The micronutrient status of long-term Home Enteral Nutrition (HEN) patients of Te Whatu Ora Counties Manukau

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Abstract

Background: Iron, folate, vitamin B_{12}, copper, and zinc are essential nutrients that play a role in metabolic processes associated with disease prevention and improving health complications and well-being. Enteral feeds contain adequate nutrients that patients may require. However, these nutrients may be digested and absorbed differently than in whole foods. Nutrient deficiencies can only be treated once recognised and confirmed, so it is in our best interest to know if deficiency is present in patients receiving long-term home enteral nutrition (HEN).

Aim: To investigate the nutritional status of long-term enterally fed patients over 18 years of age in Te Whatu Ora Counties Manukau.

Methods: Data from 42 patients receiving long-term enteral nutrition for ≥ 4 weeks were collected. The blood concentrations of iron, copper, zinc, folate and vitamin B_{12} of only 21 participants were obtained and compared to recognised cut-offs for adults. For all participants, a detailed 5 x 24-hour recall of dietary intake, including enteral nutrition (EN) and oral food sources, was determined and compared to the Recommended Daily Intake (RDI) for age appropriate. A physical assessment of nutritional signs and symptoms determined the presence of deficiency for the selected micronutrients. The Charlson comorbidity index score (CCIS) was evaluated and categorised by summing the weight of 17 comorbidities, severe (>5), moderate (3-4), mild (1-2), or no comorbidities (0), indicating the degree of mortality within the next 10-years. Descriptive statistical analyses were completed for participants' characteristics, demographics, and health characteristics by gender. Generalised linear models and binary regression estimated the association between dietary intake, biomarker status and physical signs and symptoms of deficiency. A stepwise regression method was performed on the model residuals to confirm normality (histogram and Shapiro-Wilk), independence (Durbin-Watson), equality of variance (scatter plot) and multi-collinearity (VIF and tolerance).

Results: The rates of total participants with blood results lower than the reference ranges for iron and zinc were 19.04% (n=4) and 66.7% (n=14), respectively. Folate, vitamin B_{12} and copper were all within their respective reference ranges. Dietary intake for women only was below the RDIs for iron (n=9, 47%), dietary folate equivalent (DFE) (n=3, 15.9%), vitamin B_{12} (n=3, 26%), zinc (n=1, 5.3%), and copper (n=1, 6.3%). Most participants had adequate dietary intake via EN feeds, excluding iron, with a mean±standard deviation of 17.1±8.3 mg/d (RDI <18mg/d for women). Alopecia was correlated with decreased dietary intake of iron and zinc and reduced serum zinc concentrations. The presence of eczema, dermatitis and perioral

stomatitis and the absence of wound healing and alopecia combined were associated with lower blood zinc concentration. Increased age, cerebrovascular accident (CVA) & transient ischemic attack (TIA), liver disease, solid tumour and myocardial infarction were contributor comorbidities found to decrease the risk of 10-year survival rates among the participants.

Conclusion: Blood results below the reference range values for iron and zinc were significant. Given that the total dietary intake for iron and zinc was insufficient to meet the RDIs for women, it may suggest that dietary iron and zinc are lacking in the diets of HEN women patients. Strategies to improve this, including fortification and supplementations, may positively impact the nutritional status of HEN patients and should be investigated further. The physical signs and symptoms of deficiency were largely unrelated to their biomarker status and dietary intake; however, it is essential to regularly monitor the nutrition physical examination of HEN patients to detect any clinical symptoms of nutrient-related deficiencies.

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1. Chapter 1: Introduction

1.1 Background

Eating is essential for humans, both from a biological and a social perspective (Rolandelli, 2005). When being affected by a disease, the ability and pleasure of eating can be reduced or impaired. The risk of being malnourished is apparent for patients who cannot eat and take in their daily need for nourishment (Boullata et al., 2017). Individuals who rely on nourishment through feeding tubes encounter difficulties with oral eating due to issues related to chewing and swallowing. These challenges often stem from neurological and gastrointestinal diseases, cancer, and brain injuries (Rolandelli, 2005; Santos, Fonseca, Carolino, & Guerreiro, 2016).

Malnutrition implies deviations from normal nutrition (Morrow & Raymond, 2021), but there is no common consent about a uniform definition (Soeters et al., 2017). In addressing malnutrition, the treatment can range from oral support, including food fortification and oral supplements (Boullata et al., 2017), to more intensive measure. For severe cases, the treatment may be initiated with a feeding tube. If enteral feeding fails, parenteral nutrition may be required (Baxter, Speight, & Weir, 2021). For patients unable to meet their nutritional needs orally (i.e., patients with eating problems having their gut preserved), enteral feeding tube is the preferred mode for delivery of nourishment (Boullata et al., 2017; Liley & Manthorpe, 2003).

Historically, feeding through a tube has been around as early as 1800 (Minard, 2006). During the 1950s and 1960s, attention was paid to developing feeding formulas containing complete nutrition, which were shown to positively impact the patient's wellbeing (Phillips, 2006). Enteral nutrition (EN) is one of the most efficient nutritional methods in medical nutrition therapy and refers to the intake of food directly into the gastrointestinal tract bypassing the oral cavity (Boullata et al., 2017; Nishiwaki et al., 2011; Oliver, Allen, & Taylor, 2005).

Worldwide, enteral nutrition is considered to be the act of providing nutrition intake through the gut, orally or through an enteral access device (Boullata et al., 2017; Ferrie et al., 2018). There are several routes for the delivery of feeding tubes. The most frequent tube methods used in long-term HEN include; percutaneous endoscopic gastrostomy (PEG), PEG with jejunal extension (PEG-J), nasojejunal tube (NJT), percutaneous endoscopic jejunostomy (PEJ), radiologically inserted gastrostomy (RIG), and jejunostomy tube (JJ) (Boullata et al., 2017; Pearce, 2002). These feeding tubes appear safe, well-tolerated, and effective in treating various nutrition-related deficiencies (Boullata et al., 2017; Oliver et al., 2005; Pearce, 2002).

According to guidelines from the European Society for Clinical Nutrition and Metabolism (ESPEN), PEG is a safe and comfortable long-term enteral feeding method for patients considered appropriate for PEG (Löser et al. 2005). However, there are still different methods for hospitals for the choice of route for enteral feeding.

Home enteral nutrition (HEN) significantly improves patients' quality of life in community healthcare settings (Ojo & Brooke, 2016). Home enteral nutrition is considered short-term when administrated for up to 4-6 weeks, beyond which time it is regarded as a long-term feeding strategy (Bischoff et al., 2020). In previous studies, micronutrient deficiency is said to have become evident during HEN treatment. The writers (Kang, Baik, & Chung, 2014; Nishiwaki et al., 2011; Oliver et al., 2005; Vural, Avery, Kalogiros, Coneyworth, & Welham, 2020) have identified trace elements such as zinc (Zn), iron (Fe), copper (Cu), vitamin B_{12}, and folate to be in deficit in a patient receiving HEN over different timeframes. According to Khan & Jialal (2022), iron and zinc biomarker status is decreased within 1-2 months. This author discovered an increase in types of micronutrients becoming deficient after 2-6 months of HEN, including iron, zinc, copper, and folate, decreased subsequent to vitamin B_{12} deficiency and became evident within 8-16 weeks.

It is well known that a deficiency of micronutrients can lead to physical signs and symptoms. However, few studies have explored the relationship between HEN, dietary intake, and the nutrition physical signs and symptoms of micronutrient deficiency within a single study design. This area remains largely unexplored. To date, the existing literature indicates that significant deficiencies in micronutrients, such as Zn, Fe, Cu, vitamin B_{12}, and folate, can lead to physical manifestations of nutrient deficiency. For instance, particularly when the deficiency is prolonged and severe. Such deficits can thereby worsen health outcomes (Boland et al., 2017; Milne, 1994; Oliver et al., 2005). Physical signs and symptoms of these nutrients can become apparent when the nutritional status is deprived (MacQuillan, Ford, & Baird, 2021). The functional improvement of the body as micronutrient status improves, counteract the physical signs of deficiency. It also enhances metabolism and tissue function, which supports the reversing process of health-related disorders such as anaemia, wound healing, bone health, oxidative damage, skin and eye disease, inflammation and overall improved immune system function (Johnson, 2002; Kang et al., 2014; Okada et al., 2001; Shenkin, 2006). It is, then, vital to consider the impact of adequate nutrient intakes on health status in patients undergoing long-term HEN.

Zinc deficiency leads to skin rashes and delayed wound healing (Maxfield, Shukla, & Crane, 2022). Mild iron, vitamin B_{12}, and folate deficiency may result in hair loss, weight loss, tiredness and irritability. Severe deficiency of these nutrients is associated with anaemia and neurological defect disorders such as progressive wasting of the muscles controlling movement, depression, Alzheimer's disease, Parkinson's disease and most commonly, stroke (Ferrie et al., 2018; Khan & Jialal, 2022; Stavroulakis & McDermott, 2016; Vural et al., 2020). Patients with a copper deficiency may have sparse, brittle, and kinky hair (Altarelli, Ben-Hamouda, Schneider, & Berger, 2019).

Other factors, such as increasing age and altered gastrointestinal absorption, may attenuate the bioavailability of the selected nutrients studied in this thesis (Bailey et al., 2011). Inadequate nutrient intakes also have been associated with lower biomarkers of these nutrients (Fraser et al., 1989; Khan & Jialal, 2022; Nishiwaki et al., 2011; Oliver et al., 2005). Studies suggest that patients who cannot eat orally and rely on HEN for their daily requirements might be at a higher risk of developing micronutrient deficiency than patients who solely get their nutrients orally (Kajiyama et al., 2001; Kang et al., 2014; Okada et al., 2001; Oliver et al., 2005).

Dietary intake in the EN population is also shown to be affected by compliance and adherence to EN treatment (Alicia Gea, María, Javier, & Elsa, 2019). According to Kraft et al. (2012), patients undergoing long-term EN feeding regimes show noncompliance to EN intake. The authors did not present figures; however, their system made it possible to receive alerts such as that the patients were not taking EN supplements since they disliked the flavour and texture of EN formulas. Alicia et al. (2019), in agreement, state that the EN population commonly experience a decrease in appetite caused by nausea sensations, barriers of beliefs, ignorance and forgetfulness or carelessness, which can lead to neglecting compliance with EN and reduction of dietary intake (Boland et al., 2017; Boullata et al., 2017). This ultimately has implications affecting dietary patterns, thereby lowering serum biomarker concentrations of Zn, Cu, Fe, vitamin B_{12} and folate (Gille & Schmid, 2015; Kang et al., 2014; Osland, Polichronis, Madkour, Watt, & Blake, 2022). Trace elements such as Fe, folate and vitamin B_{12} are intertwined in the production of red blood cells in the haematopoietic system; in particular, they are the most common nutrient deficiencies associated with anaemia (Snow, 1999). Copper plays many essential roles in the body by acting as a cofactor for the enzyme function of ceruloplasmin and assisting in haemoglobin formation, cell signalling, and cellular respiration (Myint, Oo, Thein, Tun, & Saeed, 2018; Osland et al., 2022). Zinc's role within the

human body is extensive in reproduction, immune function, and wound repair (Maxfield et al., 2022).

The Nutrient Values Reference Values (NRVs) for Australia and New Zealand refer to the levels of the recommended intake of essential nutrients, such as vitamins and minerals (NHMRC, 2017). The NRVs include a level known as the recommended dietary intake (RDI), which is the average daily intake level of a particular nutrient sufficient to meet the requirements of nearly all healthy individuals in a specific life stage and gender group. These include zinc, iron, vitamin B_{12} and Folate. For Cu, an adequate intake (AI) is used when an RDI cannot be determined, which estimates of nutrient intake of apparently healthy people that are assumed to be adequate (NHMRC, 2017).

Inflammatory markers, C-reactive protein (CRP) and albumin are pentameric proteins synthesised by the liver (Morrow & Raymond, 2021). For decades, these markers have been used as indicators of inflammation or malnutrition, especially in patients in clinically unstable conditions. During the acute phase response, serum levels of negative proteins (e.g., albumin and pre-albumin) decrease, whilst positive acute-phase proteins (e.g., CRP) increase. This shift in protein concentrations can be attributed to the body prioritising the production of immune mediators during stress, and downregulating the production of non-essential proteins for immune function. Since albumin levels can decline in inflammation regardless of nutrition status, both CRP and albumin are used as an independent marker to assess the presence of inflammation.

The Charlson Comorbidity Index Score (CCIS) is a valuable tool that quantifies the mortality risk for patients with various comorbidities. It provides an estimate of their life expectancy, which is particularly important for HEN patients (Kobayashi et al., 2002). Patients who require feeding tubes often suffer from multiple concurrent conditions, such as cancer and organ failure (Blumenstein, 2014). Furthermore, micronutrient deficiencies can develop despite receiving HEN due to their existing medical conditions (e.g., gastrointestinal cancer). Such deficiencies can further exacerbate the comorbidities identified by the CCIS (Jorgensen et al.,2012; Voelkle et al., 2022). These comorbidities can independently lead to malnutrition, potentially reducing life expectancy (Jorgensen et al., 2012). Therefore, it is in our interest to understand the interrelation between micronutrient deficiencies and the presence of comorbidities in predicting life expectancy for this group. A deeper understanding of these factors can offer

evidence to improve quality of life, enhance clinical outcomes, and potentially extend life expectancy for these patients (Jorgensen et al., 2012; Kobayashi et al., 2002).

In this study, the population investigated are patients from Te Whatu Ora – Health New Zealand Counties Manukau. For short, Counties Manukau District (CMD). The CMD play an essential role in promoting health and wellbeing among a variety of ethnic groups in New Zealand. The most recent CMD population projection for 2020/21 anticipated a resident population of 578,658 persons serviced by CMD (Lees J, 2021). The population comes from various ethnic backgrounds: 16.3% of CMD patients identified as Māori, 22% as Pacific, and 61.8% as other ethnicities (Lees J, 2021). A study, Nutrition and Indigenous Health in New Zealand, identified that 12% of Māori women aged 15-49 showed iron deficiency compared to European/other 7% (Grant, Wall, Yates, & Crengle, 2010), which demonstrates that Māori women, a population group that is a large part of CMD, are at risk of nutrient depletion.

In summary, adequate nutrition intake is critical for people undergoing long-term HEN and assists with decreasing the risk of prolonged health outcomes that may lead to worsening clinical status (Mundi, Patel, McClave, & Hurt, 2018; Vural et al., 2020). The lack of literature comparing HEN and NFPF is evident and requires attention. Although evidence shows that clinical signs and symptoms are apparent in patients with trace elements deficiency, there is not enough evidence linking it to HEN. The population group studied in this thesis are prone to develop nutrient deficiency. The RDI and AI are used to compare adequate nutrient intake for each element and age-gender appropriately. The selected biomarker will indicate a deficiency, and the CCIS will predict life expectancy for the population.

1.2 Purpose of Study

The nutrition of patients undergoing long-term enteral nutrition hasn't been thoroughly studied and is an area that requires attention. Enteral feeding is known to help people who have trouble eating and cannot meet their nutrient requirements. Trace elements such as zinc and copper appear not to be regularly tested in clinical settings in New Zealand (Song, 2010; University of Otago, 2011a), and iron, vitamin B_{12} and folate are the three essential nutrients associated with the development of anaemia and have also been associated with the dietary patterns linked to higher malnutrition risk in older adults in New Zealand (Green, Venn, Skeaff, & Williams, 2005; University of Otago, 2011a).

Therefore, whether or not patients on tube feeding receive adequate nutrition through their feeding regimes is still uncertain. Despite the degree of health implications requiring tube feeding, patients should be aware if they are prone to micronutrient deficiency.

When patients require long-term home enteral nutrition (HEN) and have received preparatory guidance at the hospital, they can be discharged to their homes. When researching the scientific literature, it was evident that some studies described how HEN care should be performed, but few studies explored the nutrient intake and status of those patients.

Therefore, this thesis focuses on understanding more about HEN patients and their micronutrient status. This thesis, in particular, aims to investigate the effect of selected micronutrient intakes (zinc, iron, copper, vitamin B_{12}, and folate) on biomarker status and physical signs and symptoms of deficiency in patients receiving long-term HEN from Te Whatu Ora Counties Manukau. The CCIS is used to evaluate the level of comorbidities that may affect the life expectancy of HEN patients. It also helps compare if there is an association between CCIS and nutritional status, particularly regarding micronutrient deficiencies.

To our knowledge, this is the first study conducted in New Zealand, focusing primarily on the association of micronutrient intake of zinc, iron, copper, vitamin B_{12}, and folate on biomarker status and physical signs and symptoms of long-term HEN patients.

1.3 Aims and Objectives

Aim:

To investigate the nutritional status of long-term home enterally fed patients older than 18 years in patients of Te Whatu Ora Counties Manukau.

Objectives:

1. To assess the biomarker status of the selected minerals (iron, zinc and copper) and vitamins (folate and vitamin B_{12}) in relation to recognized reference ranges.
2. To investigate the dietary adequacy of iron, vitamin B_{12}, folate, zinc and copper in relation to the RDIs.
3. To investigate the presence of the nutrition-related physical signs and symptoms of deficiency of the selected micronutrients.

4. To investigate the estimated 10-year survival rate using the Charlson Comorbidity Index Score.
5. To determine the association between the serum zinc, copper plasma and serum iron concentrations, physical signs and symptoms of deficiency and dietary intake.

1.4 Structure of thesis

This thesis includes four key chapters with additional sections for references and appendices. *Chapter one* presents the introduction and purpose of the study and outlines the scope and justification of this research regarding iron, vitamin B_{12}, folate, zinc and copper of long-term home enteral nutrition patients of Te Whatu Ora – Health New Zealand Counties Manukau. This chapter also outlines the study aims, objectives, and researcher contributions. *Chapter two* provides a review of the current literature regarding the health and nutritional status of enteral nutrition and home care, malnutrition and various enteral feeding methods of tube-fed patients, non-pathophysiological factors affecting micronutrient deficiency and investigation of micronutrient deficiency for iron, vitamin B_{12}, folate, zinc and copper, with particular focus on literature relating to adults age >18 years old. *Chapter three* provides the research design and participants recruitment, ethical considerations, dietary assessment (24-hour diet recall, biomarkers analysis, nutrition-focused physical examination, Charlson Comorbidity Index and statistical analysis, results and discussion. *Chapter four* outlines the conclusions and recommendations based on the presented research findings, including the study's strengths and limitations. *Appendices* include the supplementary methods relating to the Participant Information Sheet, the Charlson Comorbidity Index Score Assessment form, the standard operating procedures (SOPs) for the Nutrition-Focused Physical Examination Form, the 24-hour Diet Recall Assessment Form, FoodWorks Analysis for iron, vitamin B_{12}, folate, zinc and copper, and *Nutrition Journal* requirements.

1.5 Research's contributions

Table 1: Researcher's contributions

Marcos Mantovani	Master's student; - Primary researcher/writer - Data collection - Literature review - Statistics analysis - Writing of the thesis
Andrew Xia	Primary supervisor and co-investigator;

	- Research design and funding acquisition - Thesis guidance and review - Ethics application
Professor Rozanne Kruger	Co-supervisor and principal investigator; - Research design and funding acquisition Thesis guidance and review - Ethics application
Sally Pattison	Research Fellow - Data collection
Sophie Turner	Research Assistant - Data collection - FoodWorks Software data analysis
Dr Owen Mugride (Nutrition and Dietetics research trial manager, Massey University	Laboratory supervisor - Biomarker processing advisor (teaching technician)
Dr Thomas Russell George King (NZRN) Liam Perrell (NZRN) Yongsijia Wei (Research assistant, Massey University)	Phlebotomists; - Biomarker collection

2. Chapter 2: Review of Literature

2.1 Enteral nutrition and home care

Individuals who are unable to safely consume enough food by mouth to meet their nutritional requirements are at high risk of malnutrition (Sánchez-Rodríguez et al., 2019). Since it is essential to prevent and combat malnutrition, enteral nutrition (EN) should be considered when a patient is not safe for oral intake and/or oral intake cannot meet complete nutritional requirements (Bischoff et al., 2020; Boullata et al., 2017; Gramlich, Hurt, Jin, & Mundi, 2018). Enteral nutrition provides nourishment through the stomach or small intestine via an enteral access device for these patients (Ferrie et al., 2018). Enteral nutrition has been shown to be safe, cost-effective, and compatible with the body's normal processes (A. Wong, Goh, Banks, & Bauer, 2018).

Home enteral nutrition (HEN) refers to the provision of complete or partial feeding for patients whose acute medical condition has stabilised after being discharged from a healthcare facility (i.e., hospital) and continuing their enterally nutritional care at home (Gramlich et al., 2018). Often patients with long-term HEN have chronic medical conditions that reduce their ability to swallow, digest or absorb nutrients (Silver, Wellman, Arnold, Livingstone, & Byers, 2004). For example, neurological diseases, head and neck cancer, gastrointestinal cancer, cerebral palsy, non-neoplastic gastrointestinal disease (e.g., fistulae, oesophageal stenosis, inflammatory bowel disease), head injury, malabsorptive syndromes (e.g., short bowel syndrome), severe intestinal motility disorders, inherited metabolic diseases, and cystic fibrosis (Bischoff et al., 2020; Gramlich et al., 2018; Liley & Manthorpe, 2003).

According to Flood et al. (2021), the absence of a centralised registration or reporting system for HEN services in Australia and New Zealand has resulted in a lack of data to understand the HEN care process and the number of patients receiving EN in the community is currently unknown. Interestingly, a study has suggested that patients, when discharged home, may lack sufficient understanding of the feeding regime (Liley & Manthorpe, 2003). The lack of knowledge on adherence to EN treatment could lead to underestimating treatment effects, such as lower dietary intake of prescribed oral nutrition supplements. Moreover, the effectiveness of nutritional supplementation depends on patient acceptance and compliance. (Alicia Gea et al., 2019). It is still a relatively scarcely explored area regarding patients receiving preparatory guidance about the feeding regime at home following discharge from the hospital (DeBruyne & Pinna, 2020; Gramlich et al., 2018; Mcwhirter & Pennington, 1996).

Overall, HEN can offer comprehensive nourishment to long-term EN patients. However, the number of individuals receiving HEN in Australia and New Zealand is unclear (Flood et al., 2021). There is also a need for more research on patients' compliance with HEN feeding and their nutrition status. Therefore, further investigation is necessary to enhance our understanding of HEN treatment and care.

2.2 Malnutrition

Malnutrition remains to be a major public health problem worldwide. It occurs when a population's diet lacks sufficient macronutrients like protein, carbohydrates, and fat (Millward & Jackson, 2004), or is deficient in specific micronutrients such as minerals and vitamins (Mette, Olivier, Antoine, & Nawfel, 2019). Globally, about 2 billion people are affected by micronutrient deficiency. The highest prevalence is monitored in South-East Asia and Sub-Saharan Africa, but developed countries are not excluded (WHO, 2022).

In New Zealand, a study found that 56.5% of community-living older adults were at some degree of nutritional risk, and Māori were 5.2 times more likely to be at nutritional risk compared to non-Māori (McElnay et al., 2012). Also, in 2010, the Australasian Nutrition Care Survey (ANCDS) reported that 40% of the patients in acute care across New Zealand hospitals were at risk of malnutrition (Agarwal et al., 2013). Approximately 32% of them were identified as malnourished (Agarwal et al., 2013). Malnutrition, if untreated, may result in a prolonged and complicated recovery from illness or surgery (Goldstein, Katona, & Katona-Apte, 2008), an increased risk of infection, compromised wound healing and persisting functional deficits (Agarwal et al., 2013; Mette et al., 2019). This, in turn, leads to a more extended hospital stay with a possible increase in cost and a negative impact on quality of life (Volkert, Pauly, Stehle, & Sieber, 2011).

Iron deficiency anaemia is a common clinical diagnosis related to iron deficiency (WHO, 2020). To show the aggrievance of micronutrient deficiency in New Zealand, the Activity & Nutrition Aotearoa (2020) reported the prevalence of inadequate iron intake in the overall population of 5.6%. It was shown to be higher in women (9.7%) and even higher for Māori and Pacific women (18.4% and 19.9%, respectively) compared to New Zealand Europeans and Others (9.3%) (Young et al., 2020). Micronutrient deficiency, if untreated, can cause visible and dangerous health conditions, but it can also lead to less clinically notable reductions in

energy level, mental clarity and overall capacity (Shenkin, 2006). This can further lead to an increased risk of developing other diseases and health conditions and cutaneous manifestations of micronutrient deficiency (i.e., hair, nails and skin) (Dibaise & Tarleton, 2019). In New Zealand, one in fourteen adults (6.9%) (5.0% in men and 8.7% in women) in 2014/15 was shown to have iron deficiency anaemia (Young et al., 2020).

