will have had

sufficient time to understand the information and ask the investigator any questions.

The letter will be sent by Susanna Harwood, a PN pharmacist routinely involved in the care of the patients through the provision of pharmacy services involved with the HPN clinics. The participant information sheet (PIS) included in the postal invitation will give the patient the opportunity to read more about the study and help them make a decision about whether to participate.

In the PIS, patients will be informed that:

- Their decision to take part in the study will not affect their standard of care in any way and that they can choose to withdraw at any time.
- The results of the study will be used to improve the provision of HPN services, in particular, to reduce the chances of nutritional deficiencies occurring in patients maintained on this therapy long-term.
- Any of their information used in the research will be kept confidential and the only researchers directly involved will have access to this data.
- The PIS contains the relevant contact details for the researchers should the participants have any questions or complaints regarding the study.

The participant will have a copy of the consent form to sign and retain for their records, and an additional copy to send on to the researcher. By sending the signed consent form to the researcher, the patient is agreeing to permit the use of their medical information for research purposes.

### Confidentiality

Patient confidentiality will be maintained throughout the study. Participants are assured in the PIS that any information related to them will be handled in complete confidence. They are also informed in the PIS that their information will be kept anonymous, that no patient-identifying information will be used (e.g. name and address). Each patient will be assigned a code number at the point of recruitment into the study. Only the study investigators will have access to the study data, which will be kept in a locked and secure location at Cardiff School of Pharmacy and Pharmaceutical Sciences. All electronic data will be password protected.

### Welsh Language Speakers

If when the participants receive the postal invitation, should they decide that they would like the information provided in Welsh; they will be directed to contact the research team via the contact details in the PIS so that this can be arranged.

### Complaints Procedure

The likelihood of breaches of data confidentiality occurring is small. The recruitment process allows for the data to only be accessed and used in the research process with the consent and permission of the participants.

The anonymisation and coding stage will happen at St Mary's Pharmaceutical Unit, this is the site where the HPN is routinely managed for these patients. The risk of data breaches occurring here is minimal as staff have permission to access the patients' data for the purpose of their work and are conscious of the issues surrounding data security.

In the unlikely event that a complaint should be made from a patient regarding a breach of data confidentiality, it is assumed that the complaint would be identified by the patient. In the participant information sheet it directs the patient to contact an independent member of staff at Cardiff School of Pharmacy and Pharmaceutical Sciences, Prof. Mark Gumbleton (contact details included in the PIS). Potential complaints would be handled in a serious and professional manner and the rights of the patient would be respected.

## FUNDING

The study is funded by Cardiff University and the researcher is based at Cardiff School of Pharmacy and Pharmaceutical Sciences.

## PUBLICATION OF RESULTS

At the end of the study, the results may be submitted for publication and also for presentation at research conferences. A thesis will be written based on this research project for submission for a PhD.

## RESEARCH STAFF

- *Dr Allan Cosslett*: Lecturer, Disability Officer, Admission Officer, Senior Tutor and Manager of the Fresenius Kabi Stability Assessment Unit at Cardiff University
- *Dr Rebecca Price-Davies*: Lecturer in Pharmaceutics and Research Pharmacist
- *Susanna Harwood*: Specialist PN Pharmacist/Principle Investigator
- *Sean Rhys Dodington*: PhD Student and Pharmacist

## GLOSSARY

ANOVA – Analysis of Variance

BANS – British Artificial Nutrition Survey

HPN – Home parenteral nutrition

IF – Intestinal failure

LT – Long-term

NHS – National Health Service

NICE – National Institute for Clinical Excellence

PIS – Patient Information Sheet

PN - Parenteral nutrition

R&D – Research and Development

SBS – Short bowel syndrome

TE – Trace elements

UHW – University Hospital of Wales

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## APPENDIX III – CONFIRMATION OF R&D APPROVAL

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Caerdydd a'r Fro
Cardiff and Vale University Health Board

Ysbyty Athrofaol Cymru
University Hospital of Wales

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Tel: 029 20746986
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CAV_Research.Development@wales.nhs.uk