Zinc deficiency targets more than 2 billion people worldwide (Prasad, 2013). The leading causes of zinc deficiency include insufficient intake, increased requirements, malabsorption, increased losses and impaired utilization. Inadequate intake of zinc is considered to be one of the most significant determinants of the development of zinc deficiency (Maxfield et al., 2022). Zinc deficiency is commonly seen in developing regions and is attributable to malnutrition; however, it is associated with aging and many chronic illnesses and can be acquired or inherited (Maxfield et al., 2022). Zinc deficiency can occur from decreased intake, inability of absorption, increased metabolic demand, or excessive loss (Maxfield et al., 2022). Patients with an acquired form of zinc deficiency usually have a combination of various factors: inadequate nutritional intake of zinc-rich foods (e.g., meat, legumes, seeds, soy products and whole grains) and chronic illnesses (e.g., gastrointestinal diseases, liver disease, excess of alcohol or chronic infections) (Maxfield et al., 2022). A review of zinc status in Australia and New Zealand found that certain groups were at risk, including toddlers, adolescents (particularly those of Pacific Islander descent), and older people living in institutions (Anna & Samir, 2012; Gibson & Heath, 2011). While these findings may also apply to New Zealand, as the two countries share similar dietary habits, further research is needed to understand zinc intake levels among individuals in New Zealand.

Copper is involved in various physiological functions, and deficiency impairs growth and reproduction and can cause biochemical alterations, leading to health problems (Altarelli et al., 2019; Leslie, 2022). Biochemical or clinical copper deficiency is common in infants recovering from malnutrition, children with chronic diarrhea, malabsorption cases, and areas with low copper content in soil or food availability (Altarelli et al., 2019; Leslie, 2022). Medical publications revealed poor copper nutrition in over 2,500 individuals with cardiovascular, musculoskeletal, and nervous diseases (Leslie, 2022). Sixty-thousand more individuals with common disorders have abnormally low copper concentrations or compromised metabolic pathways dependent on copper, which are diagnostic of deficiency (Leslie, 2022).

A review of the magnitude of folate and vitamin B_{12} deficiency worldwide states that folate and vitamin B_{12} deficiencies have long been known to have adverse effects on health, including anemia and neuropathy (McLean, de Benoist, & Allen, 2008). This study identified folate and vitamin B_{12} status most frequently assessed in women of reproductive age in 34 countries and all adults in 27 countries. Their findings show that in most countries worldwide for which national surveys were available, folate and vitamin B_{12} deficiencies appeared to be a public health problem; six out of eight countries were deficient in folate, and five out of seven were deficient in vitamin B_{12}. Deficiency was identified in preschool children in Venezuela (33.8%), pregnant women in Costa Rica (48.8%) and Venezuela (25.5%), and older adults in the United Kingdom (15.0%) are the main groups affected by folate deficiency. Vitamin B_{12} deficiency is prevalent in school-age children in Venezuela (11.8%), pregnant women in Venezuela (10.9%) and Costa Rica (5.3%), and older adults in the United Kingdom (31.8%) and New Zealand (12.0%) (McLean et al., 2008). Deficiencies in folate and vitamin B_{12} may be of public health consequences, but it is unclear how prevalent these deficiencies are (Allen, 2008; McLean et al., 2008).

The authors Mette et al. (2019) report that the leading causes of micronutrient malnutrition include reduction of food intake, lower bioavailability and malabsorption. Many of these deficiencies are preventable through nutrition education, a healthy diet containing diverse foods, and food fortification and supplementation as needed (DeBruyne & Pinna, 2020). Boullata et al. (2017) reports that the importance of adequate nutrition among critically ill and HEN patients is amplified by an increase in the metabolic stress response, impaired immune function and the severity of illness. This author states that it is often challenging to deliver nutrition in this population with increased requirements in the context of an altered metabolic and immune response, which lead to a cumulative energy and protein deficit, resulting in muscle wastage (Millward & Jackson, 2004), as well micronutrient deficiency (Osland et al., 2022).

The influence of co-existing illnesses on prognosis, therapy and patient outcomes has been recognized since the 1970s (Lu, Barratt, Vitry, & Roughead, 2011). In epidemiological studies, clinical trials and health services research, controlling for additional co-existing diseases or comorbidity is essential (Feinstein, 1970). Comorbidity refers to the presence of one or more other health conditions co-occurring with a primary condition (Lu et al., 2011). The Charlson index (CCI) was developed in 1984 by Charlson et al. and categorizes patients' comorbidity

conditions based on medical diagnoses and has successfully predicted mortality in various patient populations (Lu et al., 2011). This instrument identifies comorbid conditions, which may, singly or in combination, affect the short-term mortality risk for patients (Feinstein, 1970; Lu et al., 2011). Mortality rates by the CCI can be evaluated by categorising the sum of 17 comorbidities (Lu et al., 2011) into four categories of the Charlson Comorbidity Index Score (CCIS): severe (>5), moderate (3-4), mild (1-2), or no comorbidities (0) (Huang et al., 2014). The CCIS indicates the degree of mortality within the next ten years. The CCIS has been used to evaluate the long-term prognosis of EN and the parenteral nutrition population (Kobayashi et al., 2002). A study discovered using the CCIS instrument that of 3.548 participants, 2,384 (67%) died within 730 days after the initiation of gastrostomy (GS) and nasogastric tube feeding (NGT) and parenteral nutrition (PN) in those with malignancies and enteral feeding including secondary GS, primary GS, and NGT had a better 2-year prognosis than PN in older patients with and without malignant disease (Kobayashi et al., 2002).

Thus, trauma and critically ill patients receiving HEN might be at risk of micronutrient malnutrition. Vitamin and mineral deficiencies are widespread, affecting the vulnerable population and worsening clinical status.

2.3 Various enteral feeding methods

Enteral Nutrition is a valuable intervention for patients of all ages in various care settings and enters the body through various routes (Boullata et al., 2017). Different tubes differ based on how the tube is inserted, through the nose or abdomen and where the tube ends in the digestive system, stomach or intestine (Bischoff et al., 2020).

The most commonly used enteral feeding delivery methods include nasogastric tube (NG), percutaneous endoscopic gastrostomy (PEG), and percutaneous endoscopic gastrojejunostomy (PEGJ) (Gossum, 2005). Several studies have looked at the benefits and challenges of various delivery methods, including NG and PEG. Though NG has been mostly used for short-term treatment, whilst PEG is reported as a long-term pathway (Boullata et al., 2017; Crosby & Duerksen, 2005; Ferrie et al., 2018). According to ESPEN (2020), PEG is a safe and comfortable method for long-term HEN patients (Bischoff et al., 2020).

Enterally feeding methods can be described as intermittent (allocated in shorter or longer periods throughout the day) (Ferrie et al., 2018). A continuous feeding regime is defined as

feeding for 24 hours continuously either by gravity drip or feeding pump method (Bischoff et al., 2020; Ferrie et al., 2018). Bolus feeding delivers a liquid meal for a short feeding time (100-400ml over 15-60 minutes) using a syringe and may be repeated at intervals to achieve the required intake (Ferrie et al., 2018). Bolus feeding mainly delivers food to the stomach due to the higher capacity to tolerate a larger feed volume. The literature shows no consensus found on which method is preferred (Minard, 2006; Pearce, 2002; Phillips, 2006).

Studies show that enterally fed patients are prone to under-feeding due to improper use of the tubes and gastric intolerance, leading to negative implications for nutritional status (Boullata et al., 2017; Crosby & Duerksen, 2005). The results of a prospective cohort study show that patients often do not take their enteral nourishment, causing a lack of nutrition intake (De Jonghe et al., 2001). This study concluded that, out of the 100 kcal that were required, 78.3% were prescribed; however, only 71.2% were taken. This means there was a difference between the theoretical requirement and the actual delivery of calories. The difference resulted from under-prescription, which accounted for about two-thirds of the difference, and under-delivery, which accounted for about one-third, attributed to wasted volume through interruptions caused by digestive intolerance, airway management and diagnostic procedures (i.e., tracheal tube repositioning) and mechanical issues (i.e., electric feeding pump dysfunction, gastric tube occlusion or malposition). Interestingly, other researchers (Boullata et al., 2017; Crosby & Duerksen, 2005; Pearce, 2002) agree that enteral nutrition may have shortcomings, including underfeeding, perceived intolerance, aspiration, access-related complications, vomiting and diarrhoea, which leads to under intake of nutrition. To exemplify, a previous study by Layec et al. (2011) found that patients who receive exclusive feeding through a tube in the jejunum are at a high risk of copper deficiency. This is because the duodenal and gastric mucosa are critical in copper absorption. When the duodenal site is bypassed during jejunal feeding, the absorption area of copper is reduced, leading to impaired absorption and potential copper deficiency. It has been observed that jejunal supplementation is ineffective in correcting the deficiency, but using the gastric route has been a successful and definitive solution.

2.4 Non-Pathophysiological Factors Affecting Micronutrient Deficiency

Several factors may influence the dietary intake of micronutrients such as iron, zinc, copper, vitamin B_{12} and folate. Studies in older adults have shown a positive correlation between dietary intake of iron, zinc, copper, vitamin B_{12} and folate and their respective serum biomarkers (Kang et al., 2014; Shi et al., 2020; Suchdev et al., 2016). However, numerous

factors, including age-related changes to the gastrointestinal tract, gender, oral health, enteral feeding formulations, bioavailability, and cooking methods, may hinder this association (S. Lee, Choi, Jeong, Lee, & Sung, 2017; Milne & Johnson, 1993; Russell, 2001; WHO, 2020).

Non-pathophysiological factors such as poverty, lack of access to a variety of foods, and lack of knowledge of optimal dietary practices are some factors that lead to deficiency. Inadequate intake of these micronutrients can be seen with strict vegetarian diets, anorexia nervosa and when receiving exclusively enteral nutrition. Research confirms that inadequate enterally intake leads to micronutrient deficiency due to inconsistency between the prescribed and delivered nutrition methods (De Jonghe et al., 2001; Flood et al., 2021; Gregg, Reddy, & Prchal, 2002; Kang et al., 2014; Myint et al., 2018).

2.4.1 Age

Micronutrient intake in older persons can be affected by physiological changes associated with ageing and changes in health status and lifestyle (Organization, 2020; Shenkin, 2006; WHO, 2020). The KORA-Age Study conducted in Germany suggests that micronutrient deficiency tends to increase beyond age 65 and even more so after 80. Their population study identified that older adults aged 85 and over were two times more likely to have low folate and low vitamin B_{12} levels than those younger than that age due to problems with the acids and stomach enzymes needed to process the vitamins (Snow, 1999). As a result, reduced gastric acid secretion prevents the release of vitamin B_{12} from foods, affecting absorption (Conzade et al., 2017). Epidemiological evidence suggests that subclinical micronutrient deficiencies in older adults are associated with chronic age-related diseases and adverse functional outcomes (Morrow & Raymond, 2021). Absorption and the bioavailability of nutrients decrease with ageing due to changes in the gastrointestinal tract and polypharmacy, leading to malabsorption of macronutrients and micronutrients (Morrow & Raymond, 2021; Saboor, Zehra, Qamar, & Moinuddin, 2015). Plasma copper has been identified as affected by age; men and women older than 50 showed higher concentrations than men and women under 40 (Milne & Johnson, 1993). The ZENITH study assessed zinc intake and status in European males and females of age 55-70 years (middle-aged group) and older-aged subjects (>70 years). They documented that individuals presenting a serum zinc concentration below the cut-off level were 4.8% in middle-aged and 5.6% in older subjects (Andriollo-Sanchez et al., 2005).

Research indicates that older adults aged ≥70 years are more likely to require EN care as they experience higher levels of disability and chronic diseases explained by the demographic transition associated with aging (Menezes & Fortes, 2019; Okada et al., 2001). Okada et. (2001) found that 44 bed-ridden patients who received tube feeding had a higher incidence of protein malnutrition than 41 free-eating elderlies. Their findings indicate a decrease in arm muscle circumference (<80% of normal) and hypoalbuminemia (<35 g/L) in EN patients higher than in healthy elders. Interestingly, the study also revealed that orally fed bed-ridden patients were malnourished, indicating that bed-ridden patients are susceptible to malnutrition despite receiving energy and protein similar to calculated predicted values. Therefore, ageing becomes a risk factor for developing diseases, and older adults have the highest risk of being at nutritional risk or becoming malnourished.

2.4.2 Gender

Recognising nutritional differences between men and women is critical to prevent gender-related micronutrient deficiency. There has been a lack of research on how gender differences impact nutrient status in tube-feeding patients. This is likely because men and women have different nutrient needs. However, we attempted to connect existing research on the relationship between gender differences and nutrient status in patients receiving EN. For example, the KORA-Age Study found that subclinical deficiencies were more common in women for folate in gender-specific analysis, 9.4% vs. 8.0 % for men. They also identified a high prevalence of deficiency in vitamin B_{12} (28.5% vs. 26.0%) and iron (13.5% vs. 8.4%) for women and men, respectively. Folate deficiency was also identified to be lower in the male participants, 8.2%, compared to 9.2% in females (Conzade et al., 2017).

A study stated that the overall range of plasma copper concentrations was 8.8-17.5 μmol/L for men, 10.7-26.6 μmol/L for women who were not taking oral contraceptives, and 15.7-31.5 umol/L for women who were taking oral contraceptives or who were on oestrogen therapy (Milne & Johnson, 1993). In agreement, an investigation study on gender effects on plasma and brain copper discovered that plasma copper was higher in women, 1008 ± 51 ng/mL, than in male (836 ± 41) control subjects (Quinn et al., 2011). Lower serum zinc was identified in the ZENITH study showing in the middle-age group 55-70 years (6.4% men, 3.1% women) and the older group aged 70 + years (9% men and 6.2%), showing zinc deficiency higher in men compared to women despite the age difference (Andriollo-Sanchez et al., 2005).

Based on the results, the difference in nutrient status between gender is evident and may be translated to EN patients; however, it still needs further study to comprehend gender-specific and EN.

2.4.3 Mouth Health

Dental health is a key indicator of overall health, well-being and quality of life. Dental health has been shown to reduce the nutritional intake of foods essential for optimal health and plays a critical role in a person's ability to consume adequate nutrition (Sheiham et al., 2001). The absence of oral health is known to cause avoidance of some nutritional foods such as whole grains, fruits, vegetables and meat (Beaudette, Fritz, Sullivan, & Ward, 2017). Other issues associated with oral health include loose and painful teeth, decreased saliva production, and changes in sensory perceptions of taste and smell (Su, Yuki, Hirayama, Sato, & Han, 2020). In elderly subjects, tooth deterioration is not necessarily due to aging but the oral disease (dental caries, periodontal diseases), usage of drugs, and cumulated physical and psychic disorders (such as loss of autonomy, mobility, or dexterity).

As reported in The British National Diet and Nutrition Survey and the Dentistry Journal, foods containing iron, zinc, vitamin B_{12}, folate and copper are less consumed by elderly with fewer teeth or adults with dentures due to these nutrients being found primarily in meat, seafood, nuts and seeds, vegetables and green leaves (Beaudette et al., 2017; Sheiham et al., 2001). Individuals with poor chewing abilities or ill-fitting dentures have been proven in the literature to consume smaller amounts of veggies and have overall reduced nutrient intake (Sheiham et al., 2001; Su et al., 2020).

Enterally fed patients, such as those with head and neck cancers and dysphagic patients, usually lose swallowing function, subsequently leading to the inability to meet nutritional requirements (Santos et al., 2016). Dysphagia may occur due to an obstructive disease or in the setting of a neurological disorder and can affect oral intake (Lopes et al., 2022). It reduces oral intake by decreasing swallowing efficacy and safety, leading to depletion of nutrient intake and, consequently, to malnutrition and decreased quality of life (Lopes et al., 2022). When oral intake is insufficient, an individual cannot eat or drink safely, and there is no other digestive tract disorder, tube feeding is the obvious feeding option (Lopes et al., 2022; Nerina, Marco, & Elvio, 2013). It is worth noting that many patients undergoing percutaneous endoscopic gastrostomy (PEG) have limited ability to care for themselves (Lopes et al., 2022). As a result,

those responsible for caring for long-term enteral feeding patients, including caregivers and professional teams, often overlook the significance of dental health as a critical aspect of overall health (Lopes et al., 2022) . Maintaining good dental health can help prevent low-level oral inflammation linked to adverse outcomes in various systemic disorders (Lopes et al., 2022; Touger-Decker & Mobley, 2013).

Therefore, all of these features may contribute to poor oral care and impaired oral health in patients unable to adequately use their mouth for food consumption, leading to an incapacity to meet nutritional requirements.

2.4.4 Enteral feeding formulations

The content of standard enteral mixtures should contain a micronutrient content that aligns with the nutrient reference range values (NRVs) for a healthy population (Iacone et al., 2015). These mixtures are designed for people on standard enteral formulas; as outlined in the guidelines on foods for special medical purposes, these formulas are intended for individuals who cannot meet their nutritional needs through regular food consumption due to their medical condition and who do not have any specific nutritional requirements (Gazette, 2015) .

A study compared Sixty-two nutritionally complete enteral formulas (Iacone et al., 2015). This study evaluated the micronutrient content of enteral formulas grouped as standard- and disease-specific at 1500 and 2000 Kcal/day. Their findings were that micronutrients supplied in EN mixtures were often above the NRVs for a healthy population, below the upper limit (UL), and within the range of the relevant European standards; it was reported as suitable for patients on long-term total EN. As an interest of our research, the micronutrient content for some enteral formulas showed a greater zinc content than the tolerable UL levels. They conclude that it would be more appropriate to keep zinc content at the limit set by the European Commission (Iacone et al., 2015). The literature shows that most patients with long-term EN are in stable clinical conditions, and metabolic diseases are reduced, making micronutrient requirements similar to the general population under a standard diet (Iacone et al., 2015). Enteral formulas supply sufficient micronutrient intake for patients with long-term HEN.

2.5 Micronutrients Assessment

2.5.1 Iron

Iron is an essential micronutrient that plays a crucial role in erythropoiesis, oxidative metabolism and cellular immune function (Munoz, Villar, & Antonio Garcia-Erce, 2009). In addition, it is essential for growth, neurodevelopment, myelination of neurons, and neuronal energy metabolism (López & Martos, 2004; Thomas, Raghavendra, Phu, & Michael, 2020). Iron exists in states of reduced ferrous form (Fe^{2+}) and oxidised ferric form (Fe^{3+}); however, ferrous iron is more bioavailable than ferric iron (Palacios, 2012). According to Anderson et al. (2005), iron is also present in the diet in two forms, haem iron and inorganic, non-haem iron. The authors complement that non-haem iron is the most abundant in the diet; however, it is poorly absorbed compared to haem iron, derived primarily from haemoglobin and myoglobin and thus primarily associated with meat intake.

Iron deficiency (ID) is the most common micronutrient deficiency, affecting approximately two billion people worldwide (WHO & CDC, 2007). Iron deficiency is characterised by a state in which there is a reduction in iron stores and a disparity between serum iron levels and cellular requirements but adequate iron for erythropoiesis (WHO & CDC, 2007). According to a critical review, iron deficiency anaemia is associated with underlying gastrointestinal pathogenic conditions. The study states that mucosal malabsorption, such as chronic pancreatitis, cystic fibrosis, Zollinger-Ellison syndrome and obstructive jaundice, can lead to iron deficiency (Saboor et al., 2015). Iron status in an average population exhibits varying body iron levels ranging from replete stores to overt iron deficiency anaemia. From a clinical standpoint, three stages of iron deficiency can identify a decrease in iron storage. The first stage is reduced iron stores, measured by ferritin concentration (storage depletion). The second stage is early iron deficiency, identified by reduced transferrin saturation or excess free protoporphyrin or transferrin receptors (mild deficiency). The most severe phase is when erythropoiesis is impaired, leading to iron deficiency anaemia (Brito et al., 2020; Kang et al., 2014).

A study of 44 patients who received tube feeding longer than four weeks showed that 13 patients presented lower blood iron (38.5%) (Kang et al., 2014). This study identified that significant iron deficiency in long-term tube-fed patients could increase after two or >6 months (Kang et al., 2014). (Santos et al., 2016) showed similar results and identified that n=69 (47%) of (n=146) participants receiving HEN longer than 3-4 weeks showed low serum iron concentration of 7-44 mg/dl (normal range: 45-160 mg/dl).

With such results, the most common reference range for iron ranges between 50 to 120µg/dl (equivalent to approximately 10 to 30µmol/L) (WHO & CDC, 2007). The Canterbury Health Laboratories used the cut-off value <10-30µmol/L as sufficient serum iron (Canterbury Health Laboratories, 2023). From a clinical standpoint, a decrease in storage iron can be identified by a reduction in serum ferritin concentration and a decrease in stainable iron in the bone marrow. When iron stores are exhausted by low ferritin levels (lower than 157µg/L), and demand continues to exceed supply, it is considered diagnostic of iron deficiency (R. D. Lee & Nieman, 2007).

Hemoglobin in the blood primarily depends on the number of red blood cells and, to a lesser extent, on the amount of hemoglobin in each red blood cell. The results of impaired hemoglobin synthesis lead to a concentration below the widely accepted threshold level for iron deficiency of 135g per litre for men and 120g per litre for women (R. D. Lee & Nieman, 2007). In the case of iron-deficiency anaemia, blood iron is severely depleted, resulting in decreased red blood cell production and low hemoglobin concentrations.

Serum transferrin distinguishes between iron deficiency anaemia and iron deficiency due to an acute phase response. Total iron-binding capacity (TIBC) represents the total number of iron atoms binding on transferrin, and it is a more stable indicator of iron status; however, it only appears once iron stores are depleted (WHO & CDC, 2007). The normal range for TIBC levels is 45-71µmol/L, increasing when iron stores are depleted. Transferrin saturation is calculated using serum iron and TIBC, a measure of the iron supply to increase red blood cells. The WHO defines the normal range of serum transferrin as 1.9–2.58 g/L. The transferrin concentration may increase to 9mg/L in non-anaemic iron deficiency and reach higher levels of 25mg/L in iron deficiency anaemia. Therefore, studies suggest that transferrin saturation of <15% is insufficient to meet standard red blood cell production requirements and, coupled with TIBC, indicates iron deficiency (WHO & CDC, 2007).

The Biomarkers Reflecting Inflammation and Nutrition Determinants of Anaemia (BRINDA) established guidelines on assessing anaemia and micronutrient status, including serum iron. This project identified a correlation between iron status for determining iron deficiency which involves the acute phase response triggered by infection and trauma. They stated that during the inflammation response, there is an increase in the concentration of some positive acute phase protein (APPs) in plasma, and the increased APPs lead to an over- or under-estimation of iron deficiency. Examples of such positive APPs include CRP, AGP, and ferritin levels.

Negative APPs such as albumin is suppressed in the presence of inflammation (Suchdev et al., 2016; WHO, 2020). During infection and inflammation, iron is necessary for the synthesis of new red cells and for replenishing iron enzymes and transferrin transports myoglobin to the bone marrow or body tissues in the plasma (Hurrell, 2012). Iron primarily enters the plasma through macrophages when senescent red cells are destroyed, and the remaining amount is absorbed through mucosal cells or taken from the stored ferritin in the hepatocytes during times of necessity. Iron enters the plasma through the transport protein ferroportin on the cell membrane (Donovan et al., 2005). The hormone hepcidin regulates its entry and maintains adequate iron levels in the body. When iron levels are sufficient, the liver secretes hepcidin, inhibiting iron transport from macrophages and intestinal cells into the plasma (Ganz, 2005). Hepcidin binds ferroportin on the cell membrane, leading to its internalization and degradation (Ganz, 2005). In contrast, hepcidin release from the liver decreases when iron levels are low and iron absorption is maximize (Nemeth et al., 2004). When the body is infected with microbes, the innate immune response increases hepcidin through an inflammatory response, which restricts microbial growth by limiting iron entry into the plasma (Wander, Shell-Duncan, & McDade, 2009). The anaemia of infection occurs when red cell iron recycling is disrupted. Macrophage iron is not released, leading to a shortage of iron for erythropoiesis. The outcome of many infectious diseases depends on preventing the invading pathogen from accessing its iron supply. Providing high doses of iron to an infected patient can worsen the infection since the inflammatory response prevents iron release from macrophages and mucosal cells, thus restricting iron absorption (Hurrell, 2012). Recent findings suggest that the inflammatory response to malarial parasitemia reduces iron absorption from iron-fortified sorghum gruel (Cercamondi et al., 2010), and inflammation associated with obesity and overweight reduces iron absorption and diminishes the effectiveness of iron-fortified foods (Zimmermann et al., 2008), which shows how inflammation in the individuals' body can decrease iron absorption efficacy.