From: Professor C Fegan
R&D Director
R&D Office, 2nd Floor TB2
University Hospital of Wales
Cardiff
CF14 4XW

09 July 2015

Susannah Harwood
St Mary's Pharmaceutical Unit
20 Field Way
Cardiff
CF14 4HY

Dear Ms Harwood

**Cardiff and Vale UHB Ref and Study Title : 15/CMC/6168 : Evaluation And Assessment Of Nutritional Status In Home Parenteral Nutrition (HPN) Patients**

**IRAS Project ID: 169411**

The above project was forwarded to Cardiff and Vale University Health Board R&D Office by the NISCHR Permissions Coordinating Unit A Governance Review has now been completed on the project.

Documents approved for use in this study are:

| Document | Version | Date |
|---|---|---|
| Revised R&D Form | 4 | Received 06/07/2015 |
| Revised SSI Form | 4 | Received 06/07/2015 |
| Participant Consent Form | 5 | 17/04/2015 |
| Invitation Letter | 6 | 24/02/2015 |
| Participant Information Sheet | 7 | 15/06/2015 |
| Protocol | 8 | 17/04/2015 |

I am pleased to inform you that the UHB has no objection to your proposal and that this study has been classed as pathway-to-portfolio. You have informed us that Cardiff University is willing to act as Sponsor under the Research Governance Framework for Health and Social Care.

Please accept this letter as confirmation of permission for the project to begin within this UHB.

May I take this opportunity to wish you success with the project and remind you that as Chief / Principal Investigator you are required to:

- Inform the R&D Office if this project has not opened within 12 months of the date of this letter. Failure to do so may invalidate R&D approval.
- Inform NISCHR PCU and the UHB R&D Office if any external or additional funding is awarded for this project in the future
- Submit any substantial amendments relating to the study to NISCHR PCU in order that they can be reviewed and approved prior to implementation
- Ensure NISCHR PCU is notified of the study's closure
- Ensure that the study is conducted in accordance with all relevant policies, procedures and legislation
- Provide information on the project to the UHB R&D Office as requested from time to time, to include participant recruitment figures

Yours sincerely,

**Professor Christopher Fegan**
**R&D Director / Chair of the Cardiff and Vale Research Review Service (CaRRS)**

**CC** **R&D Lead: Dr Sarah Hiom**
Sean Doddington
Anthony Williams, Finance
Clinical Board, Assistant Head of Finance: Rebeka Warren
Sponsor Contact: Chris Shaw, Research Innovation Enterprise Service
Academic Supervisor: Dr Rebecca Price-Davies, School of Pharmacy and Pharmaceutical Sciences
Academic Supervisor: Dr Allan Cosslett, School of Pharmacy and Pharmaceutical Sciences

## APPENDIX IV – INTRODUCTORY LETTER OF INVITATION



Susanna Harwood
Specialist PN Pharmacist
St Mary's Pharmaceutical Unit
20 Field Way
Cardiff CF14 4HY
Tel: 02920 746393
Email: Susanna.Harwood@wales.nhs.uk

Date:

**Invitation to Study**

***Study Title: Assessment and Evaluation of Nutritional Status in Long-term Home Parenteral Nutrition (HPN) Patients***

Dear patient

I would like to invite you to participate in a research study I am undertaking in conjunction with Cardiff University. The study is being carried out by a research colleague from Cardiff University, Sean Dodington. You may have already met him whilst attending the clinic in recent months.

The study will be investigating the nutritional status of patients who are on parenteral nutrition over long periods of time. This research hopes to help us improve the services we provide.

I have enclosed a participant information sheet to give you more information about the study. Please read this information sheet and make a decision about whether you wish to participate in the study. If you decide to partake in the study, please could you sign the enclosed consent form and return it to Sean in the prepaid envelope.

The information sheet also gives the contact details for the researchers involved in the study should you want any further information or have any questions. Also, should you require the information contained in this package in Welsh, please contact the research team so that this can be arranged for you.

I would be very grateful if you could take time to consider your involvement in this study. The findings of this research could prove beneficial for patients on long-term parenteral nutrition in the future.