Anaemia was defined as HB <120 g/L (>18years old female–non-pregnant) and 120 g/L (18 years old male) (R. D. Lee & Nieman, 2007). Depleted iron stores were defined using common cut-offs, including serum iron <10-30 μmol/L, serum ferritin 20-400 μg/L, transferrin 2.0-3.5 g/L and transferrin saturation 16-45% (Canterbury Health Laboratories, 2023).

In conclusion, inflammation is known to affect iron biomarkers and can lead to incorrect diagnosis and overestimation or underestimation of the prevalence of deficiency, and serum ferritin is poor marker of iron stores in chronic inflammation.

Table 2: Iron status measure and cut-offs.

Status	Measures and cut-offs	
Iron stores	Serum Iron 10-30µmol/L Transferrin 2.0-3.5 g/L Transferrin saturation 16-45% Serum Ferritin 20-350 ug/L (>15yr-30yr men) 20-400 ug/L (>30yr men) 20-150 ug/L (>15yr-30yr women) 20-300 ug/L (>30yr women)	
Haemoglobin	Haemoglobin (men) < 130 g/L	Haemoglobin (women) < 120 g/L

References for cut-offs: iron stores and haemoglobin (Canterbury Health Laboratories, 2023).

2.5.2 Vitamin B_{12}

Vitamin B_{12} is the term used to describe the group of compounds that exhibit the biological activity of cyanocobalamin. Many cobalamin compounds are required to synthesise fatty acids in myelin and, together with folate, for DNA synthesis (NHMRC, 2017). Vitamin B_{12} is synthesised only in certain bacteria and is subsequently concentrated in the tissue of ascending organisms in the food chain. As such, animal-based foods, including milk, fish, and egg, are the main contributors of vitamin B_{12} to the human diet (Watanabe & Bito, 2018). Certain strains of bacteria in the human colon also produce vitamin B_{12}; however, the contribution of bacterial synthesised vitamin B_{12} to human physiology is unknown (Ankar & Kumar, 2021; Koury & Ponka, 2004; Watanabe & Bito, 2018).

The median intake of vitamin B_{12} as part of a Western diet contains 5-30µg of vitamin B_{12} per day, which covers the average requirement of 1-4µg lost daily through normal physiological processes (Finglas, 2000). Vitamin B_{12} can also be stored in the liver and be sufficient for up to 4 years without further supply (NHMRC, 2017). Vitamin B_{12} is bound to food proteins, serving as a coenzyme. Many events need to occur for successful vitamin B_{12} absorption, and any interruption results in malabsorption (Nielsen, Rasmussen, Andersen, Nexø, & Moestrup, 2012). Inside the stomach, vitamin B_{12} is liberated from the protein by the action of stomach acid and pepsin, allowing vitamin B_{12} to bind to small proteins secreted by the stomach called R-binders (Russell, 2001). Vitamin B_{12}, bound to the R-binders, is then transported to the proximal small intestine, where the R-binders are removed from the vitamin through the action of pancreatic proteases (Russell, 2001). Vitamin B_{12} is then bound to a small glycoprotein secreted by the stomach called intrinsic factor, which transports vitamin B_{12} to the terminal ileum, where it can be actively absorbed (Nielsen et al., 2012; Russell, 2001).

The average bioavailability of vitamin B_{12} is commonly said to be around 50%; therefore, it may be better for older adults to have multiple low-vitamin B_{12} dietary sources, such as milk and dairy, rather than one large source of dietary vitamin B_{12} from liver or meat (Gille & Schmid, 2015). The absorption of vitamin B_{12} can be reduced due to impaired gastrointestinal absorption due to decreased gastric acid and intrinsic factors (Ankar & Kumar, 2021).

The authors of Clinical Nutrition Enteral and Tube Feeding (Rolandelli, 2005) state that generally, humans maintain a significant vitamin B_{12} reserve that can last 3 to 5 years in individuals with optimal status. A review article states that, except for strict vegetarians, vitamin B_{12} deficiency implies the presence of an absorptive problem (Snow, 1999). This study indicates that the body stores a large amount of vitamin B_{12} (2-5 mg) relative to daily requirements, and it takes 2 to 5 years to develop vitamin B_{12} deficiency in the presence of severe malabsorption (Snow, 1999).

There is currently no universally accepted serum vitamin B_{12} cut-off to define deficiency; however, several have been proposed in the literature ranging from 148-250pmol/L (Ankar & Kumar, 2021; De Benoist, Darnton-Hill, Davidsson, Fontaine, & Hotz, 2007; Devi, Rush, Harper, & Venn, 2018; Wong, 2015). The literature suggests that the cut-off values used to define vitamin B_{12} deficiency is <148 pmol/L and depletion was defined as 148–221 pmol/L (Devi et al., 2018), since value is widely used in the epidemiologic setting to indicate insufficiency (Carmel, 2011). This thesis applied cut-off value based on the Canterbury Health Laboratories (Canterbury Health Laboratories, 2023).

Table 3: Vitamin B_{12} status measure and cut-off.

Status	Measures and cut-offs
Vitamin B_{12}	80-675 pmol/L

References for cut-off: vitamin B_{12} (Canterbury Health Laboratories, 2023).

2.5.3 Folate

Folate, a water-soluble B vitamin, has many roles within the body, with one of the most important roles as a coenzyme within single-carbon metabolism (Koury & Ponka, 2004). Folate comprises a base structure of a pteridine ring linked to para-aminobenzoic acid, with a different number of glutamate residues attached. Folic acid is one of several forms of folate. It is a synthetic folate isomer most commonly used for supplementation and food fortification. As such, it is required for the synthesis of DNA and is a key nutrient for growth, making it a nutrient of great concern for reproductive-aged females (Koury & Ponka, 2004; Snow, 1999).

Folate is abundant in dark leafy greens, lentils, and the liver. Other sources include fortified foods, supplementation and the folate produced by the gut microbiome (Medicine et al., 2002). Folate present in dietary supplements and fortified foods is in the form of folic acid. The folate fortification of foods continues to be a voluntary process in New Zealand, with approval to fortify breakfast cereals, bread, and fruit juices (MOH, 2003).

Generally, the bioavailability of natural food folate and synthetic folic acid is estimated to be 50 and 85%, respectively. Folate turnover results from losses due to catabolism, excretion and skin (Suh, Herbig, & Stover, 2001). Due to low storage capacity, serum folate decrease after only a few weeks of inadequate dietary intake and deficiency can develop in weeks to months (Jialal., 2021; Sriram & Lonchyna, 2009). Jialal (2021) state that folate deficiency can become evident in 8-16 weeks. A negative folate balance reduces pyrimidines and purines and results in an accumulation in homocysteine levels, leading to adverse effects on health. Megaloblastic anaemia is a common feature of folate deficiency (Koury & Ponka, 2004). The reduced cell division causes significant, nucleated erythrocyte precursors called macrocytes and hypersegmented neutrophils, resulting in decreased DNA synthesis and delayed maturation of bone marrow. Common characteristics of anaemia are weakness, fatigue, irritability, breathlessness and lack of concentration. Folate deficiency will also affect other areas with higher cell turnover rates, like the intestinal epithelium. This is shown as megaloblastic of the enterocytes, which can cause increased problems such as malabsorption and diarrhoea (Datta Mitra, Gupta, & Jialal, 2016; Jialal., 2021).

Folate status can be assessed by directly measuring serum folate and red blood cells. Red blood folate concentration indicates long-term status, while serum folate indicates folate status when the blood sample is collected. Serum folate of <7nmol/L mmol/L indicates negative folate balance (R. D. Lee & Nieman, 2007), which value was used in this research to indicate deficiency (Canterbury Health Laboratories, 2023).

Table 4: Folate status measure and cut-off.

Status	Measures and cut-offs
Folate	>7nmol/L

References for cut-off: serum folate (Canterbury Health Laboratories, 2023).

2.5.4 Zinc

Zinc plays a role in T-cell activation and natural killer (NK) cell and beta cell production (Prasad, 2009). Zinc deficiency may reduce immunity response by decreasing NK cell and B-

cell function. These actions are seen to be associated with infections such as tuberculosis, pneumonia, pulmonary infections, rough skin and delayed wound healing (Lowe, Fekete, & Decsi, 2009; Prasad, 2009). However, trials in older people have observed zinc supplementation assists with wound healing time and infection resistance (Barney & Perkinson, 2015). This study showed that in older populations, normal zinc levels (>70ug/dL) were associated with a decrease in the incidence and duration of pneumonia (Barney & Perkinson, 2015).

A study on serum trace elements in dysphagic gastrostomy candidates before endoscopic gastrostomy for long-term enteral feeding identified that 122 (84%) patients showed low zinc, while 24 (16%) presented normal values, zinc in the range (normal range: 70-120 mg/dl) (Santos et al., 2016).

According to the International Zinc Nutrition Consultative Group **(**ZiNCG), <10.7 μmol/L of serum zinc is recommended to assess zinc status at the population level (De Benoist et al., 2007). For the matter of assessing zinc status, the cut-off value used in this research is based on the Canterbury Health Laboratories reference range of total zinc 10.0-17.0 μmol/L (10 μmol/L = 650 ug/L (1 ppb), being with <10.0 μmol/L considered deficiency (Canterbury Health Laboratories, 2023).

Table 5: Zinc status measure and cut-off.

Status	Measures and cut-offs
Serum zinc	10.0-17.0 μmol/L

References for cut-off: serum zinc (Canterbury Health Laboratories, 2023).

2.5.5 Copper

As one of the essential minerals, copper (CU) plays many important roles in the body by acting as a cofactor for the enzyme function of ceruloplasmin (Cp) and superoxide dismutase 1 (SOD1). It also assists in haemoglobin formation, cell signalling, and cellular respiration (Myint et al., 2018; Osland et al., 2022). Copper is absorbed as Cu+ forms at the apical membrane of the enterocyte. A metalloreductase localised in the brush border membrane and involved in this process is expressed in the duodenum. Intestinal absorption is influenced by the chemical form that Cu is in and by interactions that Cu has with other components of the diet (Harvey & McArdle, 2008). Most healthy adults meet dietary Cu requirements because it is found in commonly consumed foods such as mushrooms, leafy green vegetables, and even cocoa (Medicine et al., 2002). However, copper deficiency in humans is rare but has been found in particular conditions, such as a result of long-term tube feeding (Ferrie et al., 2018; Kang et

al., 2014). To exemplify, individuals who rely solely on jejunal tube feeding are at significant risk of developing copper deficiency due to the duodenal and gastric mucosa's crucial role in copper absorption.

Serum copper levels are used to diagnose a deficiency. During the inflammatory response, ceruloplasmin, an acute-phase protein that increases during inflammation and transports 80-95% of copper, can lead to elevated blood copper levels (Altarelli et al., 2019). This author suggests using low serum ceruloplasmin (<20 mg/dL) in addition to low serum copper levels with an elevated C-reactive protein to diagnose deficiency (Altarelli et al., 2019). Per the present value, this study uses a serum copper concentration range of 11.0-20μmol/L and a lower of <11.0μmol/L to diagnose copper deficiency.

Table 6: Copper status measure and cut-off.

Status	Measures and cut-offs
Copper (plasma)	11.0-20.0 μmol/L

References for cut-off: copper plasma (Canterbury Health Laboratories, 2023).

2.6 24-hour Diet-Recall Assessment

Traditionally, food intake and behaviours have been assessed using subjective methods such as 24-hour diet recalls, food frequency questionnaires, and food records. All of these require participants to remember their past food intake or record it as it occurs (Gemming, Utter, & Ni Mhurchu, 2015). In the 24-hour diet recall, the respondent is asked to list and quantify all foods eaten during the previous day. The 24-hour diet recall has many advantages: it is inexpensive, easy to implement and has a low respondent burden (Bailey, 2021). However, the 24-hour recall is more feasible among some people than others. For example, older individuals must have sharp memories, honesty, and willingness to record and remember what they consumed. It can be burdensome and has the potential for social desirability bias, leading to intentional or unintentional misreporting of food intake and dietary habits. Therefore, there is potential for both researcher and technical errors in the data collected from using these methods (Bailey, 2021; Boushey, Spoden, Zhu, Delp, & Kerr, 2017).

Technological devices, such as mobile phones, have been used to increase the accuracy of dietary data collection. Mobile phones are reported to eliminate recall bias and reduce participant burden by prompting them to record and send their food intake at typical eating times. However, all these methods are prone to error and bias and require considerable time

and effort from the participants and researchers (Boushey et al., 2017). According to Martin et al. (2014), the 24-hour diet recall over the phone is a valuable method for when participants forget to record their food intake by reminding them when no recent information had been received at a specific time to confirm types and amounts of foods in poor quality images received and to prompt uncertain details. This researcher concluded that using mobile phone calls to ask questions and prompt participants regarding their 24-hour diet recall resulted in a compliance rate of over 95% (Martin et al., 2014). In agreement, a systematic review by Schembre et al. (2018) found that using a mobile phone to report intake and images of their food reduced participant burden and recall bias due to less reliance on participants remembering to record their food intake. This highlights an opportunity for using images of food to estimate portion sizes and food intake without burdening participants to remember everything they consume.

In summary, the 24-hour diet recall is an efficient interview method that captures detailed information regarding food intake. Using images to estimate portion sizes and food intake is an advantage for more precise dietary recall data collection. Using a mobile phone for prompting information, with images sent to the researchers, decreases bias and burden on participants and improves high-quality information from participants. However, it is still challenging for researchers to estimate portion sizes and the food's nutritional composition.

2.7 Nutrition-Focused Physical Assessment

The nutrition-focused physical exam (NFPE) is a system-based examination of each body region to evaluate nutrition status conducted by a registered dietitian, which involves inspection, palpation, percussion, and auscultation of physical parameters (Esper, 2015). The examination focuses on anthropometric measurements, visualization, subcutaneous fat and muscle stores, and assessment of the hair, eyes, oral cavity, skin, and nails for micronutrient deficiencies (Esper, 2015; MacQuillan et al., 2021). In contrast, the nutrition-focused physical assessment (NFPA) interprets the NFPE findings (Desjardins, Brody, & Touger-Decker, 2018).

The NFPE involves a head-to-toe evaluation for micronutrient deficiencies, malnutrition, digestive health, and functional status (MacQuillan et al., 2021). According to Esper (2015), it is the clinician’s job to gather all the patient’s clinical characteristics, relate them to nutrition status, and use clinical judgment to determine the severity of malnutrition. Compared with

other techniques or the use of biomarker values, clinical histories such as 24-hour diet recall and physical examination provide more accurate information about the nutrition status of patients (MacQuillan et al., 2021). Malnutrition and micronutrient abnormalities can widely affect organs and tissues, resulting in physical sequela that can quickly be detected by a trained practitioner using NFPE (Esper, 2015). Fischer et al. (2015) state that unlike physical examinations conducted by nurses or physicians, the NFPE primarily focuses on changes to muscle and fat stores and physical signs that can result from micronutrient deficiencies or excesses.

Currently, micronutrient assessment is not part of the American Society of Parental and Enteral Nutrition and Academy of Nutrition and Dietetics (ASPEN/AND) clinical characteristics to diagnose malnutrition (White, Guenter, Jensen, Malone, & Schofield, 2012). Although, NFPE should also consider the various parts of the body where high cell turnover occurs (e.g., hair, skin, mouth, tongue) because they are among the most likely to rapidly show signs of possible nutrient deficiencies (Jensen, Hsiao, & Wheeler, 2012). Symptoms of nutrition abnormalities may be non-specific, so other possible causes need to be considered (Fischer et al., 2015). For example, anaemia is associated with reduced serum albumin in haemodialysis patients in an inflammatory response setting, but this is not related to nutritional adequacy (Behzad, Hasan, Karimollah, Mehdi, & Roghayeh, 2015).

In the NFPE, system components-based examination of each body area includes the following: general inspection, vitals, skin, nails, head/hair, eyes/nose, mouth, neck/chest, abdomen, and musculoskeletal (Esper, 2015).

2.7.1 Skin

When observing the skin, the clinician should inspect for changes in colour, texture, temperature, moisture, lesions, mobility and turgor. The physical finding of pallor can be noted in overall appearance, conjunctiva (lower eyelid), nail beds, and tongue. These findings correlate with iron or B-complex vitamin deficiencies (they are involved in hematologic processes) (Esper, 2015). Researchers also complements that depigmentation of skin, hair and anaemia are common manifestations of copper deficiency (Dibaise & Tarleton, 2019; Esper, 2015; Vanek et al., 2012). Seborrheic dermatitis (red, inflamed spots on the skin) is seen in zinc deficiency (Aktas Karabay & Aksu Cerman, 2019) and correlates with B-complex vitamins (Esper, 2015). A vitamin B deficiency is rarely seen alone and is often accompanied

by other B-complex deficiencies (R. D. Lee & Nieman, 2007). Eczema, a reddish, scaly rash found on the face, neck, and hands, is a physical finding correlated with zinc deficiency (R. D. Lee & Nieman, 2007; Osland et al., 2022).

2.7.2 Nails

Inspection and palpation of the nail should be performed to assess for colour and texture. Nail textures of thinness, brittleness, or rigidity can indicate iron deficiency anaemia or inadequate dietary protein intake. In the koilonychia condition, the nail will appear concave and flat, like a spoon-shaped fingernail, and the central ridge (appearance of vertical ridge lines) indicates iron deficiency (Montgomery, Streit, Beebe, & Maxwell, 2014). Beau's line, for instance, is related to acute and severe disease and can occur in cases of zinc deficiency (Debra & Shari, 2022).

2.7.3 Hair/Head

Physical findings of hair loss or patchy areas on the scale can relate to protein deficiencies, malnutrition, and iron and zinc deficiency (Dibaise & Tarleton, 2019; Esper, 2015). Sparse hair (alopecia) and hair loss can be caused by the depletion of zinc and iron (Samer, Abdulla, & Ausama Ayob, 2018). These authors explain that zinc is a structural or regulatory factor in the hair follicle by accelerating recovery and inhibiting hair follicle regression. Iron, for instance, is essential in forming part of the enzymes implicated in DNA synthesis and cell respiration. A study found that the mean ferritin level in patients with androgenetic alopecia was significantly lower than that in normal individuals without hair loss (Park et al., 2013).

2.7.4 Mouth

According to Touger-Decker & Mobley (2013), the mouth has a three-to-seven-day turnover rate of most oral mucosal cells. Vitamin and mineral deficiencies can manifest within the oral cavity relatively quickly. These researchers complement that cellular changes can also occur from periodontal disease, infections, viruses, injury, or trauma, which should be considered when noting physical changes in the oral cavity (Touger-Decker & Mobley, 2013). Angular stomatitis or cheilitis (bilateral cracks and redness at the corners of the lips and mouth) are common deficiencies of B-complex vitamins and iron (Radler & Lister, 2013). Glossitis (a beefy, red tongue) and atrophied papillae can also be significant findings for these micronutrient deficiencies (R. D. Lee & Nieman, 2007). Hypogeusia (decreased sensitivity to

taste), dysgeusia (unpleasant perception of taste) and ageusia (complete loss of taste) are conditions that are characterized by changes in taste or total loss of tastiness (Rathee & Jain, 2022). If changes in taste and dryness are reported, the possibility of zinc deficiency can be investigated since zinc deficiency can affect cell structure and function (Dibaise & Tarleton, 2019; Gooding, Packer, & Pensiero, 2019).

2.7.5 Eyes

Pallor conjunctiva is a traditional sign used in the physical diagnosis of anaemia (Sheth, Choudhry, Bowes, & Detsky, 1997). A study suggests that pallor conjunctiva may be a more accurate indicator of the presence or absence of anaemia and has been reported to appear more frequently in patients with severe anaemia. It may be more sensitive than other signs, such as pallor of the palms or nail beds (Sheth et al., 1997), a physical sign related to iron and vitamin B_{12} deficiency due to anaemia (Sheth et al., 1997). Vitamin B_{12} deficiency anaemia and pallor conjunctiva are probably due to the common pathology of capillaries disruptions (Sheth et al., 1997), whether the iron deficiency is commonly associated with microcytic anaemia results from processes that impair haemoglobin synthesis in the RBC (Chai, Huang, Rakočević, & Chung, 2021). Thus, the present evidence suggests that severe anaemia causes the conjunctiva to appear abnormally pale due to reduced amounts of red-coloured oxyhaemoglobin that circulate in the dermal and subconjunctival capillaries.

2.8 Te Whatu Ora Counties Manukau

Counties Manukau Health is one of twenty District Health Boards (DHBs) in New Zealand and was established in 2001 and is primarily funded by the government (Lees J, 2021).

On 1 July 2022, the public health Counties Manukau District Health Board (CMDHB) were merged into Te Whatu Ora – Health New Zealand (all health services, including hospitals, specialist services, and primary and community care). Counties Manukau DHB is now called Te Whatu Ora – Health New Zealand Counties Manukau. For short, Counties Manukau District (CMD) (Commissioner, 2023).

Counties Manukau District is responsible for providing strategic direction for health and disability services in the northern region of NZ in collaboration with other Te Whatu Ora, services providers, the community, and other stakeholders. The CMD provides and funds most health and stability services through contracts with health and disability providers and non-governmental organizations (CMH, 2021). Counties Manukau District offers hospital-based

services for its population, and some patients referred from other Te What Ora have access to specialist or highly complex services. It is committed to promoting, protecting and improving the health of its population through public health promotion, health education, and evidence-based public health initiatives (Lees J, 2021).

The CMD has the most prominent and growing diverse population in NZ with strong cultural values, expected to increase to 17% Māori, 23% Pacific, 33% Asian, and 26% NZ European and other ethnicities by 2030 (Lees J, 2021). It is home to New Zealand's second-largest Māori and the largest Pacific people and Asian communities. Communities within the CMD have different health outcomes and subsequent experiences of health, with thirty-seven per cent of the CM population living in the most socioeconomic deprived. Counties Manukau District is divided into four localities Maangere/Ōtara, Manukau, Eastern and Franklin (Lees J, 2021).

Long-term conditions (LTCs) are illnesses that progress slowly, limit daily living activities, require ongoing medical treatment, are rarely cured, and last over twelve months. Long-term conditions are a significant cause of death and disease in CM ("Long term Conditions and their Impact in Counties Manukau," 2015). Long-term conditions (i.e., cancers, cardiovascular diseases, respiratory diseases and dementia) have become the most significant cause of death and illness globally and are strongly associated with long-term enteral feeding (Mendis et al., 2014). In CMD, LTCs are a major cause of death and illness with the most significant impact on diseases such as cardiovascular disease, chronic respiratory diseases, chronic obstructive pulmonary disease and asthma, diabetes, arthritis, including gout congestive heart failure ("Long term Conditions and their Impact in Counties Manukau," 2015). Common contributing factors for LTCs are smoking, unhealthy diet, lack of physical activity and harmful use of alcohol. Also, reports from CMD show that Māori, Pacific and older New Zealanders in the CMD catchment area are disproportionately affected and tend to have lower incomes, greater exposure to health risks and lower access to health services, which may result in poorer health and lead to malnutrition, especially due to LTCs ("Long term Conditions and their Impact in Counties Manukau," 2015). For example, low income is more common in the CM Heath district than in the rest of the NZ population, with approximately 49% of the CM Heath population aged 15 years and over having a personal income of $30,000 or less per year, compared to 35% for of the rest of the NZ population for the same age (Lees J, 2021).

Overall, CMD provides health services for a large population in the Northern region of New Zealand, with a large population being Māori, Pacific and Asians. Long-term conditions appear

to be a significant problem among CMD, and unhealthy diet and income contribute as risk factors for developing diseases.

3. Chapter 3: Research Study Manuscript

The micronutrient status of long-term Home Enteral Nutrition (HEN) patients of Te Whatu Ora Counties Manukau.

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3.1 Abstract

Background: Long-term home enteral nutrition patients from Te Whatu Ora Counties Manukau District may be at increased risk for iron, folate, zinc, copper and vitamin B_{12} deficiency. These micronutrients are essential for disease prevention, improving health complications and wellbeing. Enteral feeds contain adequate nutrients but may be digested and absorbed differently than whole foods. Nutrient deficiencies can only be treated once recognised and confirmed; therefore, it is essential to investigate if deficiency is present in patients receiving HEN.

Method: Forty-two participants completed a detailed 5x 24-hr dietary assessment of enteral nutrition (EN) and oral food. The nutrition-focused physical findings (NFPF) were conducted to identify micronutrient deficiency. The Charlson Comorbidity Index Score (CCIS) determined the estimated 10-year survival rate for all participants based on the summing weight of 17 comorbidities. Adequacy of zinc, copper, folate, and vitamin B_{12} were determined using the Recommended Daily Intake (RDI) for adults >18 years. Biomarkers of folate, vitamin B_{12}, copper, zinc, iron, ferritin, transferrin saturation, transferrin and haemoglobin were measured in 21 participants and compared to adult reference ranges. Descriptive analyses were conducted for participants' health characteristics and demographics. Statistical regression evaluated dietary intake, the NFPF and biomarkers. A stepwise regression was performed to confirm normality, and the models met all assumptions for regression.

Results: Nine (47%) participants had iron intake below the RDI, dietary folate equivalent (n=3, 15.9%), vitamin B_{12} (n=3, 26%), zinc (n=1, 5.3%) and copper (n=1, 6.3%) copper. Enteral intake for iron was below the RDI in women with a mean±standard deviation of 17.1±8.3 mg/d. Serum iron (n=4, 19.4%) and zinc (n=14, 66.7%) were below the reference range. Alopecia was correlated with decreased dietary intake of iron and zinc and reduced serum zinc. Eczema dermatitis, perioral stomatitis, and the absence of wound healing and alopecia combined were

associated with lower blood zinc concentration. Age, CVA/TIA, liver disease, and myocardial infarction were contributor commodities to decrease the risk 10-year survival rate.

Conclusion: Dietary intake was generally adequate, and iron and zinc stores were largely below the cut-off values in this population. Strategies to optimise micronutrient intake and bioavailability via EN and oral intake may be beneficial.

3.2 Background

Eating is essential for humans, but when affected by a disease, the ability and pleasure of eating can be reduced or impaired. Studies suggest that patients who cannot eat orally and rely on HEN for their daily requirements may be at a higher risk of developing micronutrient deficiency than those who solely get their nutrients orally (Boullata et al., 2017). For those needing nourishment feeds, eating problems related to chewing and swallowing are commonly associated with neurological and gastrointestinal diseases, cancer and brain injury (Rolandelli, 2005; Santos et al., 2016). For patients unable to meet their nutritional needs orally (i.e., patients with eating problems having their gut preserved), enteral tube feeding is the preferred mode for delivery of nourishment (Boullata et al., 2017; Liley & Manthorpe, 2003). Worldwide, enteral nutrition provides nutrition intake through the gut, orally or through an enteral access device (Boullata et al., 2017; Ferrie et al., 2018). The most frequent, safe and well-tolerated tube feeding methods used in long-term HEN include; percutaneous endoscopic gastrostomy (PEG), PEG with jejunal extension (PEG-J), nasojejunal tube (NJT), percutaneous endoscopic jejunostomy (PEJ), radiologically inserted gastrostomy (RIG), and jejunostomy tube (JJ) (Boullata et al., 2017; Pearce, 2002). Home enteral nutrition (HEN) is the method of delivering nutrition via tube feeding outside the hospital setting. It can significantly improve a patient's quality of life in community healthcare settings (Ojo & Brooke, 2016) and is referred to as a long-term feeding strategy when administrated for up to 4-6 weeks or longer (Bischoff et al., 2020).

Previous reports show that trace elements such as zinc, iron, copper, vitamin B_{12}, and folate become deficient in a patient receiving HEN over different timeframes due to eating problems related such as chewing or swallowing difficulties and gastrointestinal diseases (Kang et al., 2014; Nishiwaki et al., 2011; Oliver et al., 2005; Vural et al., 2020). Trace elements such as iron, folate and vitamin B_{12} are intertwined in the production of red blood cells in the haematopoietic system (Mette et al., 2019). Copper plays many essential roles in the body by

acting as a cofactor for the enzyme function of ceruloplasmin and assisting in haemoglobin formation, cell signalling, and cellular respiration (Jaiser & Winston, 2010). Zinc's role within the human body is extensive in reproduction, immune function, and wound repair (Maxfield et al., 2022). Therefore, a severe deficit in dietary intake of micronutrients may lead to developing physical signs and symptoms, worsening health status (Aktas Karabay & Aksu Cerman, 2019; Altarelli et al., 2019; Dibaise & Tarleton, 2019). The longevity of survival rate by the Charlson Comorbidity Index has been reported to have a higher mortality rate after HEN initiation (Tsugihashi et al., 2021), and whether it affects this study population group is of concern. Charlson Comorbidity Index performs equally well in primary and secondary care data over the long-term (10 years), and whether combining information would provide a better measure of survival rate (Kobayashi et al., 2002).

Under this study, the demographics investigated are Te Whatu Ora Counties Manukau District, for short Counties Manukau District (CMD) patients receiving long-term HEN. Te Whatu Ora Counties Manukau District plays an essential role in promoting health and wellbeing among a variety of ethnic groups in New Zealander, including Māori, Pacific, Asians and European people. A previous study in New Zealand reported that 12% of Māori women aged 15-49 years presented iron deficiency compared to European/other 7%, showing deficiency of nutrients in the population of this study.

Therefore, in this study, we aim to assess the micronutrient status of long-term HEN patients (≥18 years) of CMD patients by assessing biomarker results, dietary intake based on the recommended dietary intake (RDIs), and the physical signs and symptoms of deficiency as well the longevity of survival rate by CCIS of this population.

3.3 Materials and Methods

3.3.1 Study Design and Participant's Recruitment

This study was carried out as an exploratory observational study of serum zinc, iron, copper, vitamin B_{12} and folate concentrations to determine nutritional intake, biomarker status and physical signs and symptoms in patients of CMD receiving long-term HEN treatment (over four weeks of tube-feeding). C-reactive protein and albumin were used as inflammatory markers. Participants have required a total commitment of 1-2 visits to complete data collection. Blood sample collection, physical signs and symptoms examination, and the first

24-hour diet recall interview were conducted face to face and the remaining over the phone. The data used in this study were collected over three months (August-October 2022).

The sample size was calculated based on the Kang et al. study (Kang et al., 2014), with an estimated prevalence of iron, copper, zinc and selenium deficiencies of 22.7%, 4.5%, 26.6%, and 9.1%, respectively. Based on the prevalence of iron as the most compelling nutrient, a minimum sample size of ±66 to estimate a deficiency of 22.7% with a 10% level of precision and 95% confidence Interval.

The inclusion and exclusion criteria were applied to identify potential participants. Inclusion criteria: HEN patients in CMD that were ≥18 years old; receiving enteral tube feeding for >4 weeks. Exclusion criteria: all patients that received supplemental parenteral nutrition and were palliative. All participants for this study were recruited using the CMD data system. Seventy-nine patients were eligible to participate in the study; however, only 42 were willing to participate in the partial or complete data collection. Some reasons included refusal to respond to particular questions, refusing the physical assessment, and refusing or being unable to give blood samples due to personal reasons or failure of the blood draw. Therefore, only 21 participants provided blood samples.

3.3.2 Ethical considerations

Prior to their inclusion in the study, written informed consent was obtained from all participants. Following consent to participate, all participants were given a study ID number to ensure confidentiality.

Massey University Human Ethics Committee (MUHEC) granted ethical approval for the "The health, wellbeing and nutritional outcomes of long-term enterally fed patients – Home Enteral nutrition Performance (HELP)" study on the 26th of June 2022 (HEC: Southern A Application SOA 22/20). Also, approval was granted by the ANZCTR in July 2022 (registration number: ACTRN12622001044718) and by Te Whatu Ora – Health New Zealand Counties Manukau (research registry 1631).

3.3.3 Dietary assessment – 24-hour diet recall

All participants were required to complete five random 24-hour food recalls. Both enteral feeding regime and oral food intake were collected over four weeks, including weekdays and weekends. Participants were asked to recall all enteral feeding regimes and food and drink items consumed in the last 24 hours on the previous day between midnight and midnight.

The first 24-hour recall was carried out in person (at the participant's home) with the participant, a family member or carer giver when required due to the complexity of cases. The four other diet recalls were carried out via telephone/video calls, text messages and emails. A standard operating procedure was used to guide performing consistent 24-hour recalls for both enteral feeding and food intake. Before data collection, all data collectors, including the candidate, attended several training sessions for dietary and anthropometric data collection.

The 24-hour recalls followed a 3-stage multiple pass method using a standardised protocol. In the first stage, a quick list was made based on the recollection of all the food and drinks participants consumed on the previous day (between midnight and midnight). The second stage gathered detailed information (e.g., brand name, cooking method and recipes) about the food and drinks consumed and their time of consumption. Amounts of each food and beverage consumed were noted using household measures (e.g., cups, spoons) to quantify the food and drink intake with the help of other probes (food models, measurement aids, photographs). The enteral feeding regime was recorded based on the patient-specific daily prescribed feed volume. Both enteral feeding and oral food intake for the nutrients of interest were analysed using FoodWorks 10 (Xyris Software (Australia) Pty Ltd, 2019).

3.3.4 Biomarker analysis

Biomarker analysis for the minerals (iron, zinc, copper), vitamins (vitamin B_{12}, folate), full blood count (haemoglobin, transferrin, transferrin saturation and ferritin) and inflammatory markers (C-reactive protein and albumin) were collected in a non-fasting state. Blood samples were collected by phlebotomists, a medical doctor and/or by an NZRN participating in the study, using EDTA and heparin-coated tubes and processed per pathology laboratory protocols. All samples were snaped-frozen in separate aliquots in Eppendorf tubes and stored at -80 ºC until analysis. Biomarker analysis was carried out by Canterbury Southern Community Laboratories, a fully internationally accredited medical diagnostic laboratory in Christchurch, New Zealand.

3.3.5 Nutrition Focused Physical Examination

A trained researcher assessed the physical signs and symptoms of micronutrient deficiency face-to-face during the first visit using a SOP (refer to Appendix D). The designed SOP used detailed instructions and images to guide the interviewers to perform consistent physical signs and symptoms examinations. The investigation included specific trace elements of deficiencies

(zinc, iron, copper) and vitamins (vitamin B_{12}, folate). The physical signs and symptoms examination of the selected micronutrients included hair, eyes, mouth, tongue, taste, nails, skin and wound healing (see Table 12).

3.3.6 Charlson Comorbidity Index Score

An online form of *the Charlson Comorbidity Index Score* was used to determine the severity of the clinical situation and the estimated 10-year survival rate of each participant. The diagnostic form was completed by the research candidate or an authorised person part of the research team. Seventeen conditions were assessed, and each condition was assigned a weight from zero to six based on the estimated 10-year mortality risk. These weights were summed to produce a patient-level score of an estimated 10-year survival rate. Scores were categorised using the following cut-points: severe (>5), moderate (3-4), mild (1-2), or no comorbidities (0). Higher numerical weighting appropriate to its severity indicates a more severe condition and a worse prognosis. By using the CCIS tool, we can gain insight into the life expectancy of our participants and identify comorbidities that may predict mortality. Patients who require feeding tubes may also have multiple concurrent conditions, such as cancer, organ failure and malnutrition, which can develop at various stages of long-term diseases, such as gastrointestinal cancer. Malnourished patients may have a higher likelihood of low levels of specific micronutrients, leading to nutrient deficiencies that could contribute to more severe complications in patients diagnosed with CCIs. We are interested in understanding the deficiency of micronutrients and how life expectancy is predicted for this population to improve their quality of life, enhance their clinical status, and increase their life expectancy.

3.3.7 Statistical Analysis

Statistical analyses were performed using IBM SPSS statistics package version 28 (SPSS Inc., Chicago, IL, USA). Multiple linear regression was used to determine associations between biomarkers (blood levels of zinc, iron and copper) and nutrition-focused physical signs. A stepwise method was used to build a final model. The independent variables included in each model are shown in Table 7. Tests were performed on the model residuals to confirm normality (histogram and Shapiro-Wilk), independence (Durbin-Watson), equality of variance (scatter plot) and multi-collinearity (VIF and tolerance). Descriptive statistics (i.e., means, median and frequencies) were calculated to summarise data frequency for:

1. Demographics, participants' and health characteristics.

2. Charlson Comorbidity Index Score.
3. Dietary intake and adequacy in relation to NRVs and reference ranges.
4. Biomarker assessment of iron, vitamin B_{12}, folate, zinc and copper.

Frequency tests and (%), in particular, were used to analyse yes/no questions. The independent-Sample T-test (2-tailed) analysis was completed to determine the difference between EN intake and oral food source. Pearson correlation tests were used to assess the correlation coefficient between blood levels of micronutrients by C-reactive protein, serum albumin and haemoglobin. Linear regression T-test ANOVA was used to determine the odds ratio (likelihood) of deficiency between biomarkers status according to their nutrition-focused physical signs and symptoms of deficiency. A *P* value <0.05 was considered statistically significant, and all tests were two-tailed.

Table 7: Table of variables in each model

Physical signs and symptoms of deficiency	**Zinc**	**Iron**	**Copper**
Seborrheic dermatitis	ü		
Hypogeusia	ü		
Dysgeusia	ü		
Ageusia	ü		
Eczema dermatitis	ü		
Beau's line	ü		
Wound healing	ü		
Perioral stomatitis	ü		
Alopecia	ü	ü	
Pallor (skin)		ü	
Pallor conjunctiva		ü	
Glossitis		ü	
Pale tongue		ü	
Koilonychia		ü	
Vertical ridges		ü	
Hypopigmentation			ü
Diet Recall	ü	ü	ü

3.4 Results

Forty-two participants were enrolled into the study, and completed the demographic questionnaire, dietary assessment, physical findings assessment, Charlson comorbidity index score (CCIS). However, of these, only 21 participants were able to provide an adequate blood sample for analysis. Data analysis was distinguished by the total number of participants and gender when appropriate.

3.4.1 Demographics and participants' characteristics

Of the 42 participants, 19 (45%) were women with a mean age of 45 [20, 62] years and 23 (55%) men mean age of 49 [27, 66] years. Eight women (42.1%) and 19 (42.1%) men self-reported the questionnaires and assessments. Predominantly, 27 (64%) participants identified as European and only one (2%) male participant identified as Māori. A total of (36%) of the participants resided in the Eastern area; the others were from the Franklin (26%), Māngere/Ōtara (14%), and Manukau (24%) localities. Approximately a half of the participants (47%) had a household income less than $60,000 per year and only six (14%) had >100,000.

The majority [29 (69%)] of the participants did not take additional supplements, and those that did, used mainly vitamins and men only used minerals, amino acid and omega-3 fatty acid supplements. Thirty-seven participants reported the duration of EN treatment (M = 18.76, SD = 12.02) years.

Table 8: Participant characteristics, demographics, and health characteristics by gender.

Participant characteristics		**Total**	**Women**	**Men**
Number of participants[2]		42	19 (45)	23 (55)
Self-reported[2] (myself/support person)	Myself	27 (64.3)	8 (42.1)	19 (82.6)
	Someone else	15 (35.7)	11 (57.9)	4 (17.4)
Demographics				
Age (years)[1]		47 [26,64]	45 [20,62]	49 [27,66]
Ethnicity[2]	European	27 (64)	12 (63)	15 (65)
	Māori	1 (2)	-	1 (4)
	Pacific people	9 (21)	5 (26)	4 (17)
	Asian	5 (12)	2 (10)	3 (13)
	Other ethnicity	-	-	-
Locality[2]	Eastern	15 (36)	7 (36.8)	8 (34.8)
	Franklin	11 (26)	5 (26.3)	6 (26.1)
	Māngere/Ōtara	6 (14)	3 (15.8)	3 (13.0)
	Manukau	10 (24)	4 (26.1)	6 (26.1)
Household income[2]	<20,000	9 (21)	4 (21.1)	5 (21.7)
	20,001 – 40,000	6 (14)	3 (15.8)	3 (13)
	40,001 – 60,000	5 (12)	1 (5.3)	4 (17.4)
	60,001 – 80,000	4 (9)	1 (5.3)	3 (13)
	80,001 – 100,00	7 (18)	4 (21.1)	3 (13)
	≥ 100,000	6 (14)	2(10.5)	4 (17.4)
	Prefer not to answer	5 (11.9)	4 (21.1)	1 (4.3)
Health characteristics				
Dietary supplements[2,3]	No	29 (69)	15 (79)	14 (61)
	Yes	13 (31)	4 (21)	9 (39)

Type of dietary supplement[2]	Vitamins	7 (17.7)	2 (11)	5 (22)
	Minerals	1 (2.4)		1 (4.3)
	Amino Acid	1 (2.4)		1 (4)
	Omega 3 – Fatty Acids	2 (4.8)		2 (9)
Timeframe of EN[4] treatment (n=37, missing=5)	Minimum (years)	Maximum (years)	Mean (years)	Std. Deviation
	1	50	18.76	12.02

[1]Age expressed as mean [IQR]. [2]Data expressed as number and (%). [3]Include intake of additional vitamins (Clinician Booster, Multivitamin, Thiamine, Berroca, Centrum Men, Vitamin C, Mvite tablets), mineral (Iron tablets), amino acids (Creatine), omega-3 fatty acids (Krill oil, Fish oil). [4]Enteral Nutrition.

3.4.2 Charlson Comorbidity Index Score by Gender

Figure 1 shows the total distribution of CCIS, and Figure 2 shows the total percentage of the 10-year survival rate, both for all participants.

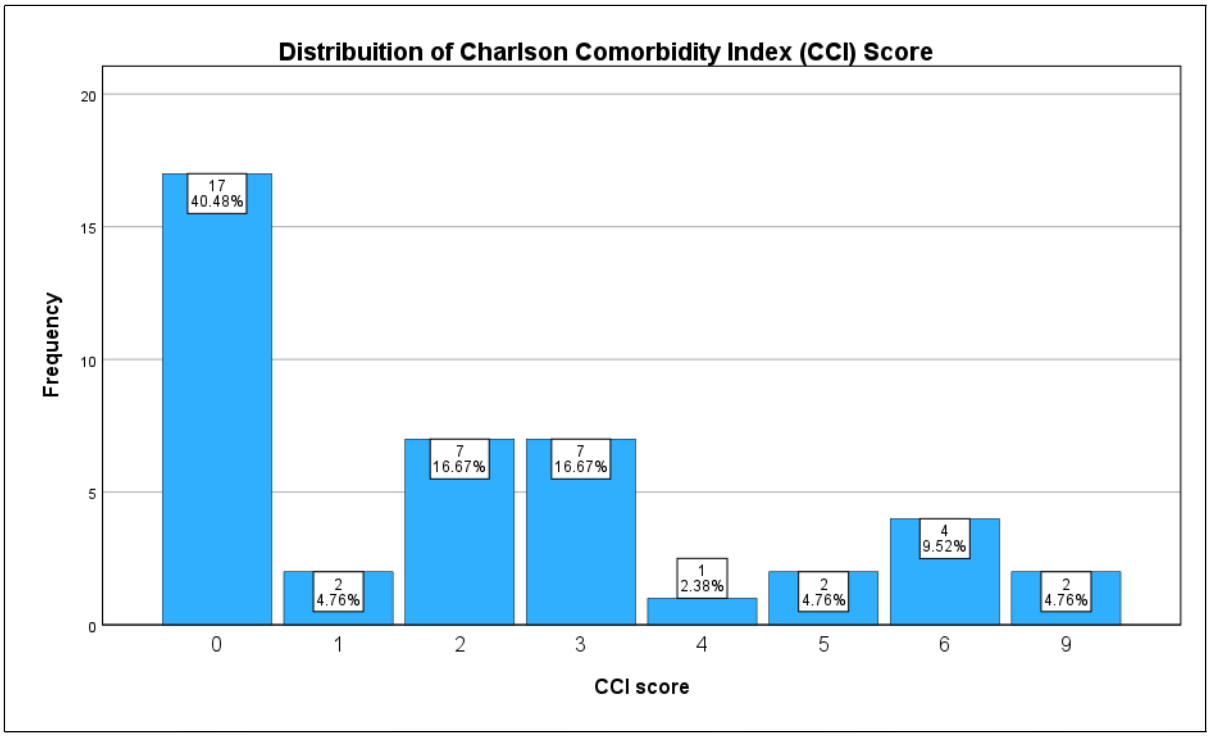

Figure 1. Distribution of Charlson Comorbidity Index Score for all participants.
Data demonstrating the weighting of Charlson comorbidity index scores (0-9). Highest score = more likely the predicted outcome results in mortality. Score zero = indicates no risk of mortality based on the sum of comorbidities

Figure 1 shows the total CCIS, which consists of a simple sum of the weights, with higher scores indicating a greater mortality risk and more severe comorbid conditions (see Table 9). The mean score for all participants showed that 17 (40.5%) participants scored zero points, meaning no risk of mortality, and two (4.8%) participants had the highest score of 9 points

which is associated with the highest risk of mortality. The CCIS 2 and 3 had equal results [7 (16.67%)], indicating a moderate mortality risk.

Prevalence of comorbid conditions in each category of the Charlson Comorbidity Index.

The Charlson Comorbidity Index Score (CCIS) are presented in Table 9. Each comorbidity category has an associated weight (from 1 to 6), and the sum of all weights results in a single comorbidity score for a patient. The higher the score, the more likely the predicted outcome will result in mortality, and a score of zero represents no comorbidity found. Seven-teen different categories were used to identify comorbidity, but only five diagnoses were found in this study.

The most predominant comorbidity found was solid tumor CCIS 2 for both women [3 (15.8%)] and men [8 (34.8%)]. The comorbidity age was a predictor factor contributing to mortality, and it was found ranging between the CCIS 1 to 4.

Table 9: Prevalence of comorbid conditions in each category of the Charlson Comorbidity Index.

Gender	**Comorbidity**	**CCIS 0**	**CCIS 1**	**CCIS 2**	**CCIS 3**	**CCIS 4**	**CCIS >4**
Women (n=19)	Age	-	3 (15.8)	4 (21.1)	1 (5.3)	1 (5.3)	0
	CVA & TIA	-	1 (5.3)	0	0	0	0
	Liver disease	-	1 (5.3)	0	0	0	0
	Solid tumour	-	0	3 (15.8)	0	0	1 (5.3)
Gender	Comorbidity	CCIS 0	CCIS 1	CCIS 2	CCIS 3	CCIS 4	CCIS >4
Men (n=23)	Age	-	5 (21.7)	3 (13)	2 (8.7)	2 (8.7)	0
	Myocardial infarction	-	1 (4.3)	0	0	0	0
	CVA & TIA	-	4 (17.4)	0	0	0	0
	Solid tumour	-	0	8 (34.8)	0	0	2 (8.7)

Comorbidities expressed as number and (%). CCIS: Charlson Comorbidity Index Score; cerebrovascular accident (CVA); transient ischemic attack (TIA). CCIS: Weighting of CCIS (1-6), the sum of all the weights, results in a singles comorbidity score. The higher the score, the more likely the predicted outcome result in mortality. A score of zero indicates no comorbidity found.