Yours sincerely

Susanna Harwood

Version 6 24/06/2015

## APPENDIX V – PARTICIPANT INFORMATION SHEET



# Participant Information Sheet

**Study Title: Evaluation and Assessment of Nutritional Status in Long-term Home Parenteral Nutrition (HPN) Patients**

*Your invitation to participate*

You are being invited to take part in a research study. Before you decide whether to participate it is important for you to understand why the research is being carried out, and what participating will involve.

We are carrying out a study to investigate the effects of long-term parenteral nutrition (PN) on patients. We think that the levels of some nutrients in the body might be affected when people are on PN for a long time. We would like to look at notes from people who receive PN to see if there are changes in their levels of nutrients over time and to try to work out why it happens.

This research study is being carried out as part of a PhD project.

Please take time to read the following information carefully and decide if you would like to take part.

If you have any questions about the study or if there is anything you find unclear, please do not hesitate to contact the research team (contact details at end of this document).

***Please contact us if you require this information in Welsh***

Thank you in advance for taking time to read this information sheet.

This sheet has been separated into two sections:

- SECTION 1 - Briefly explains the purpose of the study and explains what will happen if you decide to take part
- SECTION 2 - Gives further information about how we undertake the study

Version 7 15/06/2015

SECTION 1

### *What is the purpose of the study?*

Your PN bags are designed especially for you. When you go to the HPN clinic, your weight and blood biochemistry is checked, which helps the team decide what needs to go into your bag. Sometimes the amounts of ingredients need to be changed as your weight and biochemistry changes. Although it is normal for changes to happen, we think that the levels of some nutrients in the body might be more affected when people are on PN for a long time. This study will use information from people's notes to try and find out how some of these changes happen.

### *Why have I been invited?*

You have been invited because you are an adult patient who has been receiving home parenteral nutrition for at least six months and the hospital you attend is included in this study.

### *Do I have to take part?*

Taking part is voluntary and it is up to you to decide whether to participate or not. We have given you this information sheet to read and once you have considered your options, you can make a decision regarding your participation in the study. If you decide to participate there is a consent form to sign (enclosed). It is important to note that you can decide to withdraw from the study at any time, even if you have signed a consent form. If you decide not to take part in the study, you do not have to give a reason. Please be reassured that your decision will not affect the standard of care you receive.

### *What will happen if I decide to take part in the study?*

If you choose to take part in the study, this will allow the researcher (Sean Dodington) to have access to the information from your visits to the clinic. This includes your medical notes, biochemical blood tests and parenteral nutrition prescriptions. You will not need to do anything else other than give permission for the researcher to use your hospital notes and test results for research purposes. Please be assured that any personal information that allows identification of you as an individual will be not be used (e.g. name, address).

### *What will I have to do?*

We need you to give written consent to allow the researcher to use your information. For this we need you to sign a consent form (enclosed).

### *How long will I have to decide whether or not to participate in the study?*

There is no set time limit for you to make a decision regarding participation in the study. However, we would be grateful if you could reply to us within two weeks.

### *What are the possible risks/disadvantages associated with participating?*

There are no risks associated with participating. The study only requires giving consent to allow the researcher to use medical information from your routine clinic visits.

### *What are the possible benefits/advantages associated with participating?*

We cannot promise the study will help you personally. However the information gathered from the study will help in finding out more about why changes in blood biochemistry occur in patients on long-term home PN. The findings of this research will help to improve the quality of service to patients receiving long-term PN and shape recommendations for the future.

### *Will information I provide be kept confidential?*

Any information related to you will be handled in complete confidence as we follow ethical and legal practice, further details of which are outlined in Section 2.

***This completes Section 1. If the information you have read so far interests you and you are considering participating in the study, please read the additional information in Section 2 before making any decisions.***

## SECTION 2

### *What will happen if I decide to withdraw part way through the study?*

If you decide to withdraw from the study, we will not continue to use the information obtained from your medical notes, blood tests or prescriptions. All the information used up to the point you withdraw will then be removed.