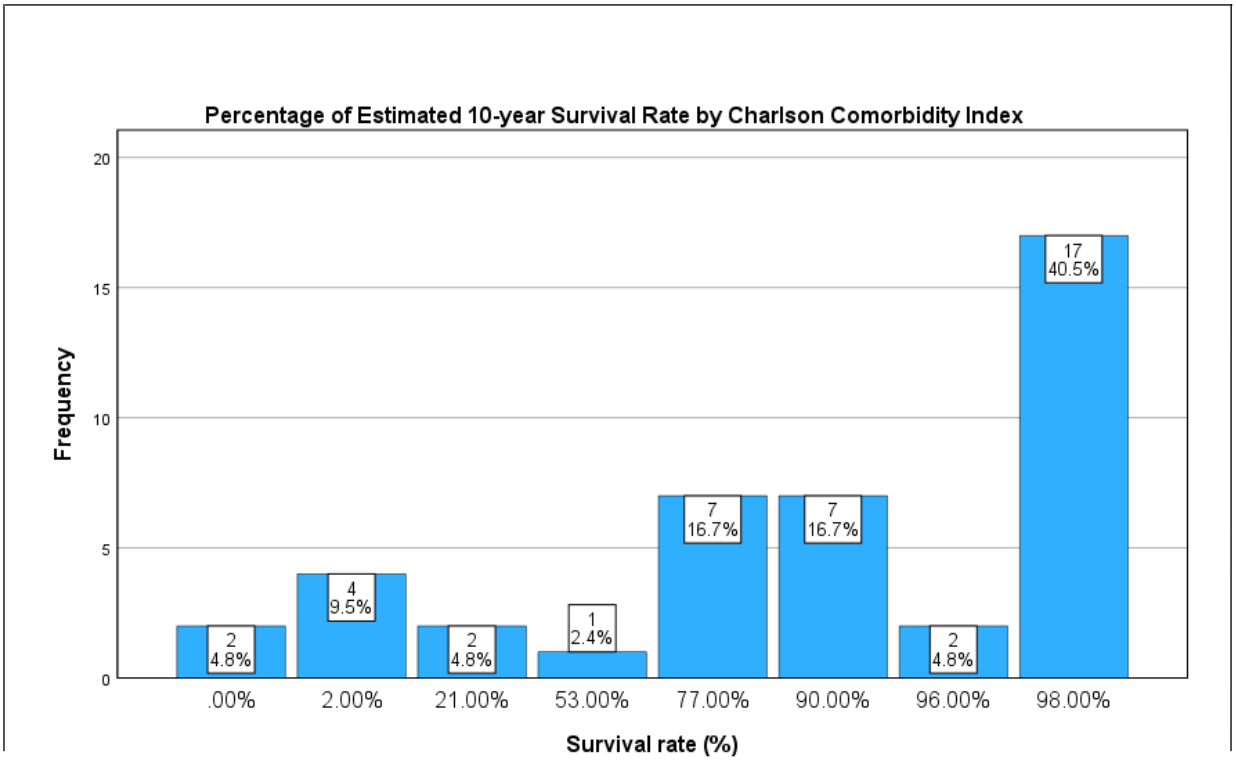

Figure 2. Comparison of Estimated 10-year Survival Rate by CCIS for all participants.

Data demonstrating the (%) of 10-years mortality rate based on the sum of total Charlson comorbidity score. 0.00% = lowest survival rate, and 98% = highest survival rate.

Figure 2 shows the mortality rate based on the scores of comorbidity conditions, indicating survival time is decreased when CCIS increases. Two (4.8%) participants were estimated to have a zero per cent of 10-year survival rate, while 17 (40.5%) participants had a 98% survival rate in the next ten years according to the CCI percentage rating.

3.4.3 Total dietary intake of Energy, iron, folate, vitamin B_{12}, copper and zinc.

Table 10 provides details on the actual intakes in relation to the recommended dietary intake (RDI) and biomarker results. The average dietary intake (oral food sources and EN) among the participants for iron was 25.30mg/d, total folate 479µg/d, dietary folate equivalent (DFE) 744µg/d, vitamin B_{12} was 5.3mg/d, copper 2.7mg/d, and zinc 20.3 mg/d.

3.4.4 Biomarker status

Biomarkers for iron, folate, vitamin B_{12}, copper and zinc in relation to recognised cut-offs are reported in Table 10. Deficiency of the selected micronutrients is identified when biomarkers

are below the cut-off values. Serum iron was below the cut-off (<10g/L) in 4 (9.6%) participants. Four (9.6%) and three (7.2%) participants were low in transferrin saturation and serum ferritin, respectively. Only three (14.3%) of men were below their respective haemoglobin reference ranges (<130g/L).

Approximately seven (43.2%) men and seven (37.1%) women were below the cut-off value for serum zinc. No men and women were below the cut-off for serum folate, serum vitamin $B_{12,}$ and copper. C-reactive protein was found higher in 5 (12%) participants and only two (4.8%) participants had a low albumin level.

Table 10: Iron, folate, vitamin B_{12}, copper and zinc: total intake and nutritional biomarkers in relation to cut-off values.

	Total	**Women (n=8)**	**Men (n=13)**	**P – value***
Energy intake (KJ)[1] (n=42)	8488 [6497,10354]	7273 [5251,9133]	9492 [7751,11340]	0.004
Iron (n=21)				
Intake (mg/d)[1]	25.3 [18.8, 29.8]	19.20 [14.0, 24.1]	27.4 [25.2, 32.7]	<0.001
Haemoglobin (g/L)[1]	149 [134, 163]	134 [135, 149]	157 [132, 172]	0.267
HB <130g/L (men)[2] <120g/L (women)	3 (14.3)	0	3 (14.3)	
Serum iron (µmol/L)[1]	13 [11, 18.0]	12 [9.3, 16]	14 [11, 18.0]	0.327
<10 (µmol/L)[2]	4 (19.04)	2 (9.5)	2 (9.5)	
Transferrin (g/L)[1]	2.4 [2.2, 2.7]	2.6 [2.4, 3.0]	2.4 [2.2,2.5]	0.029
<2.0 (g/L)	0	0	0	
Transferrin (% Saturation)[1]	21 [21 [16.5, 29.5]	18 [12, 28]	22 [19, 31.5]	0.237
<16%[2]	4 (19.04)	3 (14.28)	1 (4.76)	
Serum ferritin[1,2]	51 [28.5, 127.5]	36 [18.5, 51.0]	118 [47.5, 185.5]	0.052
20-350 ug/L (>15yr-30yr men) 20-400 ug/L (>30yr men) 20-150 ug/L (>15yr-30yr women) 20-300 ug/L (>30yr women)	3 (14.28)	2 (9.52)	1 (4.76)	
Folate (n=21)				
Total Intake (µg/d)[1]	470.7 [379, 564]	393 [294, 486]	550 [481, 578]	0.001
Total Folate (DFE) (µg/d)[1]	744.2 [603.4, 904.4]	757.0 [538.4, 897.7]	733.6 [606.2, 924.0]	0.001
Serum folate (n/mol/L)[1]	43 [33, 56]	48 [34.75,57.5]	41 [33,56]	0.492
<7 (nmol/L)	0	0	0	
Vitamin B_{12} (n=21)				
Intake (mg/d)[1]	5.3 [3.5, 5.9]	4.9 [3.2, 5.6]	5.7 [3.8, 7.3]	0.343
Serum vitamin B_{12} (pmol/L)[1]	294.5 [216, 461]	349 [244, 639]	259 [181, 461]	0.384
<80 (pmol/L)	0	0	0	
>675 (pmol/L)[2]	3 (14.28)	2 (9.52)	1 (4.76)	
Copper (n=21)				
Intake (mg/d)[1]	2.7 [2.2, 3.3]	2.2 [1.6, 2.7]	3.1 [2.6, 3.5]	<0.001
Copper plasma (µmol/L)[1]	17.4 [15.2, 18.6]	18.1 [17.4, 18.7]	16.9 [14.7, 19.0]	<0.161
<11 (µmol/L)	0	0	0	
>20 (µmol/L) [2]	3 (14.28)	1 (4.76)	2 (9.52)	

	Total	Women	Men	P – value*
Zinc (n=21)				
Intake (mg/d)[1]	20.3 [16.2, 24.6]	17.7 [12.8, 22.1]	22.4 [18.5, 25.7]	0.015
Serum zinc (μmol/L)[1]	9.0 [8.2, 9.8]	9.4 [7.8, 9.8]	9.0 [8.2, 9.6]	0.645
<10 (μmol/L)[2]	14 (66.7)	7 (33.33)	7 (33.33)	
>17 (μmol/L)	0	0	0	
Inflammatory Markers (n=21)				
Albumin <32 g/L[2]	2 (9.52)	1 (4.76)	1 (4.76)	
CRP >5 g/L[2]	5 (23.8)	1 (4.76)	4 (19.04)	

[1]Expressed as mean [IQR]. [2]Data expressed as number and (%). Biomarker results (n=21 participants); dietary intake results (n=42 participants).
References: RDI (recommended dietary intake) and AI (adequate intake) (NHMRC, 201).

3.4.5 Enteral nutrition and oral intake food sources by gender

Total mean and standard deviation (SD) dietary intake was differentiated into the contribution by enteral nutrition (EN) and food sources independently for Energy (KJ), iron (mg/d), folate (µg/d), vitamin B_{12} (mg/d), copper (mg/d) and zinc (mg/d) (see Table 11). Overall, EN sources contributed the most towards the total dietary intake compared with oral food sources.

All men met the RDI for iron, folate, vitamin B_{12}, zinc and AI for copper. A large proportion of women did not meet their RDIs, this being for folate (DFE) three (15.9%), for iron [9 (47.2%)], vitamin B_{12} [3 (26%)], copper [1 (6.3%)] and zinc [1 (5.3%)].

Of 42 dietary recall assessments, 23 participants were receiving EN feeding solely, and 19 participants received a combination of EN and oral food sources

Table 11: Comparison of micronutrient intake distribution, oral intake food sources vs enteral feeding sources by gender in relation to RDIs.

	Women (n=19)			**Men (n=23)**		
	Total intake (x̄±SD)	Oral intake (x̄±SD)	EN intake (x̄±SD)	Total intake (x̄±SD)	Oral intake (x̄±SD)	EN intake (x̄±SD)
Energy						
Energy (KJ)	7273.1±2554.1	2456.7±3134.6	4816.4±2219.8	9492.7±2134.8	2091.9±2994.0	7400.8±2491.4
Iron						
Iron (mg/d)	19±7.3	2.9±4.5	17.1±8.3	28±5.7	3.5±5.9	24.5±8.2
<RDI[1] (8mg/d, >19yr men); (18mg/d, 19-50yr women); (8mg/d, >51yr women)	Inadequate 9 (47)	-	-	Adequate	-	-
Folate						
Total folate (µg/d)	393±125.8	83.8±119.4	310.0±138.3	550±113.2	94.0±139.4	456.6±160.2
Folate (DFE) (µg/d)	757±231.8	96.3±162.8	660.7±225.5	733.6±198.6	134.2±162.7	599.3±278.7
<RDI [Folate (DFE)][1] (400µg/d, >18yr women and men)	Inadequate 3 (15.9)	-	-	Adequate	-	
Vitamin B_{12}						
Vitamin B_{12} (mg/d)	4.9±3.3	1.9±3.2	3.0±1.4	5.7±3.7	1.0±1.5	4.8±3.9
<RDI[1] (2.4µg/d, >18yr women and men)	Inadequate 3 (15.8)	-	-	Adequate	-	-
Zinc						
Zinc (mg/d)	17.7±6.3	4.1±5.8	13.6±6.2	22.4±4.9	2.6±3.8	19.8±6.5
<RDI[1] (13mg/d,18yr boys); (14mg/d,>19yr men); (7mg/d, 18yr girls); (8mg/d, >19 yr women	Inadequate 1 (5.3)	-	-	Adequate	-	-
Copper						
Copper (mg/d)	2.2±0.74	0.3±0.4	1.9±0.9	3.1±.61	0.3±0.5	2.8±0.9
<AI[1] (1.5mg/d, ≥ 18yr men); 1.1mg/d, 18yr girls); (1.2mg/d, >19yr women)	Inadequate 1 (6.3)	-	-	Adequate	-	-

[1]Expressed as number and (%). x̄: Mean value of intake, $p<0.05$. SD: Standard deviation. Folate (DFE): Dietary folate equivalents. Adequate/Inadequate in relation to RDIs. EN: Enteral Nutrition (sources).

3.4.6 Nutrition-Focused Physical Assessment

Assessment of zinc, iron, vitamin B_{12}, folate and copper status by its physical signs and symptoms of deficiency are presented in Table 12 in terms of the frequency of micronutrient deficiency symptoms found among the participants.

Most physical signs and symptoms of deficiency were found in women compared to men. Of all participants, the average for women was 12.8%, indicating some diagnosis of physical signs of deficiency and for men was 10.3%. Analysis of trace elements by gender with its physical signs and symptoms of deficiency revealed that zinc deficiency was predominant in women by seborrheic dermatitis [7 (36.8%)] and by wound healing problems for men [6 (26.1%)]. Alopecia was found in six (31.6%) women and two (8.7%) men, which may be related to either zinc and/or iron deficiencies. Hyperpigmentation was found in one (5.3%) woman associated with Vitamin B_{12} deficiency. Angular stomatitis/cheilitis was higher in women [2 (10.5%)] than in men [1 (4.3%)]. Hypopigmentation was found in one each male and female participant.

Overall, the physical signs and symptoms of deficiency related to their micronutrients were predominantly higher in women than men, more abundant for zinc and iron.

Table 12: Frequency of micronutrient deficiency: Nutrition focused physical findings by gender.

Nutrient	Physical examination	Women (n=19) (n, %)	Men (n=23) (n, %)
Zinc	Seborrheic dermatitis	7 (36.8)	5 (21.7)
	Hypogeusia	3 (15.8)	6 (26.1)
	Dysgeusia	2 (10.5)	4 (17.4)
	Ageusia	0	1 (4.3)
	Eczema dermatitis	5 (26.3)	1 (4.3)
	Beau's line	1 (5.3)	5 (21.7)
	Wound healing	1 (5.3)	6 (26.1)
	Perioral stomatitis	1 (5.3)	1 (4.3)
Zinc, Iron	Alopecia	6 (31.6)	2 (8.7)
Iron, Vitamin B_{12}	Pallor (skin)	5 (26.3)	3 (13)
	Pallor conjunctiva	4 (21.1)	3 (13)
	Glossitis	0	0
	Pale tongue	1 (5.3)	1 (4.3)
Vitamin B_{12}	Hyperpigmentation	1 (5.3)	0
Folate	Angular stomatitis/ Angular Cheilitis	2 (10.5)	1 (4.3)
	Perioral stomatitis	1 (5.3)	1(4.3)
Iron	Koilonychia	1 (5.3)	0
	Vertical ridges	3 (15.8)	2 (13)
Copper	Hypopigmentation	1 (5.3)	1 (4.3)
Total (average) (%)		(12.8%)	(10.3%)

3.4.7 Association analysis between C-reactive protein, albumin, and haemoglobin with blood levels for iron, folate, vitamin B_{12}, copper and zinc.

The correlation analysis results (Table 13) showed that serum iron and serum zinc concentrations had a moderate positive correlation with serum albumin concentrations (r=0.433, p<0.01; r=0.453, p<0.01), respectively, while showing a negative correlation with copper plasma concentrations (r= -0.570, p<0.05). However, there was no significant correlation between C-reactive protein and haemoglobin with these nutrients. Ferritin levels did not correlate with C-reactive protein, albumin, or haemoglobin.

Table 13: Correlation coefficients between blood levels of micronutrients, c-reactive protein, albumin, and haemoglobin levels.

(n=21)	Ferritin	Iron	Folate	VitaminB_{12}	Copper	Zinc
C-reactive protein	-1.35	.382	-.243	-.113	-.362	-.113
Serum albumin	-0.040	0.433*	-0.128	-0.361	-0.570**	0.453*
Haemoglobin	-0.138	0.269	-0.069	0.100	-0.402	0.277

Data are presented as the Pearson's presented as the Pearson's correlation coefficients which were acquired by correlation analysis. Biomarkers results (n=21 participants).
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

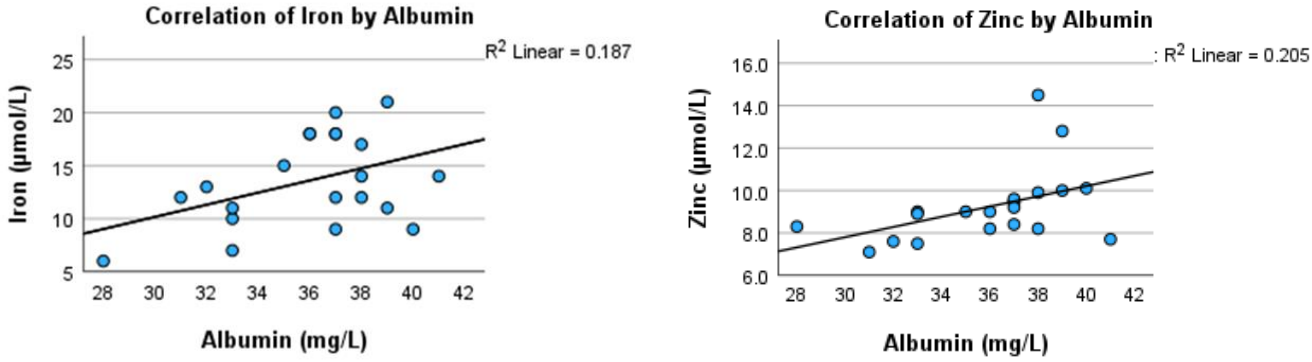

Figure 3: Positive linear regression by albumin with iron and zinc.

Albumin was found as a significant predictor of iron and zinc levels (p<0.05) (R^2 Linear = 0.187, R^2 Linear = 0.205), meaning 18.7% for iron and 20.5% for zinc can be accounted for by albumin correlation.

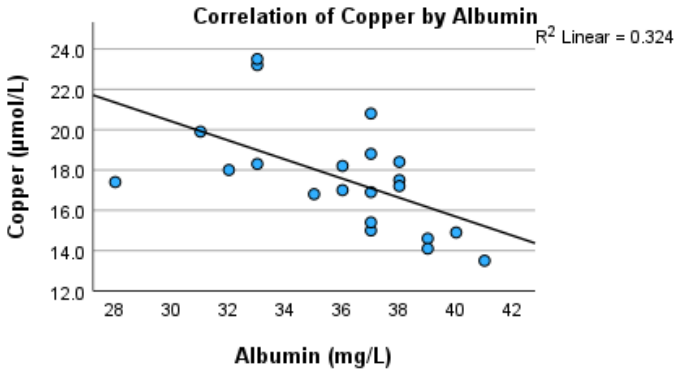

Figure 4: Negative linear regression by albumin with copper.

Albumin is not a significant predictor of copper levels ($p<0.05$), (R^2 Linear = 0.324), meaning that 32.4% cannot be accounted for by albumin and copper correlation showing a negative slope downtrend as albumin levels increase copper decreases leading to no correlation.

3.4.8 Association between blood test results of zinc, iron, vitamin B_{12}, folate and copper by their nutrition-focused physical signs and symptoms of deficiency.

Regression results of trace elements and their physical signs and symptoms of deficiency are presented in Table 14.

Hypothesis: The hypothesis tests if low blood levels (iron, folate, vitamin B_{12}, copper and zinc) carry a significant impact on physical signs and symptoms of deficiency of these trace elements.

Coefficients were assessed to ascertain the influence of each deficiency on criterion variables (blood result tests) of zinc, iron, vitamin B_{12}, folate and copper. Notable results showed a positive correlation between the blood result test of zinc and alopecia (β=3.866, t =-2.606, p=.035), meaning that lower serum zinc correlated with diagnosis of alopecia. There was no positive correlation under this study for the remaining blood test results and their respective physical signs and symptoms.

Table 14: Regression analysis of blood results: zinc, iron, vitamin B_{12}, folate and cooper with its expected physical signs and symptoms of deficiency.

Hypothesis (H) Dietary intake by NFPF (n=21)	**Regression weights**	**β**	**t-value**	**p-value**
H_1 (multiple regression) Zinc	Zn ↔ Seborrheic dermatitis	1.613	.798	.451
	Zn ↔ hypogeusia	-.309	-.222	.831
	Zn ↔ dysgeusia	1.204	.75	.478
	Zn ↔ ageusia	.809	.276	.791
	Zn ↔ eczema dermatitis	-4.468	-1.439	.193
	Zn ↔ Beau's lie	-.336	-.204	.844
	Zn ↔ wound healing	3.168	1.838	.109
	Zinc ↔ alopecia	3.866	2.606	.035
	Zinc ↔ PS	-1.087	-.845	.409
H_2 (multiple regression) Iron	Iron ↔ alopecia	-.484	-134	.896
	Iron ↔ pallor (skin)	3.516	.974	.349
	Iron ↔ pallor conjunctiva	-1.226	.370	.718
	Iron ↔ glossitis[1]	-	-	-
	Iron ↔ pallor tongue	-	-	-
	Iron ↔ Koilonychia	6.500	1.239	.239
	Iron ↔ vertical ridges	.274	.083	.935
H_3 (multiple regression) Vitamin B_{12}	Vitamin B_{12} ↔ pallor (skin)	-21.269	-.159	.876
	Vitamin B_{12} ↔ pallor conjunctiva	-192.769	-1.053	.311
	Vitamin B_{12} ↔ glossitis[1]	-	-	-
	Vitamin B_{12} ↔ pallor tongue	-	-	-
	Vitamin B_{12} ↔ hyperpigmentation	356.0	1.719	.103
H_4 (simple regression) Folate	Folate ↔AS/AC	-1188.3	-.496	.627
H_5 (simple regression) Copper	Copper ↔ hypopigmentation	-1.703	-.849	.406

Note. $p < 0.05$.
[1] Glossitis and pallor tongue, dependent variable with "no diagnosis" for all participants; statistics cannot be computed.
AS/AC: Angular stomatitis/ Angular Cheilitis
PS: Perioral stomatitis

3.4.9 Association between dietary intake of zinc, iron, vitamin B_{12}, folate and copper by their nutrition-focused physical signs and symptoms of deficiency.

Regression results of dietary intake of zinc, iron, vitamin B_{12}, folate and copper and their physical signs and symptoms of deficiency were analysed in Table 15.

Hypothesis: The hypothesis tests if low dietary intake significantly impacts physical signs and symptoms of deficiency of their selected micronutrients.

Coefficients were assessed to ascertain the influence of each deficiency on criterion variables' dietary intakes (zinc, iron, vitamin B_{12}, folate and copper). The result analysis showed a positive correlation between the dietary intake of zinc with alopecia (β=-11.384, t =-3.64, p=.002) and iron with alopecia (β= -9.919, t =-3.157, p=.004), meaning that lower dietary iron

and zinc intake was correlated with alopecia. This study had no positive correlation between the remaining dietary intake and their respective physical signs and symptoms.

Table 15: Regression analysis of total dietary intake of zinc, iron, vitamin B_{12}, and folate and cooper with its expected physical signs and symptoms of deficiency.

Hypothesis (H) Dietary intake by NFPF (n=42)	**Regression weights**	**β**	**t-value**	**p-value**
H_1 (multiple regression) Zinc	Zn ↔ Seborrheic dermatitis	-2.07	-.574	.574
	Zn ↔ hypogeusia	.481	.143	.888
	Zn ↔ dysgeusia	-1.663	-.39	.701
	Zn ↔ ageusia	-.769	-.112	.912
	Zn ↔ eczema dermatitis	6.545	1.249	.229
	Zn ↔ Beau's lie	3.555	.832	.417
	Zn ↔ wound healing	-5.264	-1.476	.158
	Zinc ↔ alopecia	-11.384	-3.64	.002
	Zinc ↔ PS	1.583	.347	.733
H_2 (multiple regression) Iron	Iron ↔ alopecia	-9.919	-3.157	.004
	Iron ↔ pallor (skin)	-2.659	-.83	.413
	Iron ↔ pallor conjunctiva	-1.072	-.288	.775
	Iron ↔ glossitis[1]	-	-	-
	Iron ↔ pallor tongue[1]	-	-	-
	Iron ↔ Koilonychia	-15.015	-1.93	.063
	Iron ↔ vertical ridges	2.339	.588	.561
H_3 (multiple regression) Vitamin B_{12}	Vitamin B_{12} ↔ pallor (skin)	.011	.009	.993
	Vitamin B_{12} ↔ pallor conjunctiva	-1.285	-.777	.443
	Vitamin B_{12} ↔ glossitis[1]	-	-	-
	Vitamin B_{12} ↔ pallor tongue	-	-	-
	Vitamin B_{12} ↔ hyperpigmentation	.616	.194	.848
H_4 (simple regression) Folate	Folate ↔AS/AC	-46.267	-.556	.581
H_5 (simple regression) Copper	Copper ↔ hypopigmentation	.296	.513	.611

Note. $p < 0.05$.
[1] Glossitis, dependent variable with "no diagnosis" for all participants; statistics cannot be computed.
AS/AC: Angular stomatitis/ Angular Cheilitis
PS: Perioral stomatitis.

3.4.10 Linear regression analysis to predict zinc, iron and copper blood levels using combined physical signs and symptoms of deficiency and dietary intake.