### *What if I have questions or if there is a problem?*

If at any point you have concerns regarding the study, please feel free to contact the research team via the contact details at end of sheet, who will make every effort to address the question or problem.

If your query remains unresolved and you wish to complain formally, you can do so by contacting Professor Mark Gumbleton, who is independent of the research team, at Cardiff University.

- Email: Gumbleton@cardiff.ac.uk
- Tel: 02920 875449

### *Confidentiality*

Each patient will be assigned a unique number, which will then be used to replace any identifiable information (e.g. name) to ensure the information used during the research is kept anonymous. All study data will be kept in a secure locked place within the Cardiff School of Pharmacy and Pharmaceutical Sciences at Cardiff University. All those involved in the research will have a duty of confidentiality to you as a research participant and only key researchers will have access to the data. The data collected from the study will not reveal any information that can lead to your identification and will be stored for 3 years before it's secure disposal.

### *What will happen to the results of the research?*

The results of the study may be published in a scientific journal or presented at a conference, in addition to being used to form part of a Ph.D thesis. The information presented will be an overview of many people and so will not show any identifiable information about individual study participants. If you would like a copy of any publications once the study finishes, you can contact the researchers at the address shown at the end of this information sheet and they will forward on a copy for you.

### *Who is organising and funding this study?*

The School of Pharmacy and Pharmaceutical Sciences at Cardiff University is conducting this study in collaboration with your hospital and the multidisciplinary practitioners responsible for your care. The project is being funded by Cardiff University.

### *Who has reviewed the study?*

The protocol of this study has been independently peer reviewed and Cardiff University is the official sponsor of the study. All research within the NHS is looked at by an independent body called a Research Ethics Committee for the safety, rights, wellbeing and dignity of study participants. This study has obtained a favourable opinion from the "Wales REC 7".

### *Contact details:*

Should you have any further questions about the study, please do not hesitate to contact one of the investigators.

The contact details of the study investigators are as follows:


1. Dr Rebecca Price-Davies
   Chief Investigator — Tel: 02920 874952
   Email: PriceR@cardiff.ac.uk
2. Susanna Harwood
   Principle Investigator — Tel: 02920 748109
   Email: Susanna.Harwood@wales.nhs.uk
3. Sean Rhys Dodington
   Student Investigator — Tel: 02920 874987
   Email: DodingtonSR1@cardiff.ac.uk

Correspondence address:

Cardiff School of Pharmacy and Pharmaceutical Sciences
Redwood Building
King Edward VII Avenue
Cardiff University
CF10 3NB


***Thank you for taking the time to read this information sheet. We are grateful for your contribution to this study if you choose to participate.***


Version 7 15/06/2015

## APPENDIX VI – PARTICIPANT CONSENT FORM

### Patient Consent Form

*Version 5 - Dated 17/04/2015*

**Full Study Title: Evaluation and Assessment of Nutritional Status in Long-term Home Parenteral Nutrition (HPN) Patients**

***Please put your initials in the box next to each statement***

| | |
|---|---|
| I confirm that I have read the participant information sheet (Version 7 - dated 15/06/2015) and understand the intent of the study. | |
| I confirm that I have considered the information provided. If I have had any questions, these have been answered satisfactorily by the research team (see contact details in participant information sheet) | |
| I understand that relevant sections of my medical notes, test results and parenteral nutrition details may be looked at by individuals from Cardiff University or from the University Local Health Board; where it is relevant to my taking part in this research. I give permission for these individuals to have access to my confidential records and information. | |
| I understand that some of my medical information may be copied into another format for data research purposes and that it will be non-identifiable. This includes medical notes, test results and parenteral nutrition prescriptions. | |
| I understand that my participation in the study is voluntary and I have the right to withdraw at any point without justification. I also understand that any anonymised data already used in the study will be removed. | |
| I understand that the results and findings from this study will be published and included in part for submission of a Ph.D. | |
| I hereby give written consent to participate in the above study. | |

**Participant Name:** .....
**Date:** .....
**Signature:** .....

***Please sign both copies of this form. One to keep for yourself and one to return in the envelope provided***

## APPENDIX VII – CONFIRMATION OF SERVICE EVALUATION APPROVAL

Cardiff and Vale University Health Board

**12.0 Statement by Project leader**

I agree to carry out the project as set out in this plan

I confirm that I have read the UHB Data Protection guidance issued by the UHB and agree to ensure that all data for this project will be collected, collated and stored in accordance with the principles outlined in this guidance.