Multiple linear regression results for associations between blood levels (zinc, iron and copper) and dietary intake and physical signs and symptoms of deficiency are shown in Table 16 and Appendix A. The models met all assumptions for regression. From the multiple linear regression, the presence of eczema dermatitis and perioral stomatitis and the absence of wound healing and alopecia, combined, were associated with lower blood zinc levels (p=.004).

Iron and copper blood levels were not associated with dietary intake and physical signs and symptoms (p=0.634), (p=0.696) respectively; (see appendix A).

Table 16: Multiple linear regression analysis for association between blood zinc, copper and iron level and physical signs of deficiency and dietary intake.

	Coefficient (β)	**95% CI β**	**Standard error β**	**Standardized β**	**P-value**
Model 1 Zinc	**F(1,20) = 6.059, R2 = 0.60, Adj R2 = 0.50**				<0.01
(Constant)	8.5	5.0, 12.1	1.7		
Eczema dermatitis (Yes)	-3.9	-6.1, -1.6	1.1	-0.674	
Wound healing (Yes)	3.1	1.2, 5.1	0.9	0.652	
Perioral Stomatitis (Yes)	-2.2	-4.3, -0.1	1.0	-0.386	
Alopecia (Yes)	3.2	1.6, 5.1	0.8	0.687	
Model 2 Iron	**F(2,17) = 0.699, R2 = 0.23, Adj R2 = 0.23**				0.63
(Constant)	-7.1	-40.2, 25.9	15.0		
Alopecia (Yes)	2.5	-8.0, 13.1	4.8	0.200	
Pale Conjunctiva (Yes)	-0.7	-8.1, 6.8	3.4	-0.052	
Pallor (Skin) (Yes)	4.0	-4.0, 12.1	3.7	0.322	
Koilonychia (Yes)	9.1	-3.9, 22.1	6.0	0.524	
Vertical ridges (Yes)	0.2	-7.1, 7.5	3.3	0.020	
Diet hx. Iron (Yes)	0.2	-0.2, 0.5	0.1	0.374	
Model 3 Copper	**F(3,20) = 0. 369, R2 = 0.04, Adj R2 = -0.07**				0.69
(Constant)	19.0	12.8, 25.3	2.9		
Diet Hx. Copper (Yes)	0.1	-1.3, 1.6	0.7	0.053	
Hypopigmentation (Yes)	-1.7	-6.0, 2.6	2.0	-.195	

3.5 Discussion

This study investigated the nutritional status of long-term HEN patients within the Te What Ora Counties Manukau District. Assessing the biomarker status of iron, zinc, copper, folate and vitamin B_{12} in relation to recognized cut-offs. Our analysis of blood concentrations revealed lower biomarker results in our participants on long-term tube feeding, particularly in serum iron (n=4, 19.04%) and serum zinc (n=14, 66.7%), both falling below acceptable ranges. In contrast, copper plasma, vitamin B_{12}, and folate biomarkers were within the acceptable range, indicating sufficiency in these nutrients. A noteworthy finding emerged in our examination of dietary adequacy concerning iron, vitamin B_{12}, folate, zinc, and copper in relation to the RDIs. Specifically, we observed that a considerable proportion of female participants (n=9, 47%) fell short of meeting their iron requirements. Interestingly, it was observed that the iron content within the EN formulations failed to provide adequate intake to

fulfil the RDIs for females. However, when considering the overall nutritional sources, EN sources alone managed to provide sufficient folate, copper, vitamin B_{12}, and zinc levels for all participants. On a positive note, the male participants in our study exhibited a different trend. They met their RDIs for all the selected nutrients, suggesting a more favourable dietary profile in this regard.

Focused on identifying physical signs and symptoms of micronutrient deficiency. This study identified participants with deficiencies in iron, copper, zinc, vitamin B_{12}, and folate; these clinical signs were not consistently linked to their respective micronutrient deficiencies and were often attributed to other medical complications. Our analysis did reveal associations between alopecia and serum zinc levels and dietary intake of zinc and iron. Evaluating 17 comorbidities using the CCIs, we identified age, CVA & TIA, liver disease, and solid tumours as factors potentially lowering the 10-year survival rate. Approximately 14% of participants with a comorbidity score of ≥ 4 may face reduced chances of long-term survival, although the exact timeframe for potential mortality remains uncertain. The results of the association between blood zinc, copper, and iron levels, physical signs and symptoms of deficiency, and dietary intake revealed correlations between eczema dermatitis, perioral stomatitis, absence of wound healing, alopecia, and lower blood zinc levels. However, no such associations were found for iron and copper concerning dietary intake and physical signs and symptoms.

Furthermore, our study unveiled an essential finding regarding the duration of Enteral Nutrition (EN) intake among the participants. Participants received EN treatment for approximately (M=18.7, SD=12.02) years. A report by Kang et al. (2014), has indicated that the duration of EN treatment plays a significant role in the development of micronutrient deficiencies. Kang et al. (2014) found that signs of deficiency could become apparent within a relatively short timeframe, typically within 1-2 months of commencing EN treatment. For instance, iron as an example, it was observed that (n=4, 22.7%) of patients had sufficient iron levels in the first and second months of EN treatment. However, this figure dropped to only (n=1, 5.3%) after six months of EN treatment. Intriguingly, zinc levels followed a different pattern, with (n=1, 8.3%) of patients experiencing zinc deficiency within the initial two months, which increased to (n=6, 31%) after six months. These results show that micronutrient deficiency may occur over varying durations of treatment. Overall, this research's results confirmed that lower biomarker values for some trace elements exist among long-term home enteral feeding patients.

3.5.1 Iron

Total dietary iron intake was found inadequate in nine of the women in this study compared with their respective RDIs. The women's average age ranged from 26 to 62 years old, with most falling into the 18mg/d category of RDI. The average iron intake for women who received EN was 17.73 mg/d, with a standard deviation of 8.3 mg/d. However, those who could consume food sources had sufficient iron intake to meet the RDI. Dietary iron intake is subject to issues with bioavailability (Fairweather-Tait & Teucher, 2002). Iron obtained from animal sources (haem-iron) is more easily absorbed by the body and less influenced by dietary factors like phytates, polyphenols, calcium, and existing iron stores. In contrast, non-haem iron from plant sources and fortified foods is less bioavailable (Fairweather-Tait & Teucher, 2002; López & Martos, 2004). Studies show that the body absorbs more haem iron (over 15%) compared to non-haem iron (less than 5%)(López & Martos, 2004). Consequently, measuring serum levels may not accurately reflect dietary iron intake. Additionally, inflammation can affect ferritin, serum iron, TIBC, and transferrin saturation, making it hard to interpret these markers without knowing the inflammatory status (Fairweather-Tait, Wawer, Gillings, Jennings, & Myint, 2014; WHO & CDC, 2007). This is especially relevant in older adults and EN patients with chronic low-grade inflammation (Shi et al., 2020). Furthermore, diurnal variation may also impact serum iron and transferrin saturation levels (Dale, Burritt, & Zinsmeister, 2002), which could explain why iron biomarkers may not relate to dietary intake.

Concerning iron biomarkers, of all the participants, only three had lower serum ferritin. Of these three participants, only one had lower iron levels with low/normal transferrin, suggesting a compromised body's iron stores (Cooper, Greene-Finestone, Lowell, Levesque, & Robinson, 2012). We found that the a portion of the participants had inadequate levels of serum iron (n=4, 19.0%), normal serum transferrin, and inadequate transferrin saturation (n=4, 19.0%), indicating mild iron deficiency (Cooper et al., 2012). Lower haemoglobin concentration was found in three participants independently of other biomarker blood test results, which may be formally defined as iron deficiency anaemia. Since our participants had lower serum ferritin, transferrin saturation, and serum iron, the result indicates lower iron stores and a possible prognosis of iron deficiency anaemia. Results agree with the findings by Kang et al. (2014), which showed a prevalence of (n=10, 22.7%) of 44 participants with iron deficiency among EN patients showing a relationship between blood iron levels and low haemoglobin.

This study also investigated the physical signs and symptoms of iron deficiency. Iron deficiency anaemia is associated with developing physical signs and symptoms such as alopecia (Park et al., 2013), which was investigated and positively correlated with dietary intake in this study. Previous studies also found that alopecia was detected alongside patients suffering from anaemia (Park et al., 2013; Salinas, Flores, López-Garrigós, Leiva-Salinas, & Leiva-Salinas, 2020).

3.5.2 Zinc

In our study, serum zinc was the predominant biomarker, showing lower concentrations of 14 (66.7%) among the 21 participants who provided blood samples. These findings are in line with a previous research in patients undergoing long-term enteral feeding (Kang et al., 2014; McWhirter, Hambling, & Pennington, 1994). Kang et al. (2014) reported among 44 tube-fed patients in their study, 12 (27%) exhibited lower serum zinc concentration, indicative of deficiency (Kang et al., 2014). Similarly, Oliver et al. (2005) noted comparable results, with zinc deficiency most prevalent among long-term tube-fed patients.

Zinc plays a crucial role as a component of metalloenzymes and is essential for various physiological processes, including immune function, stress response, wound healing, and glucose control. Previous studies have established a correlation between serum zinc levels and disease severity, with regular zinc supplementation being associated with reduced infectious complications and mortality (Cander, Dundar, Gul, & Girisgin, 2011; Heyland, Jones, Cvijanovich, & Wong, 2008). These findings suggest that zinc deficiency in long-term tube-fed patients may exacerbate clinical outcomes. Therefore, it is imperative to implement proactive zinc monitoring to prevent potential complications.

The results of the investigation on the dietary intake of the participants in our study show that only one woman, age 53 years, receiving EN solely, had a dietary zinc intake below the RDI, whilst all the remaining participants had adequate zinc intake and met the RDIs. A case-control study compared the zinc status among outpatients receiving EN (mean age >70 years) (Pereira et al., 2021). Their results indicated that the mean serum zinc concentration of outpatients was 0.76 mg/ L, which was considered normal; however, the lower quartile was a mean of approximately 0.68 mg/L, indicating a deficiency in 25% of 191 participants due to possible association with insufficient zinc intake, altered intestinal absorption, inadequate chewing, drug interactions, and disorders in zinc transporters (Pereira et al., 2021). Based on our research, most participants had an adequate zinc intake, with only one person consuming less

than the recommended daily intake (RDI) of 8mg. Zinc deficiency can have various causes. A study states that individuals with conditions causing chronic diarrhoea like inflammatory bowel disease, celiac disease, short bowel syndrome, or enterocutaneous fistulas are at a higher risk of developing a specific type of zinc deficiency (Jhangiani, Prince, Holmes, & Agarwal, 1986)

Zinc, as a cofactor of metalloenzymes, has considerable effects on nearly all aspects of the metabolism in the body's organs, including skin and hair (Samer et al., 2018). With zinc deficiency, hair growth is either decreased or stopped (alopecia), the decisive factor being the degree of deficiency. In our study, both lower dietary zinc intake and zinc biomarker status correlated with the presence of alopecia, which is similar to a previous report investigating changes in serum zinc levels and alopecia (H. Park, Kim, Kim, & Park, 2009).

3.5.3 Copper

Dietary copper intake was found largely adequate (M = 2.7 mg/d, SD = 0.807) in relation to RDIs, except for one woman who had an inadequate copper intake of 1.15 mg/d (RDI <1.2 mg/d). Enteral nutrition sources alone provided adequate copper intake for all participants. Our study used a copper plasma range of 11-20 μmol/L. Ceruloplasmin levels alone were not measured. This study did not use the ceruloplasmin biomarker test since we aimed to observe the overall copper plasma status of the population studied. Ceruloplasmin, a blood protein responsible for copper binding, plays a vital role in copper transport and metabolism (Altarelli et al., 2019). However, when individuals are acutely unwell and experiencing inflammation, solely relying on serum copper measurements may not accurately assess their copper status(Jaiser & Winston, 2010). Inflammation can significantly impact ceruloplasmin levels and its copper-binding capacity. Consequently, in such cases, measuring ceruloplasmin levels in conjunction with serum copper is imperative to gain a more precise understanding of copper status (Jaiser & Winston, 2010). For generally healthy individuals not experiencing acute illness or inflammation, serum copper concentration alone can often suffice for assessing their copper status (Grażyna et al., 2023). Serum copper concentration denotes the copper content within the liquid component of the blood, providing a reasonably accurate reflection of copper status under typical circumstances (Grażyna et al., 2023). C-reactive protein serves as a marker for inflammation within the body (Patel, 2023) .When evaluating copper status in individuals with acute inflammation, it becomes crucial to account for their CRP levels (Grażyna et al., 2023; Jaiser & Winston, 2010). Therefore, the approach to assessing copper status in individuals varies depending on their health condition. Serum copper levels alone may suffice for healthy individuals. However, in cases marked by acute illness and inflammation, it is

imperative to incorporate ceruloplasmin and CRP levels alongside serum copper measurements. This comprehensive approach allows healthcare professionals to gain a deeper insight into how inflammation and illness affect copper metabolism in these individuals.

Although, this study did not find copper plasma concentrations below 11 μmol/L, reflecting adequate copper stores. Contrarily, three (7.2%) participants had copper above the 20 μmol/L marker. These findings differ from a previous study showing copper deficiency in two (4.5%) of 44 tube-fed patients studied (Kang et al., 2014), demonstrating a lower presence of copper deficiency.

This study investigated hypopigmentation as a nutrition-related physical finding to identify a copper deficiency. Lower serum copper causes malfunction of essential copper-dependent enzymes manifesting as one of the symptoms of skin hypopigmentation (Menkes, 1988); however, a correlation was not found between either dietary copper and/or zinc biomarker status and hypopigmentation. This may be because a copper deficiency can take several years before it depletes the body's copper stores and physical signs become apparent (Kumar, Ahlskog, & Gross, 2004; Shoaib & Ibrahim, 2017).

Although two participants presented with hypopigmentation of the skin, observed in the NPFE, these participants did not present with abnormalities of copper plasma concentrations or dietary intake. The identification of hypopigmentation in this study may have been related to non-nutrition-related causes, such as genetic and autoimmune skin disorders (e.g., vitiligo) (Poon & Beach, 2018). Medical specialist dermatologists critically observed in a case study report of localised hypopigmentation diagnostic study that hypopigmentation disorders are challenging as multiple forms exist; therefore, diagnoses may seem unclear (Poon & Beach, 2018). Their statement agrees with the findings of this study, as hypopigmentation did not correlate with copper deficiency but probably to other health factors. Overall, the mean timeframe of our participants receiving HEN was of (M = 18.7, SD = 12.02) years since most participants had a tube feeding placed from birth; this indicated that with a longer timeframe in HEN patients, copper stores were still sufficient in our group of participants, and hypopigmentation was undetected.

3.5.4 Vitamin B_{12}

The serum vitamin B_{12} levels in the study did not fall below the established cut-off value of 80 pmol/L. This finding suggests that the participants had sufficient stores of vitamin B_{12}. This observation aligns with previous research, indicating that vitamin B_{12} deficiency is unlikely to occur unless individuals have diets lacking in vitamin B_{12}-rich foods or people with gastrointestinal conditions or are taking medications (e.g., Metformin) that impact vitamin B_{12} absorption (Fiona & Samir, 2010; McLean et al., 2008).

Dietary vitamin B_{12} intakes in the present study were below the RDI (2.4 mg/d) in three participants. Positively, the total EN intake for vitamin B_{12} was adequate for all participants. A study by Brito et al. (2020) explains that the total body's store of vitamin B_{12} is typically in the range of 2 to 5 mg, which is sufficient to supply needs for years. Even when vitamin B_{12} intake reduces, deficiency does not develop for at least one or two years or longer (Brito et al., 2020). Since vitamin B_{12} deficiency takes a significant time to develop, we found no correlation between serum vitamin B_{12}, dietary intake and the NFPF. To complement, Brito et al. (2020), in their study of 472 patients with head/neck or oesophageal disorder with suggestive anaemia, only 1.3% (n=6) presented vitamin B_{12} deficiency, which shows that a very small proportion of patients may develop vitamin B_{12} deficiency in cases of severe deficiency. Our study found that even participants receiving EN for over a decade or since birth showed no deficiencies in serum vitamin B_{12} levels below the cut-off value of 80 pmol/L.

Therefore, with adequate EN, deficiency of vitamin B_{12} is unlikely to occur in HEN patients over the medium term since EN formulations are designed to provide sufficient vitamin B_{12} to meet physiological needs (Berger et al., 2022). In fact, even if the minimum RDI is not met, the body's store of vitamin B_{12} is still sufficient, as shown in the biomarker test.

3.5.5 Folate

Folate in the body's store is estimated to be approximately 0.5 to 20 mg, and the capacity to supply needs to an individual is significantly lower (Brito et al., 2020). However, studies suggest that if folate intake decreases, deficiency may develop within weeks to months (Bhattacharya, 2023; Brito et al., 2020). Our research found that the serum folate levels of all participants were normal, with a range above 7 nmol/L. However, we discovered that three women had a combined dietary folate (DFE) intake below the RDI of 400 µg/d. Of these three females, two participants were close to the cut-off value of 400 µg/d. *Participant one* presented

a total DFE intake (374.8 µg/d), oral intake (104.72 µg/d) and EN intake (270.08 µg/d); *participant two* had a total DFE intake (358.9 µg/d) oral intake (34.9 µg/d) and EN intake (324.1 µg/d) and *participant three* had a total DFE intake (240 µg/d) oral intake (173 µg/d) and EN intake (67. µg/d) since this participant was able to consume most food orally. Iacone et al. (2015) found in their comparison study of micronutrient content in EN formulas that long-term EN patients receive adequate folate intake in relation to the RDIs and that it meets the same standards as a healthy population. Since folate is a marker of long-term status, it is possible that the consumption of folate-rich foods was insufficient among those who could consume oral food sources, despite their sufficient EN dietary intake. To explain, folic acid supplements taken on an empty stomach are almost 100% bioavailable; however, absorption decreases to 85% when taken with food, while the bioavailability of food folate is around 50-60% (Caudill, 2010).

Therefore, food preparation and cooking may explain the inadequacy of meeting the RDI for those able to consume oral food sources. This is consistent with Allen (2008) statement that food folates are relatively unstable to oxidation and heat; therefore, during food preparation and cooking, folate losses can occur.

Overall, DFE was largely adequate among the participants, results that align with the ESPEN recommended guidelines suggesting 300-400 ug DFE per 1500 kcal formula (Berger et al., 2022), which shows that our participants were receiving adequate DFE intake based on a mean folate (DFE) intake of 744.2 µg/d. This study found no correlation between dietary intake, folate biomarker status and the NFPF.

3.5.6 Inflammatory markers

Clinical biomarkers of inflammation, as measured by CRP and albumin, were used to determine inflammation patterns. In this study, CRP was found to be higher than the reference range (>5 g/L) in five participants, indicating the presence of inflammation. Our findings indicate that individuals with higher CRP concentrations also exhibited one or a combination of abnormal statuses in other biomarkers. For instance, among five cases with elevated CRP, three showed both reduced zinc and copper plasma levels, while two exhibited lower zinc plasma concentrations exclusively. High CRP levels may have been shown in the biomarker results during acute conditions of inflammatory or infectious processes. The levels of CRP rise and fall rapidly with the onset and removal of the inflammatory stimulus (Patel, 2023). In fact, Kang et al. (2014) reported that CRP levels decreased in patients receiving feeding tubes longer

than six months (M = 2.1 mg/dL, SD = 3.5 mg/dL) when compared to the first and second month of the feeding tube (M = 3.1 mg/dL, SD = 2.3 mg/dL). C-reactive protein elevation is associated with falsely lowering serum zinc levels (Jung et al., 2015). This occurs because the body's cells tend to sequester zinc to limit its availability to pathogens during inflammation. As a result, serum zinc levels can decrease, which may not accurately reflect the body's overall zinc status (Jung et al., 2015). For instance, inflammation can affect the body's zinc transport and metabolism pathways through elevated CRP levels, which may lead to changes in zinc-binding proteins and enzymes, potentially reducing zinc availability for various cellular functions. However, this is generally transient and should not be confused with a true zinc deficiency (Jung et al., 2015; Maxfield et al., 2022).

Similarly, the literature reports that CRP elevation leads to a false elevation in serum copper levels (Galloway, McMillan, & Sattar, 2000). During infection and inflammation, serum copper concentration and ceruloplasmin activity increase due to interleukin-1-mediated increases in liver ceruloplasmin synthesis and release, an acute-phase protein (Yamei et al., 2022). Therefore, high CRP levels affect copper-binding proteins, potentially leading to changes in the distribution and availability of copper in the body. The result is an increase in serum copper levels, even if there is no increase in copper intake or absorption (Galloway et al., 2000; Yamei et al., 2022). An effect is generally a consequence of inflammation and does not necessarily indicate increased copper stores in the body (Yamei et al., 2022). Copper plasma binds only to high-affinity sites on albumin (~15%), which may explain the inverse relationship between copper and albumin in this study. Also, of the two low albumin results, one had lower serum iron levels. This could be due to inadequate nutrition intake since hypoalbuminemia is one of many malnutrition parameters. Patients with severe protein-energy malnutrition and hypoalbuminemia have low serum albumin levels due to decreased amino acid supply to the liver and other nutritional deficiencies, including iron and zinc (Jialal, 2022).

In our study, the assessment of biomarkers revealed that serum albumin levels of two participants fell below the normal reference range of 32-48 g/L. Notably, these individuals also displayed low serum zinc levels. A strong relationship can be assumed between these markers since albumin is involved in zinc transport in the body due to its high binding capacity to zinc (Katayama et al., 2018). Album and zinc are both associated as a marker for nutrition status (Keller, 2019; King et al., 2001). Low serum albumin can indicate malnutrition (Keller, 2019), whilst lower serum zinc indicates inadequate zinc-rich foods (Maxfield et al., 2022). Zinc

deficiency typically occurs in cases of prolonged inadequate zinc intake (e.g., due to malnutrition or eating disorders), malabsorption issues, or increased zinc loss (e.g., chronic diarrhea or chelation therapy) (Jhangiani et al., 1986). Thus, serum albumin has been associated with a poor prognosis; however, it may not fully reflect the impact of nutrient-dense supplemental nutrition. This limitation arises from the relatively long half-life of albumin (approximately 18-21 days), which means that significant changes in liver function may not be immediately apparent in serum albumin test results (Raymond & Morrow, 2021).

As Katayama et al. (2018) reported, zinc deficiency may contribute to decreased blood albumin levels. Hypoalbuminemia leads to decreased blood zinc concentrations because zinc binds mainly to albumin and is transported in blood. In other words, zinc deficiency is a cause and an outcome of hypoalbuminemia. Thus, because of the strong association between blood zinc and blood albumin levels, a strong correlation can be assumed to exist between them.

In summary, as indicated by high CRP levels, inflammation can influence the accuracy of serum zinc and copper measurements due to temporary shifts in their availability and binding proteins in the body. These changes are typically associated with acute inflammatory processes and should be considered when interpreting these biomarkers in clinical settings. Furthermore, the findings revealed the complex relationship between serum albumin and serum zinc levels as indicators of nutritional status. We have observed that individuals with low serum albumin levels also displayed low serum zinc levels, suggesting a strong correlation between these two markers. Zinc deficiency can cause reduced blood albumin levels because zinc binds primarily to albumin and is transported in the bloodstream for the body's use. Conversely, low albumin levels can also lead to decreased blood zinc concentrations.

3.5.7 Charlson comorbidity index

This study aimed to determine comorbidities commonly found in tube-feeding patients and if these population groups are at risk of reducing lifespan based on the CCIs. Tube-feeding patients are often diagnosed with malnutrition (Agarwal et al., 2013). Malnourished patients have a higher likelihood of low levels of different specific micronutrients, leading to nutrient deficiencies that could contribute to more severe courses in patients with complications diagnosed in the CCIs. Therefore, identifying comorbidities that tube-feeding patients have

using the CCIs tool and understanding the intake patterns of this population group allow further association between deficiencies in micronutrients and severe progression of the diseases.

The comorbidities age, CVA & TIA, liver disease and solid tumour were found as diseases that may lead to a decrease in life longevity. Each comorbidity identified among the participants was represented by the sum of the CCIS, which provided an estimated mortality rate among the studied group. Our study identified that age across the CCIS was predominantly ranging from 1-2 (mild prognosis) for women [7 (36.9%)] and men [8 (34.7%)], and 3-4 (moderate prognosis) for women [2 (10.6%)] and men [4 (17.4%)]. Therefore, a cohort study of long-term prognosis of EN feeding and parental nutrition (PN) in a population aged 75 years and older identified that of 3548 observed patients who received some type of EN, 2384 (67%) died within 730 days after initiation of gastrostomy and NGT (1). Interestingly, a multivariate analysis study in 58 patients receiving PEG feeding identified the survival time related to the degree of CCIS. They found that the median survival in patients with CCIS <4 (n=37) and ≥4 (n=21) was 55 and 40 days, respectively. Based on the CCIS in our study, it has been concluded a mean survival rate of 74.5% for the next decade for our participants.