I agree to ensure that a copy of the findings and recommendations are submitted to the Assistant Director of Innovation and Improvement upon project completion.

Signature

Name (PRINT) Paul Spark

Date 13/10/16

**13.0 Statement of Support**

**I, Darrell Baker, Service Director for Pharmacy and Medicines Management support this application.**

Signature

Name (PRINT) D BAKER

Date 17/10/16

**I..MATT TEMBY.....,Clinical Board DirectorDirector/Head of Operations/ supervisor for .....support this application.**

Signature

Name (PRINT) MATT TEMBY

Date 18/10/16

## APPENDIX VIII – PREPARATION FORMULATIONS

### 1. Cernevit®

The active ingredients include: retinol (as palmitate) 3500 IU, cholecalciferol 5.5mcg (220IU), dl-alpha-tocopherol 10.2mg 11.2IU, ascorbic acid 125mg, co-carboxylase tetrahydrate 5.8mg (thiamine 3.51mg), riboflavin dehydrated sodium phosphate 5.67mg (riboflavin 4.14mg), pyridoxine hydrochloride 5.5mg (pyridoxine 4.53mg), cyanocobalamin 0.006mg, folic acid 0.414mg, dexpanthenol 16.15mg (pantothenic acid 17.25mg), d-biotin 0.069mg and nicotinamide 46mg. The other inactive ingredients are glycine 250mg, glycocholic acid 140mg, soybean phosphatides 112.5mg, sodium hydroxide and hydrochloric acid q.s.

### 2. Vitlipid N Adult®

The active ingredients are retinol (as palmitate) 990 mcg, ergocalciferol 5mcg (200IU), dl-alpha-tocopherol 9.1mg and phytomenadione (vitamin $K_1$) 150mcg. The inactive ingredients are fractionated soybean oil 1g, fractionated egg phospholipids 120mg, glycerol 225mg, sodium hydroxide q.s. and WFI to 10mL.

### 3. Additrace®

Each 1mL of Additrace® contains the active ingredients: ferric chloride 540mcg, zinc chloride 1.36mg, manganese chloride 99mcg, copper chloride 340mcg, chromic chloride 5.33mcg, sodium selenite 10.5mcg, sodium molybdate 4.85mcg, sodium fluoride 210 mcg and potassium iodide 16.6mcg. Other excipients include xylitol, hydrochloric acid (for pH adjustment) and water for injections to 10mL.

## APPENDIX IX – PUBLICATIONS

### 1. Journal articles

Triple chamber bags and meeting patient requirements. SR Dodington, R Price-Davies. *Hospital Pharmacy Europe*. 2017. Autumn Issue 86/87, pp. 48-51.

### 2. Scientific abstracts

Nutritional abnormalities in long-term parenteral nutrition patients. SR Dodington, AC Cosslett, R Price-Davies, S Hiom. *International Journal of Pharmacy and Practice.* 2016. Volume 24, Issue Suppl S3, page 93.

### 3. Conferences and meetings

#### *3.1. Poster presentations*

**April 2015:**
Cardiff School of Pharmacy and Pharmaceutical Sciences Postgraduate Research Day, Cardiff, UK.

**September 2016:**
Royal Pharmaceutical Society Annual Conference, Birmingham, UK.

#### *3.2. Oral communications*

**April 2016:**
Cardiff School of Pharmacy and Pharmaceutical Sciences Postgraduate Research Day, Cardiff, UK.

**May 2016:**
Research & Development Conference, International Clinical Trials Day, University Hospital of Wales, Cardiff, UK.

**June 2016:**
The Allied Healthcare Professional (AHP) Conference, University of Wales, Cardiff, UK