In conclusion, dietary intake for iron, copper, folate, vitamin B_{12}, and zinc was mostly adequate for all participants. Combined intakes (EN and oral food sources) were inadequate for all women in the study, with higher predominance of iron. Concerning biomarker status, all participants appeared to have sufficient blood stores for serum folate, serum vitamin B_{12} and copper plasma. However, serum iron and serum zinc were below the cut-off values. Two participants with elevated CRP concentration presented low serum zinc, and three others had a combination of lower zinc and copper levels. A correlation was identified between albumin with iron and zinc but not with folate, vitamin B_{12} and copper. Only alopecia was identified to have a correlation with the nutrition physical signs and symptoms of deficiency and dietary intake, this being with serum zinc and alopecia and dietary zinc and iron with alopecia. Also, the presence of eczema dermatitis, perioral stomatitis, and the absence of wound healing and alopecia were also associated with lower blood zinc levels. However, iron and copper were not associated with dietary intake and physical signs and symptoms. Lastly, with the CCIS assessment, we identified that age, CVA & TIA, liver disease, and solid tumours were the only comorbidities that may had an impact on the survival rate, showing that only seventeen participants had a 98% chance of survival with the next 10-years.

4. Chapter 4: Conclusions and Recommendations

This study aimed to investigate the nutritional status of long-term HEN patients (aged ≥18 years) within the Te Whatu Ora Counties Manukau District. The objectives were to assess the biomarker status of the selected mineral (iron, zinc and copper) and vitamins (folate and vitamin B_{12}) in relation to recognized cut-offs, the physical signs and symptoms of deficiency of these nutrients, the dietary intake through the enteral feeding regime and oral food sources in relation to the RDIs. Lastly, to investigate the estimated 10-year survival rate using the CCIS.

The main findings in relation to each objective are presented below:

Objective 1: To assess the biomarker status of the selected mineral (iron, zinc and copper) and vitamins (folate and vitamin B_{12}) in relation to recognized cut-offs.

Evaluation of the blood concentrations of iron, copper, zinc, vitamin B_{12} and folate revealed that some micronutrient deficiencies were substantial in patients receiving long-term tube feeding. Individuals with biomarker values for serum iron and serum zinc below the established cut-offs were receiving tube feeding for approximately 13 years. After analysing the data, we discovered that serum iron and serum zinc concentrations were below the acceptable ranges, indicating that the participants had lower concentrations of these micronutrients in the blood. On the other hand, biomarkers such as copper plasma, vitamin B_{12}, and folate were found to be within the acceptable range, indicating adequacy of these nutrients in blood.

Objective 2: To investigate the dietary adequacy of iron, vitamin B_{12}, folate, zinc and copper in relation to the RDIs.

Assessing the dietary intake by gender, we identified a combined EN and oral food sources intake to be below the RDI for folate, vitamin B_{12}, iron, zinc and copper for a small portion of female participants, most notable for iron, showing that nine (47%) were consuming less than the RDIs. The sources of enteral nutrition were sufficient in providing the RDI of folate, copper, vitamin B_{12}, and zinc for all participants. However, female participants needed an additional 0.8 mg/d of iron to meet their RDI. Male participants in this study met the total nutritional intake within the RDIs for all selected nutrients analysed. As anticipated, the studied group only met the RDIs partially through oral food sources, as it is not their primary dietary intake.

Objective 3: To investigate the presence of the nutrition physical signs and symptoms of deficiency of the selected micronutrients studied.

Through evaluating the physical nutrition signs and symptoms of deficiency for iron, copper, zinc, vitamin B_{12}, and folate, we identified participants with one or a combination of deficiencies in these micronutrients. However, the clinical signs identified were not linked to their respective micronutrients, but for other medical complications. As explained by Poon & Beach (2018), in the case of copper, identifying a participant with hypopigmentation can be difficult as medical professionals recognise that various forms of hypopigmentation exist.

After conducting statistical analysis, we discovered an association between alopecia and serum zinc and between alopecia and dietary intake of zinc and iron.

Objective 4: To investigate estimated 10-year survival rate using the Charlson Comorbidity Index Score.

Using the CCIS tool, we can comprehensively understand the life expectancy of tube-feeding patients and detect comorbidities that may predict mortality. After evaluating the 17 comorbidities using the CCIS for all participants, we discovered that only age, CVA & TIA, liver disease, and solid tumours are the diagnostics that may lower the 10-year survival rate. Based on the scores rank for each comorbidity, those four (14%) participants with a comorbidity score of ≥ 4 may have lower chances of survival within the next decade. Although, the CCIS assessment under this study did not determine the exact timeframe for potential death, whether it may occur within the next six months or beyond.

Objective 5: To determine the association between blood zinc, copper and iron levels and the physical signs and symptoms of deficiency and dietary intake.

Due to challenges with intravenous access, collecting blood samples from our participants was impractical. Instead, we used results from the nutritional physical signs, symptoms of deficiency, and dietary intake to deduce an association with blood levels of iron, copper, and zinc. Statistical analysis indicated that eczema dermatitis, perioral stomatitis, and the absence of wound healing and alopecia correlated with lower blood zinc levels. However, no such associations were found for iron and copper with dietary intake and physical signs and symptoms.

4.1 Strengths

Our study's strengths are examining iron, folate, copper, vitamin B_{12} and zinc dietary intakes, biomarker status and the physical signs of deficiency concurrently in long-term home enteral patients. During the literature review for this research, it was challenging to locate studies that considered the biomarker status, the physical indications of deficiency for the selected micronutrients studied in this thesis, and dietary intake, whether separately or in conjunction with enteral feeding. This highlights the significance of the results of our study, which can provide valuable evidence to enhance Dietetics practice for patients receiving enteral nutrition.

The recruitment method was undertaken using patients from Te Whatu Ora Counties Manukau which led to adequate coverage of the regions of southern Auckland, New Zealand.

Blood samples were rapidly processed after collection to provide appropriate blood plasma, and it was stored at the proper temperature, ensuring its stability until it could be analysed. The biomarker cut-off used in the present analysis was based according to both age group and requirements (NHMRC, 2017).

A micronutrient assessment worksheet form containing clinical images related to the selected micronutrients to assess both macronutrients and micronutrients was designed, which worked effectively to identify the nutrition-focused physical findings.

The 24-hour diet recall was collected in detail, including the enteral nutrition prescription and oral food intake, which were analysed independently and combined. FoodWorks was used to provide a detailed analysis of the intake of the selected micronutrients.

4.2 Limitations

A key limitation of this study is the small sample size. Of the 79 eligible patients from the current Te Whatu Ora Counties Manukau District database who received long-term HEN, only 42 agreed to participate in the study, which may have affected the overall results. It is important to note that those who declined to participate did so for personal reasons, which were respected.

In the recruitment process, eligible participants expressed their desire for their dietitians to be part of the research. However, due to ethical considerations, dietitians who were already involved in the patient's care were not able to participate in the recruitment process, potentially leading to participants disagreeing to participate.

Another substantial limitation of this study was the difficult vascular access of our group of participants due to underlying diseases (i.e., cerebral palsy, strokes). The patients suffering from paralyses and flexion deformities make venepuncture challenging for the doctors and nurses involved in the study, limiting the blood sample size to only 21.

4.3 Final Recommendations

- Further investigation into the selected micronutrients investigated in this study outside the Southern area of Auckland may help validate the present study's findings and allow comparison between different geographic regions of New Zealand.
- Involve dietitians responsible for the care of HEN patients as trustworthy sources to provide advice on the purpose of data collection, which can support an increased sample size.
- Implement regular nutrition-focused physical examinations for micronutrient deficiency into patients' home visits routine as standard practice, especially for iron and zinc, since we identified a relationship between these nutrients and the NPFF. This can improve the quality of patient care and clinical outcomes and may reduce healthcare costs.
- Implement regular micronutrient biomarker tests over different treatment periods as a more accurate reflection for identifying a deficiency and those who are most at risk of needing supplementation, specifically for iron and zinc, as they were discovered to be predominantly lower in the population studied.
- Ensure patients using a blenderised formula meet the nutritional requirements by assessing the nutritional intake of the mixture.
- The present study identified the prevalence of low serum zinc and serum iron; however, the cause was not. Future investigations to determine whether these lower biomarkers are related to physiological reasons may help inform preventative measures to avoid deficiency.

4.4 Conclusion

Evaluation of the blood levels of iron, copper, zinc, vitamin B_{12} and folate revealed that some micronutrient deficiencies were present in patients receiving long-term tube feeding. Copper

plasma, serum vitamin B_{12} and serum folate were found within the normal range when compared to the cut-offs, however zinc and serum iron were found notably lower.

In the assessment of the dietary intake of all participants, we discovered that, on average, they were meeting the RDI values for iron, vitamin B_{12}, folate, and copper, excluding zinc intake that was below the RDI. In the population studied, only a small group of women experienced insufficient nutrient intake. They were consuming less than the RDI of all the selected micronutrients, with iron being the most deficient trace element. Overall enteral intakes appeared sufficient despite some participants only using EN for a partial source of nutrition.

The most apparent relationship in this population concerning the nutrition focused physical findings appeared to be alopecia, given that alopecia was found to be correlated with serum zinc and dietary intake of zinc and iron. The statistical analysis also revealed that the presence of eczema dermatitis, perioral stomatitis, and the absence of wound healing and alopecia were associated with lower blood zinc levels. However, iron and copper were not associated with dietary intake and physical signs and symptoms. The CCIS indicated the comorbidities faced in this population group were age, CVA and TIA, liver disease, and solid tumours influenced mortality rate by the scores >2, suggesting an increased risk of mortality, and only seventeen participants were found to have a 98% survival rate in the next 10-years.

The results of this study may promote the necessity for routine micronutrients evaluation through blood tests, nutrition focused physical findings and dietary intake for long-term fed patients at risk of micronutrient deficiency.

Abbreviations

AC	Angular Cheilitis
AGP	Alpha(1)-Acid glycoprotein
AI	Adequate intake
AND	Academy of Nutrition and Dietetics
ANZCTR	Australia New Zealand Clinical Trial Registry
APPs	Amyloid-beta precursor proteins
AS	Angular stomatitis
ASPEN	American Society of Parenteral and Enteral Nutrition
CCI	Charlson Comorbidity Index
CCIS	Charlson Comorbidity Index Score
CM	Counties Manukau
CMD	Counties Manukau District
CMDHB	Counties Manukau District Health Board
CMH	Counties Manukau Health
CRP	C-reactive protein
Cu	Copper
CVA	Cerebra Vascular Accident
DFE	Dietary Folate Equivalent
DHB	District of Health Board
DNA	Deoxyribonucleic acid
EAR	Estimated Average Requirement
EDTA	Ethylenediaminetetraacetic acid
EN	Enteral Nutrition
H	Hypothesis
HB	Haemoglobin
HEN	Home enteral nutrition
IBM	International Business Machines Corporation
ID	Iron deficiency
ID	Identification
JJ	Jejunostomy
KJ	Kilojoules
LTC	Long term condition
MND	Micronutrient deficiency
NFPA	Nutrition Focused Physical Assessment
NFPE	Nutrition Focused Physical Examination
NFPF	Nutrition Focused Physical Findings
NJT	Nasojejunal tube
NK cell	Natural Killer cell
NRVs	Nutrient reference values
NZ	New Zealand
NZRN	New Zealand Registered Nurse
PEG	Percutaneous endoscopic gastrostomy
PEG-J	Percutaneous endoscopic gastrostomy jejunostomy
PEJ	Percutaneous endoscopic jejunostomy
PS	Perioral stomatitis
RBC	Red blood cells
RDI	Recommended daily intake
RIG	Radiologically inserted gastrostomy
SOD1	Superoxide dismutase
SOPs	Standard operating procedure
SPSS	The Statistical Package for Social Scientists
TIA	Transient ischemic attack
TIBC	Total iron biding capacity
UL	Upper limit
VIF	Variance inflation factor
WHO	World Health Organization

5. References

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6. Appendices

Appendix A: Supplementary results

Appendix B: Participant Information Sheet

Appendix C: Charlson Comorbidity Index Assessment Form

Appendix D: Nutrition Focused Physical Examination Form

Appendix E: 24-hours Diet Recall Assessment Form

Appendix F: FoodWorks Analysis (SOP)

Appendix G: Journal requirements: *Nutrition Journal*

Appendix A – Supplementary Results

Multiple linear regression analysis to predict blood zinc level using zinc's physical signs of deficiency and dietary intake.

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	Perioral_stomatitis, Eczema_dermatitis, Alopecia, Wound_healing[b]	.	Enter

a. Dependent Variable: Zinc
b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.776[a]	.602	.503	1.2112	2.365

a. Predictors: (Constant), Perioral_stomatitis, Eczema_dermatitis, Alopecia, Wound_healing
b. Dependent Variable: Zinc

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	35.554	4	8.889	6.059	.004[b]
	Residual	23.473	16	1.467		
	Total	59.027	20			

a. Dependent Variable: Zinc
b. Predictors: (Constant), Perioral_stomatis, Eczema_dermatitis, Alopecia, Wound_healing

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	8.536	1.689		5.053	<.001	4.955	12.117		
	Eczema_dermatitis	-3.852	1.077	-.674	-3.575	.003	-6.136	-1.568	.698	1.432
	Wound_healing	3.126	.910	.652	3.436	.003	1.197	5.054	.689	1.451
	Alopecia	3.290	.842	.687	3.906	.001	1.505	5.076	.804	1.243
	Perioral_stomatitis	-2.207	.984	-.386	-2.244	.039	-4.292	-.122	.838	1.193

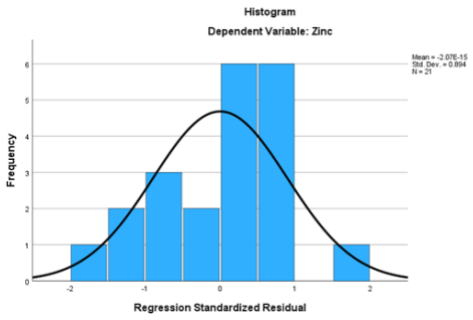
Dependent Variable: Zinc

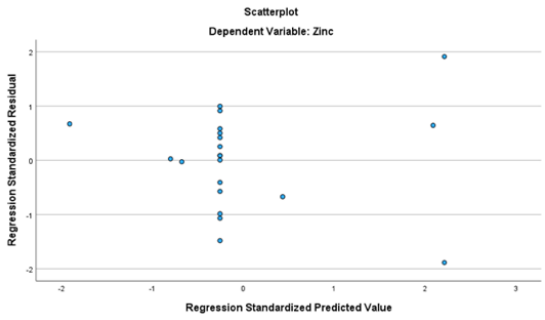
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Unstandardized Residual	.068	27	.200*	.984	27	.943

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

***Zinc** - Multiple regression analysis; Normality test. | Multiple regression analysis ; Test of equality of variable.*





Multiple linear regression analysis to predict blood iron level using iron's physical signs of deficiency and dietary intake.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
	.475[a]	.226	-.097	4.285	1.682

a. Predictors: (Constant), Vertical_ridges, Pallor(skin), Pale_conjunctiva, Alopecia, Koilonychia
b. Dependent Variable: Iron

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	64.194	5	12.839	.699	.634[b]
	Residual	220.306	12	18.359		
	Total	284.500	17			

a. Dependent Variable: Iron
b. Predictors: (Constant), Vertical_ridges, Pallor(skin), Pale_conjunctiva, Alopecia, Koilonychia

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-7.139	15.010		-.476	.644	-40.177	25.898		
	Alopecia	2.529	4.799	.200	.527	.609	-8.034	13.091	.452	2.215
	Pale Conjunctiva	-.656	3.375	-.052	-.194	.849	-8.083	6.772	.913	1.095
	Pallor (Skin)	4.067	3.668	.322	1.109	.291	-4.005	12.140	.773	1.294
	Koilonychia	9.096	5.924	.524	1.535	.153	-3.942	22.134	.558	1.792
	Vertical ridges	.209	3.322	.020	.063	.951	-7.103	7.522	.670	1.493
	Diet hx. iron	.179	.187	.374	.957	.359	-.233	.591	.426	2.348

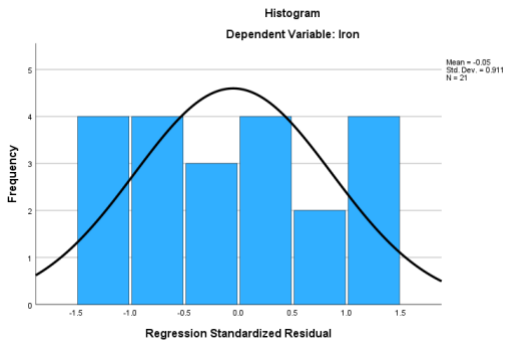
a. Dependent Variable: Iron

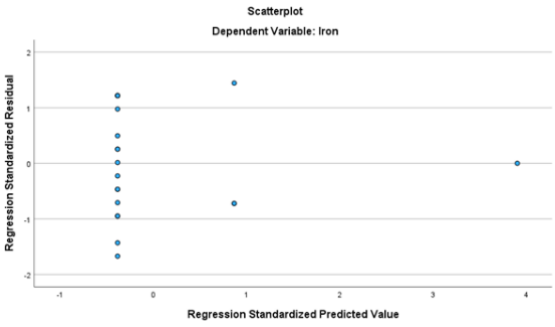
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual	.137	21	.200*	.940	21	.216

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Iron *- Multiple regression analysis; Normality test. | Multiple regression analysis ; Test of equality of variable.*





Simple regression analysis to predict blood copper levels using copper's physical signs of deficiency and dietary intake.

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	SkinHypopigment2, Diet hx_copper[b]	.	Enter

a. Dependent Variable: Copper

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.198[a]	.039	-.067	2.7667	2.450

a. Predictors: (Constant), SkinHypopigment2, Diet_hx_copper

b. Dependent Variable: Copper

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.651	2	2.826	.369	.696[b]
	Residual	137.787	18	7.655		
	Total	143.438	20			

a. Dependent Variable: Copper

b. Predictors: (Constant), Hypopigmentation, Diet_hx_copper

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	19.031	2.973		6.402	<.001	12.786	25.276		
	Diet Hx. Copper	.162	.703	.053	.230	.821	-1.316	1.640	.995	1.005
	Hypopigmentation	-1.737	2.062	-.195	-.842	.411	-6.069	2.596	.995	1.005

a. Dependent Variable: Copper

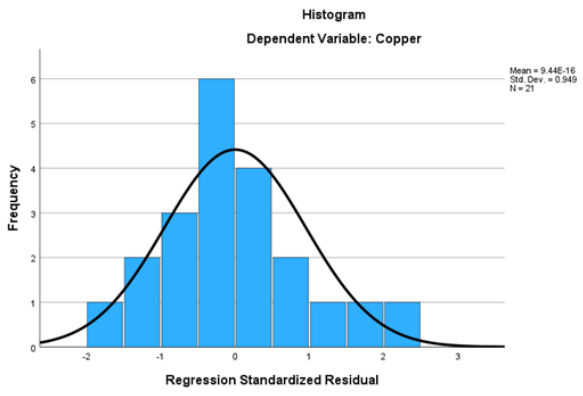
Tests of Normality

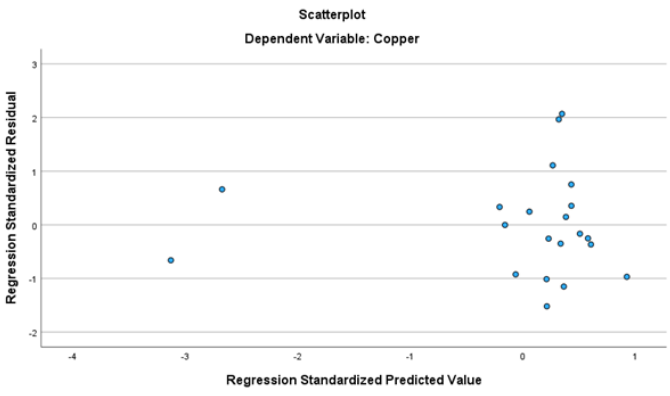
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual	.116	21	.200*	.952	21	.376

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Copper *- Multiple regression analysis; Normality test. of variabl* | *Multiple regression analysis ; Test of equality*





Length of enteral feeding received for all participants, including participants with lower biomarker for zinc and iron.

The result shows that of all 42 participants, 37 were receiving EN for 18.7 years. Those with lower biomarkers for zinc and iron were receiving EN for 13 and 14 years, respectively.

Descriptive Statistics

	N	Missing	Minimum	Maximum	Mean	Std. Deviation
EN_Timeframe	37	5	1	50	18.76	12.017
Zinc_Timeframe	20	22	1	48	13.08	12.016
Iron_Timeframe	19	23	1	48	14.05	11.903
Valid N (listwise)	17					

Appendix B: Participant Information Sheet

Participant Information Sheet

HELP (Home EnteraL nutrition Performance) Study

Kia ora,

As a patient receiving home enteral nutrition (HEN), you are invited to take part in a research study titled "**The health, wellbeing and nutritional outcomes of long-term enterally fed patients"**. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve.

WHAT IS THIS RESEARCH ABOUT?

The number of patients receiving home enteral feeding has increased considerably in recent years. It is now estimated that the number of patients receiving enteral feeding at home is increasingly higher than in hospitals.

Patients receiving home enteral nutrition may receive adequate nutrients from the feed. However, it has been shown that the nutrients in these feeds may be digested and absorbed differently than those found in whole foods. Therefore, nutrient deficiency may develop over time, leading to physical signs and symptoms associated with not having enough of a particular nutrient or a combination of them e.g., anaemia and poor wound healing due to not enough iron and zinc. It is in our best interest to know when such deficiencies may develop, and when to test for insufficiency. Therefore, we want to investigate the timeframe of developing deficiencies.

The aim of this study is to investigate the nutrition status, quality of life, care, health, and wellbeing of long-term enterally fed patients older than 18 years in CM Health.

Thus, the findings of this study can assist in determining guidelines for timely micronutrient monitoring, and potential micronutrient replacement could be established depending on the results.

Who are we looking for?

We are looking for men and women to participate in this study. To take part in this study, you need to be:

- adults ≥18 years old,
- using percutaneous endoscopic gastrostomy (PEG), percutaneous endoscopic jejunostomy (PEJ); nasojejunal tube (NJT), or jejunostomy tube (JJ),
- on this feeding regime for longer than four weeks,
- able to give a blood sample.

What is involved in the study?

If you meet the above eligibility criteria, you will be invited to participate in the study. The study will be explained to you, and the Participant Information Sheet will be provided to you to review. When you decide to participate, a visit from the researcher at your location of choice (which will take about an hour and 30 minutes) will be organised.

At the initial (first) appointment, you can first ask any questions you may have about the study. Next, you will be asked to sign a consent form for participating in the study and complete a demographic questionnaire.

During this initial visit, we will ask you to:

- Participate in a 24-hour diet recall interview.
- Complete a physical sign and symptoms examination.

- Complete electronic questionnaires about Quality of Life, HEN satisfaction, Comorbidities, and Patient experiences with assistance from the researcher including some questions on your HEN experience.
- A blood sample will be taken from a vein in your arm by a trained person (a phlebotomist) – this may happen on the same day or we will organise a suitable time for you. We will use the blood sample to measure trace element nutrients (iron studies, zinc, copper, selenium), vitamins (vitamin B_{12}, folate, vitamin D), markers of inflammation (C-reactive protein, albumin), and a Full Blood Count. There will be no requirements for fasting before the event.

After the initial appointment, we will ask you to:

- Participate in another four 24-hour diet recalls with the researcher, but this time over the phone, which we will organise at a time that suits you. It will include some week and weekend days. It will take about 15-30 minutes per phone interview. The last 24-h recall interview will also include a few short questions on the presence of other long-term conditions.

What are the benefits of taking part in this study?

There will be no charges made for any of the tests that you undertake. Taking part in this study will help to find out if you have any micronutrient deficiencies. This is also an opportunity for you to tell us about your journey in receiving HEN. Your insights will hopefully lead to further studies and may influence the optimal care of HEN patients. In recognition of your participation, you will receive a $50 supermarket or petrol voucher at the end of the study.

You will also receive a brief report summarising the main findings of the project via mail or e-mail after analysis of the data has been completed. If any of your blood results are outside normal parameters you will be advised to talk to your medical practitioner or at your request, we can send your results directly to them to ensure that you receive the required treatment.

What are the risks of taking part in this study?

There are no risks involved in taking part. Some people may fear having a blood sample taken or experience discomfort when the blood sample is taken. Occasionally, a slight bruise may result. We will take every measure to ensure you are comfortable and respected. You may also be accompanied by a support person if required.

Sample Handling and Storage

Samples will be stored in a secure laboratory freezer at the Human Nutrition Research Unit until completion of the study for a maximum of 10 years. Samples will be analysed by fully accredited laboratories in NZ. The data will be used only for the purposes of this project, and no individual will be identified. Only the investigators and administrators of the study will have access to personal information, and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number to ensure anonymity and confidentiality of these samples. After analysis, any additional blood will be destroyed following usual procedures. However, if you prefer to rather have your blood samples, it can be returned to you if you request it.

Additionally, there may be participants who identify as Māori and if specific concerns develop, the support of Dr Bevan Erueti (Taranaki, Te Ati Haunui-ā-Papārangi, Ngāti Tūwharetoa), Associate Dean Māori, will be afforded. Dr Erueti has expressed that he is happy to act in the capacity of advisor and if required will assist and facilitate the project's Māori agenda and ensure that relational aspects of trust and appreciation are upheld with Māori participants. We are also cognisant that a diversity of beliefs and cultural concerns regarding the removal, storage and transport of tissue samples exists, and because of this, it would be more appropriate to discuss this with your whānau (family) and/or seek take advisement from hapū and iwi leaders. Nonetheless, the right to decline or withdraw from the study can be done at any stage of the project.

Data Handling

The results of the study may be published in journals, presented at conferences or at other professional forums. No individual will be able to be identified. At the end of this study, the list of participants and their study identification numbers will be disposed. Any raw data on which the results of the project depend will be retained

in secure storage for 10 years, after which it will be destroyed. Results of the study will be provided to you if you wish.

Who is funding the research?

The research is funded by the Massey University Research Fund, and the CM Health TUPU Research Fund - "MAATAATUPU" Fund for new or emerging researchers.

Participant's rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Withdraw from the study at any time,
- Decline to answer any particular questions,
- Ask any questions about the study at any time during participation,
- Provide information on the understanding that your name will not be used unless you give permission to the researcher,
- Be given access to a summary of the project findings when it is concluded.

Withdrawing from the study, should you choose to do so, will not result in any disadvantage to you.

Project contacts

If you have any further questions or concerns about the project, either now or in the future, please contact the research team.
The researchers for this study include:
Marcos Mantovani, MSc candidate; Phone: 0220881493; Email: m.mantovani@massey.ac.nz
and
Sally Pattison, MSc candidate; Email: S.Pattison@Massey.ac.nz

The lead researchers for this study are Professor Rozanne Kruger and Mr Andrew Xia.
If you have any concerns, please contact Rozanne at r.kruger@massey.ac.nz; phone +649 213 6661 or Andrew at Andrew.xia@middlemore.co.nz; +64 21 510 941.

Committee approval statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, application 22/20. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone: 04 801 5799 x 63363, email: humanethicssoutha@massey.ac.nz.

The Counties Manukau Health Research Committee has also reviewed and approved this project: Registry No 1631 .

Appendix C: Charlson Comorbidity Index Form

Charlson Comorbidity Index (CCI) Assessment Form

The Charlson Comorbidity Index (CCI) will assess comorbidity level by considering both the number and severity of pre-defined comorbid conditions. This tool will provide prediction comorbidity that can predict short-term and long-term outcomes such as mortality rates. The total score is derived by summing the weight of each comorbid condition presented by each participant. Higher numerical weighting appropriate to its severity indicates a more severe condition and consequently a worse prognosis. Scores are categorized as severe (>5), moderate (3-4), mild (1-2), or no comorbidities (0).

This form has been retrieved from *https://www.mdcalc.com/charlson-comorbidity-index-cci*. This form will be accessed and completed online to facilitate the comorbidity level's sum and estimated percentage change.

Questionnaire: Charlson Comorbidity Index

Interviewer:

Name of interviewer: _____ Date:_____

Qualification (Student, NZRD, Nurse etc.):_____**Time**:_____

Participant ID: _____

Charlson Comorbidity Index (CCI) Assessment Form

Age	≤50 years = 0 50-59 years = +1 60-69 years = +2 70-79 years = +3 ≥ 80 years = +4	
Myocardial infarction (MI) History of definite or probable MI (EKG changes and/or enzyme changes)	No = 0	Yes = +1
Congestive heart failure (CHF) Exertional or paroxysmal nocturnal dyspnoea and has responded to digitalis, diuretics, or afterload reducing agents	No = 0	Yes = +1
Peripheral vascular disease Intermittent claudication or past bypass for chronic arterial insufficiency, history of gangrene or acute arterial insufficiency, or untreated thoracic or abdominal aneurysm (≥6 cm)	No = 0	Yes = +1
CVA or TIA History of a cerebrovascular accident with minor or no residua and transient ischemic attack	No = 0	Yes = +1
Dementia Chronic cognitive deficit	No = 0	Yes = +1

Chronic obstructive pulmonary disease (COPD)	No = 0	Yes = +1
Connective tissue disease	No = 0	Yes = +1
Peptic ulcer disease Any history of treatment for ulcer disease or history of ulcer bleeding	No = 0	Yes = +1
Liver disease Severe = cirrhosis and portal hypertension with variceal bleeding history, moderate = cirrhosis and portal hypertension but no variceal bleeding history, mild = chronic hepatitis (or cirrhosis without portal hypertension)	None = 0 Mild = +1 Moderate to severe = +3	
Diabetes mellitus	None or diet-controlled = 0 Uncomplicated = +1 End-organ damage = +3	
Hemiplegia	No = 0	Yes = +2
Moderate to severe CKD Severe = on dialysis, status post kidney transplant, uraemia, moderate = creatinine >3 mg/dL (0.27 mmol/L)	No = 0	Yes = +2
Solid tumour	None = 0 Localized = +2 Metastatic = +6	
Leukaemia	No = 0	Yes = +2
Lymphoma	No = 0	Yes = +2
AIDS	No = 0	Yes = +6
Is this a COVID-19 patient? For research purposes only; answer does NOT impact results.	Confirmed positive Suspected Unlikely Confirmed negative	
Total Points = sum of points.	Estimated 10-year survival = % survival rate.	

Appendix D: Nutrition Focused Physical Examination Form (NFPE)

Micronutrient Examination Form:
Nutrition Focused Physical Findings

Standard Operating Procedure (SOP)

Operation: Nutrition Focused Physical Examination (NFPE)

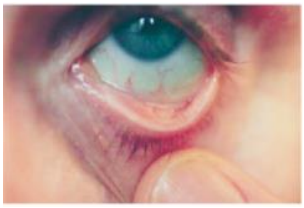
No	Main Operating Steps	Rationale
1	Ensure you and the patient is wearing an appropriate mask covering and that hands are clean and well sanitized.	To protect yourself and the patient from contagious viruses and diseases.
2	Greet the patient and Introduce yourself i.e., NZRD, student, nurse, etc.	Helps to build a good rapport with the patient and ease the assessment.
3	Confirm patient name, date of birth and study number.	Prevents examining the incorrect patient
4	Explain what the examination is and why it's being conducted.	To inform the patients on the procedures i.e., I'm examining you to check for signs of deficiency or malnutrition
5	Consent from the patient: • Ask for the patient's consent before initiating any type examination and touching the patient. For example ask: *Is it okay if I touch you to feel your muscle resistance?*	The patient has the right to make an informed choice about their care and, in most instances, and must give permission to proceed with the examination.
6	Offer a chaperone or ask for a family member to be present during the whole physical examination	For safety of the interviewer and patient: to help protect and enhance the patient's comfort, safety, privacy, security, and/or dignity during sensitive examinations or procedures.
7	Ask patient if they have any questions or concerns	Allow the interviewer to qualify any questions and concerns.
8	**Initiate examination:** 1. Begin examination from the head and move down the body towards the lower body. 2. Only examine the next part of the body after you have completed the part that you are at. 3. Ensure you use the *Score Rating* "YES-NO" approach. Mark a "X" on the "YES" or "NO" box for signs of deficiency. 4. Read each the NFPE finding's description (for *MICRONUTRIENTS*) carefully, and utilise the IMAGES provided to assist the examination. 5. Stop physical examination if patient says "NO" or "STOP" (before or during examination). 6. Complete the examination and thank the patient for their time. **Additional comments:** • Make necessary notes. • Respect patient's decision change • Show gratitude for the patients' time.	

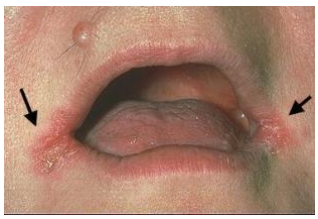
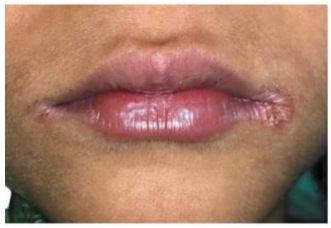
Micronutrient Assessment Form: Nutrition Focused Physical Examination

Name of interviewer: _____ **Date**:_____ **Time**:_____

Qualification (Student, NZRD, Nurse etc.):_____

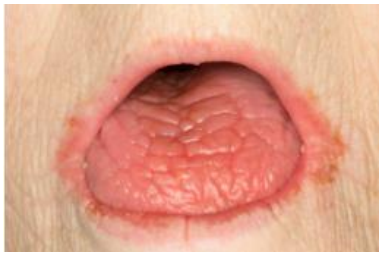
Patient study #number: _____

PHYSICAL SIGNS	PRESENCE OF DEFICIENCY		POSSIBLE NUTRIENT DEFICIENCY	POSSIBLE NON-NUTRITION-RELATED CAUSES
	Yes	No		
HAIR				
Alopecia Thin, sparce, patchy, baldness.			Zinc and Iron	At risk of alopecia: Asthma, thyroid disease, atopic dermatitis, psoriasis, vitiligo, rheumatoid arthritis, irritable bowel disease, lupus, down syndrome, hereditary.
Alopecia **Signs and symptoms**: thin, sparce, patchy, baldness.  Alopecia (male)  Alopecia (female)				
EYES	**Yes**	**No**		
Pale Conjunctiva Little or no evidence of red colour on the anterior rim.			Iron, Vitamin B_{12}	Non-nutritional anaemia
Pale conjunctiva **Signs and symptoms**: Little or no evidence of red colour on the anterior rim.  Pale Conjunctiva 				

MOUTH	Yes	No		
Angular Stomatitis or Cheilitis Redness, scars, swelling or fissures at corners of mouth.			Folate	Excessive salivation due to ill-fitting dentures; Dry skin; Dehydration; Herpes
Angular Stomatitis or Cheilitis **Signs and Symptoms**: Redness, scars, swelling or fissures at corners of mouth.    Angular Stomatitis / Angular Cheilitis (Cheilosis)				

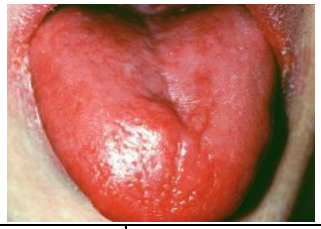
Perioral stomatitis Inflammation of the skin around the mouth. Rash around that circles the mouth.			Zinc	

Perioral stomatitis
Deficiency: Zinc
Signs and symptoms: inflammation of the skin around the mouth. Rash around that circles the mouth.



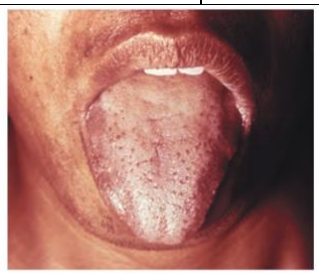
TONGUE	**Yes**	**No**		
Glossitis Sore, swollen, red and smooth tongue)			Vitamin B_{12}, Severe Iron deficiency	Crohn's; Uraemia; Infection; Malignancy; Anticancer therapy; Trauma

Glossitis
Signs and Symptoms: sore, swollen, red and smooth tongue



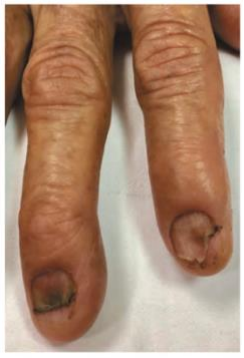
Pale Tongue Discoloured (light red/white)			Anaemia: Iron; Vitamin B_{12}	

Pale Tongue
Signs and Symptoms: discoloured (light red/white)

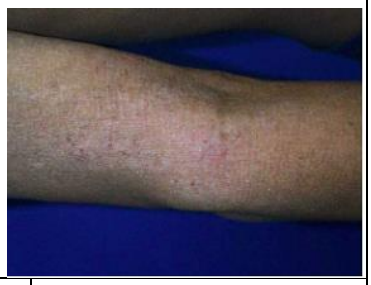


Pale Tongue

TASTE	**Yes**	**No**		
Hypogeusia - altered/diminished sense of taste (less distinct flavours – less capable of tasting sour, sweet, salty, savoury, and bitter foods; **Dysgeusia** - altered sense of taste due to bad taste in the mouth e.g., bitter taste, metallic taste; **Ageusia** - total loss of taste **Deficiency**: Zinc **Tips**: ask patient for change in taste i.e., Have you noticed any differences in flavour/taste when			Zinc	Medications such as antineoplastic agents or sulfonylureas

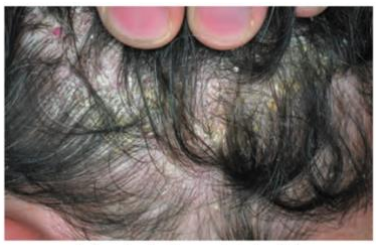
eating foods that you are familiar with? **Image**: N/A				
NAILS	**Yes**	**No**		
Beau's lines Transverse ridges, horizontal grooves on the nail.			Severe zinc deficiency	Severe illness (i.e. MI or high fevers); Immunosuppressive therapy or chemotherapy
Beau's lines **Signs and Symptoms**: transverse ridges, horizontal grooves on the nail.   Beau's Lines				
Koilonychia Spoon-shaped, concave.			Iron	Considered normal if seen on toenails only; Diabetes; Systemic Lupus; Raynaud's Disease: Hypothyroidism
Koilonychia **Signs and Symptoms**: spoon-shaped, concave.  Koilonychia				
Central ridges/vertical ridges Appearance of vertical or horizontal ridge.			Iron	Severe arterial disease
Central/Vertical ridges **Signs and Symptoms**: Appearance of vertical or horizontal ridge  Vertical Ridges				
SKIN	**Yes**	**No**		
Eczema Dermatitis Dry patched skin that rapidly evolve to a red rash (may be blistered and swollen).			Zinc	Atopic dermatitis

Eczema Dermatitis
Signs and Symptoms: dry patched skin that rapidly evolve to a red rash (may be blistered and swollen)

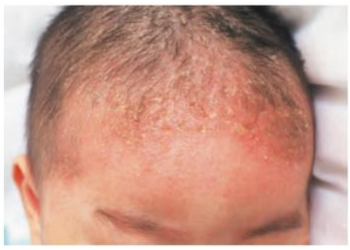


Seborrheic Dermatitis Scaliness, waxy, oiliness, crusty plaques on the scalp, lips and nasolabial folds.			Zinc,	Nasal drainage

Seborrheic Dermatitis (Scalp, Face/Nasolabial and Forehead/Eyebrows)
Signs and Symptoms: scaliness, waxy, oiliness, crusty plaques on the scalp, lips and nasolabial folds

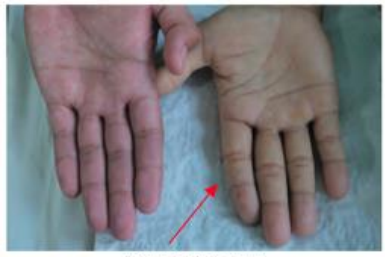


Seborrheic Dermatitis (scalp)

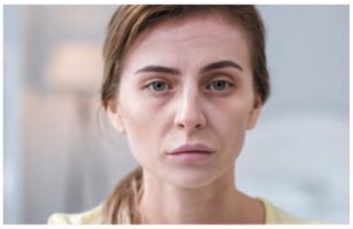


Pallor Paleness in skin, face, and/or hands			Anaemia: Iron, Vitamin B_{12}	Blood loss

Pallor Skin
Signs and Symptoms: paleness in skin, face, and/or hands



Pallor (pale hands)



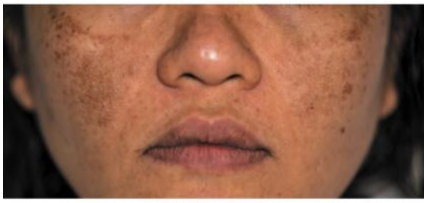
Pallor (pale skin/face)

Hypopigmentation Loss of pigment in patches (melanocytes).			Severe Copper deficiency	Genetic and autoimmune skin disorder (e.g., vitiligo).

Hypopigmentation
Signs and Symptoms: loss of pigment in patches (melanocytes).

Vitiligo

Hyperpigmentation Patches of the skin darken in colour. Most commonly on the face and extremities (dorsum of the hands and feet joint location of fingers and toes).			Vitamin B_{12}	

Hyperpigmentation **Signs and symptoms**: Most commonly on the face and extremities (dorsum of the hands and feet joint location of fingers and toes).  Hyperpigmentation				
Slow wound healing, Decubitus Ulcers delayed wound healing and appearance of pressure ulcers on the skin. **Tips**: Ask patient • How long has the wound been present? • Has the size of the wound increased? • Check common sites that are often affected by pressure ulcers (sacrum, heels, elbow). **Image**: N/A			Zinc	Poor skin care, Diabetes, Steroid use

Appendix E: 24-hours Diet Recall Assessment Form

Micronutrient Status Investigation:
24-hour Diet Recall (Assessment Form)

Standard Operating Procedure

Operation: 24-hour Diet Recall Interview

The 24h diet recall interview is expected to take 20 to 30 minutes to complete, depending on the different amount of food consumed each day.

Follow the steps below for the in-person and phone interview. The SOP will help maintain the same standard method of collecting the 24-h diet recall for each patient.

Prior to the interview ensure the operational steps are followed:

1. Interviewer and patient must wear a face mask at all the time during interview, and have hands clean and sanitized with alcohol gel.
2. Greet the patient and Introduce yourself i.e., NZRD, student, nurse, etc.
3. Confirm patient name, date of birth and study number.
4. Explain what the interview is and why it's being conducted.
5. Ask for consent to begin interview.
6. Stop interview if patient says "NO" or ask to "STOP" (before or during interview).
7. Complete the examination and thank the patient for their time.

The 24-hour diet and enteral feeding recall will be collected in three phases:

1. A quick list of foods eaten, drunk and enteral feeding.

- Begin asking the subjects to recall and describe all the foods, drinks consumed and enteral feeding (including flushes) in the previous 24 hours, from waking to sleeping or from midnight to midnight. At the end of the recall, ask patient to report additional item not initially recalled.

2. Collection of detailed information concerning the items in the quick list.

❖ For each item of food, drink and enteral feeding ask participants to provide additional details.

1. The time at which the food and drink was consumed, including all meals, snacks, drinks, "nibbles", sweets etc.
2. A full description of the food or drink, including brand name where available.
3. Any foods likely to be eaten in combination e.g. milk in coffee
4. Recipes and other combinations of foods e.g. sandwiches
5. The quantity consumed, portion sizes of foods based on participants measure e.g., 6 tablespoons, 1cup, 1slice, etc.
6. Any leftovers or second helpings

3. Participants are given an opportunity to provide additional information and for the interviewer to prompt for information about foods or drink not mentioned.

- Interviewer reviews all the food eaten and drunk in chronological order, clarifies any uncertainty regarding type of food eaten or portion size.
- Asks the participant to name the place where each food or drink item was consumed.
- Record all of the information gathered on the 24-h diet recall sheet provided.

Detailed information concerning Enteral Feeding Prescription:

- Recall the following information about the patient's daily enteral feeding routine (complete in *ENTERAL FEEDING PRESCTION* section).

  1. Enteral formula e.g., Nutrison 1.5kcal
  2. Enteral access/delivery site e.g., Gastrostomy (PEG)
  3. Method of administration e.g., Pump-assisted, gravity-assisted, bolus (syringe)
  4. Administration rate e.g., 800mL (mL feeding), 12h (feeding over), 1 time/day (times daily)
  5. Flushes e.g., 50mL water, before and after each feeding, and other times as instructed.

24-HOUR DIET AND ENTERAL FEEDING RECALL FORM

Name of interviewer: _____Date:_____ **Time**:_____

Qualification (Student, NZRD, Nurse etc.):_____

Patient study #number: _____

Day of the week/weekend: _____

Time	Food, beverage consumed, Supplements and Enteral Nutrition	Quantity/Amount consumed	Method of preparation, Brand name, Delivery site	Comments

ENTERAL FEEDING PRESCRIPTION

ENTERAL NUTRITION FORMULA:
DELIVERY SITE (ROUTE AND ACCESS):
METHOD OF ADMINISTRATION (METHOD AND RATE): mL feeding_____over_____min_____times daily_____
Amount of Water Taken/Flush feeding tube: mL of water_____times daily_____
Other/Comments:

Appendix F: FoodWorks Analysis (SOP)

FoodWorks Analysis (SOP)

General process:

- Enter as a 5-day food record*
- General tab:
  - Make name participant ID
  - Complete age/gender/weight/height
  - In description, write "entered by…"
  - In description, enter any other relevant notes from the file
- Foods tab:
  - Enter date food was consumed (DAY column)
  - Enter time food was consumed in 24-hour time (MEAL column)
  - Enter food or drink (FOOD column).
  - Enter quantity (QUANTITY column). Where possible use ml/g.
  - Record route of intake in NOTES column.
  - Record any other information in NOTES column
- Record anything that is not immediately obvious or clear in a shared file to go through later
- If the time is not clear/ cannot be assumed, put 0000
- For continuous feeds, enter total day volume at feed start time. Enter the flushes at times specified
- If no flavour of ONS specified, check photos to see if you can identify flavour. If none specified, or is via tube only – pick vanilla
- For water flushes, enter these as consumed (before/after or both)
- In FoodWorks spreadsheet note the date, person and completion status

Appendix G: Journal requirements: *Nutrition Journal*

See https://nutritionj.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research

Research

Criteria

Research reports data from original research and clinical trial outcomes. The journal doesn't consider animal studies.

Nutrition Journal supports the complete and transparent reporting of research. The Editors require the submission of a populated checklist and flow diagram from the relevant reporting guidelines, including CONSORT for randomised controlled trials, STROBE-Nut for nutritional epidemiologic studies, and SRQR for qualitative research. The checklist should be provided as an additional file and if available, the flow diagram should be included in the main body of the text; both the checklist and flow diagram should be referenced in the text. Submissions received without these elements will be requested by the Editor. A Word file of the checklists (and flow diagrams) can be downloaded via the EQUATOR Network.

It is understood that for some studies certain aspects of the report may not comply fully with the pre-specified checklist. The checklist will not be used as a tool for judging the suitability of manuscripts for publication in *Nutrition Journal*, but is intended as an aid to authors to clearly, completely, and transparently let reviewers and readers know what authors did and found. Using these guidelines to write the report, completing the checklist, and constructing a flow diagram are likely to optimize the quality of reporting and make the peer review process more efficient.

Authors are encouraged to consult guidelines regarding p-values and statistical significance and to choose, report, and interpret statistical tests and procedures appropriately. The use of an arbitrary p-value to identify significant findings is discouraged. Authors are also encouraged to consider sex and gender in study design, analyses, and interpretation.

Nutrition Journal recommends the use of **person-first language** to speak appropriately about individuals with a disability. For example, when referring to a person with a stroke or diabetes, refer to the person first using a phrase such as 'a person with a stroke' or 'a person affected by diabetes'. This also pertains to descriptions of body weight and eating disturbances, for example, refer to 'people with obesity' or 'people affected by overweight and obesity' or 'people affected by disordered eating'.

Nutrition Journal strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories. Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. A list of data where deposition is required, with the appropriate repositories, can be found on the Editorial Policies Page.